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DETECTION OF *SALMONELLA* AND *E.COLI* IN EDIBLE BIRD'S NEST RANCHED IN HOUSING SYSTEM

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DETECTION OF *SALMONELLA* AND *E.COLI* IN EDIBLE BIRD'S NEST RANCHED IN HOUSING SYSTEM

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Detection of *Salmonella* and *E.coli* in Edible Bird's Nest Ranched in Housing System", by Norfaridah binti Mohamad Razak 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-Project

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DEDICATIONS

This project paper is dedicated to the Almighty Allah, who had made all things possible,

To my beloved family,

Mother & father

Brothers

Sisters-in-law

Nieces and nephews

My late nephew

And to my friends and all my teachers and lecturers who have commited toward the noble cause of education.

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LIST OF ABBREVIATION

%	percent
BGA	Brilliant Green Agar
BPW	Buffer Peptone Water
CFU	Colony forming unit
EBN	Edible Bird's Nest
EMBA	Eosin Methylene Blue Agar
g	Gram
LIA	Lysine Iron Agar
ml	mililiter
^{o}C	Degree celsius
RV	Rappaport-Vassiliadis Enrichment Broth
SIM	Sulphide Indole Motility
SIRIM	Standard and Industrial Research Institute of Malaysia
SPC	Standard Plate Count
TSI	Triple Sugar Iron agar
XLD	Xylose Lysine Deoxycholate

ABSTRACT

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

DETECTION OF *SALMONELLA* AND *E.COLI* IN EDIBLE BIRD'S NEST RANCHED IN HOUSING SYSTEM

by

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2016

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Co-supervisor : Prof. Dr. Saleha Abdul Aziz

The swiftlet industry in Malaysia is growing very fast due to the high demand for Edible Bird's Nest (EBN) . The presence of bacteria may produce low quality of EBN that can lead to economic loss and may cause food-borne diseases. The aim of this study was to detect the presence of *Salmonella* and *E.coli* in EBN and guano of edible-nest swiftlets ranched in housing system and to enumerate the total number of bacteria and coliform in EBN. In this study, a total of 64 guano and nest swab samples were collected from three bird houses in Terengganu. The samples were pre-enriched and enriched before culturing on Brilliant Green Agar (BGA) and Xylose Lysine

Deoxycholate (XLD) for isolation of *Salmonella* and propagated in nutrient broth before culture on Eosin Methylene Blue Agar (EMBA) for isolation of *E.coli*. The isolation of the bacteria was carried out in Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, UPM. Standard Plate Count (SPC) and Coliform count using 3M® Petrifilm were done. No *Salmonella* was isolated in EBN and the prevalence of *Salmonella* in guano was 12.5% as compared to *E. coli* in EBN at 3.13% and 68.75% in guano sample. The average SPC of EBNs was 3.2 x 10^5 CFU per gram and CPC was ≤ 100 CFU per gram . *E.coli* in EBNs swab were lower than in guano samples. Hence, the absence of *Salmonella* and low number of *E.coli* in EBN is a good news for the growing swiftlet industry.

Keywords *: Edible-nest Swiftlet, Edible Bird's Nest, Housing system, Salmonella, E.coli*

ABSTRAK

Abstrak daripada kertas kerja projek yang dikemukan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian keperluan kursus VPD 4999 – Projek

PENGESANAN *SALMONELLA* DAN *E.COLI* PADA SARANG BURUNG YANG DIBIAKKAN DI DALAM SISTEM RUMAH BURUNG

oleh

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2016

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Industri sarang burung walit di Malaysia semakin pesat berkembang seiringan dengan permintaan sarang burung yang tinggi. Kewujudan bakteria mengurangkan kualiti sarang burung yang menyebabkan kerugian dan menimbulkan penyakit berpunca pemakanan. Tujuan utama penyelidikan ini adalah untuk mengenalpasti kehadiran *Salmonella* dan *E.coli* di dalam sarang burung walit dan najis burung walit yang dibiak dalam sistem rumah dan untuk mengira jumlah bakteria dan koliform di dalam sarang burung. Dalam kajian ini, sejumlah 64 sampel najis dan swab sarang

burung telah diambil daripada tiga rumah burung di Terengganu, Malaysia. Sampel dibiakkan di dalam cecair nutrisi sebelum dikultur di atas Brilliant Green Agar (BGA) and Xylose Lysine Deoxycholate (XLD) untuk isolasi *Salmonella* dan Eosin Methylene Blue Agar untuk isolasi *E.coli*. Prosedur isolasi telah dijalankan di Makmal Veterinar Kesihatan Awam, Fakulti Perubatan Veterinar, UPM. Kaedah Standard Plate Count (SPC) dan bilangan koliform dikira menggunakan 3M® Petrifilm. Tiada *Salmonella* dalam sarang burung dan 12.5 % *Salmonella* positif dalam sampel najis berbanding hanya 3.13% untuk sarang burung positif *E.coli dan* 68.75% dari sampel najis. Purata SPC dari sarang burung walit adalah $3.2x10^5$ CFU/g manakala purata CPC sebanyak 100 CFU/g. Isolasi *E.coli* dari swab sarang burung lebih rendah berbanding sampel najis. Ketiadaan *Salmonella* dan jumlah *E.coli* yang sedikit di dalam sarang burung merupakan satu berita yang baik untuk perkembangan industri sarang burung walit.

Kata kunci *: burung walit, sarang burung, sistem rumah, Salmonella, E.coli*

1.0 INTRODUCTION

Edible Bird's Nest (EBN) is a natural saliva nest (Saengkrajang and Matan, 2011), produced by white-nest swiftlet (*Aerodramus fuciphagus*) and black-nest swiftlet (*Aerodramus maximus*), which are highly traded worldwide (Babji *et al*., 2015). In Malaysia, the swiftlet industry is growing very fast due to the high demand and high value of EBN at the international market. The major demand is from the Chinese communities around the world, mainly China, Taiwan, Singapore, North America, there are new emerging markets such as Middle East, Japan and Korea (Babji *et al*., 2015). EBN has been consumed as an expensive delicacy for various health benefits. Eventhough many studies has been done to evaluate the nutritional properties in EBN. So far, the benefits, nutritional and non-nutritional contents of EBNs remain undetermine clearly (Kew *et al*., 2014). However, the demand for EBN remain high because it is not just a pleasant food to be consumed, but also it has been traditionally used to provide health benefits, such as aiding digestion, raising libido, improving the voice, alleviating asthma and improving concentration (Babji *et al*.,2015).

In 2011, China has listed EBNs from Malaysia as banned products due to the high level of nitrites (Kew *et al*., 2014). To prevent such incidence from reoccur, food safety aspects is very important to improve the sustainability of the swiftlet industry in Malaysia. However, as in other food, contaminants that are present in the EBN as well as the growth of microbes such as bacteria,virus,yeast and fungi (Oktorina *et al*., 2005) may produced low quality EBN that lead to economic loss, but also may cause food-borne diseases. There have been reports that EBN may cause side effects such as

allergic symptoms and food-induced anaphylaxis among children (Goh *et al*., 1999 ; Kemp *et al*.,2010; Kew *et al*.,2014) and adults (Thong *et al*., 2005,2007 ; Kew *et al*.,2014).

As the price of the EBN is determined by the quality of EBN, a standard guideline has been set by the Standard and Industrial Research Institute of Malaysia (SIRIM). It is mainly to ensure the acceptability of EBNs at the international market. It is stated in the guideline that EBN must not contain *Salmonella* spp. and the microbial content by Total Plate Count should be ≤ 2.5 x10^6 CFU/g, whereas the Coliforms Count should be ≤ 100 CFU/g (Kew *et al*., 2014) (Refer to Table 1).

Table 1. Microbiological requirements of raw-unclean EBN after pre-cleaning

Category	**Parameters**	**Tolerance level**	**Method of tests**
Microbiological analysis	Total Plate Count	≤ 2.5 x 10^5 cfu/g	Bacteriological Analytical manual (BAM) Method or equivalent method
	Coliform Count	≤ 1.0 x 10^2 cfu/g	
	Salmonella enteritidis	nil	
	Salmonella typhimurium		
	Salmonella pullorum		
	Salmonella gallinarum		

Following this standard, the level of food safety of EBN can be continously monitored by the authorities.

Eventhough the bird nests are made up of the nest cement produced by the salivary gland of the birds (Babji *et al*.,2015) solely, EBN also contains a lot of impurities such as feathers, eggs fraction, bird droppings, dirt fleas, and sands which may contaminate the nest (Utomo *et al*., 2014). Hence, as the EBN is intended for human consumption, it is crucial to evaluate the food from any food borne pathogens which are of major public health concern all over the world.

Thus, the main aim to study were :

1. to determine the presence of *Salmonella* and *E.coli* bacteria in EBNs and guano

2.to enumerate the total number of bacteria and coliform in EBNs.

2.0 LITERATURE REVIEW

2.1 *Salmonella*

Salmonella enterica and *S. bongori* are two species of *Salmonella* that can cause salmonellosis in human and animals (OIE, 2010). It is the normal flora of poultry and most animals which is one of the major public health concern worldwide as it may cause food borne illness (Boonmar *et al*.,1998; Forshell and Wierup, 2006 ; Kanwal *et al*., 2015). *Salmonella* may be present in the environment and may commonly be found in farm effluents, human sewage and in any material subject to faecal contamination (OIE,2010).

The main route of disease transmission is from water or food contaminated with faeces of infected animals. In addition, the direct contact between susceptible and infected animals (Kuroki et al., 2013) also may cause the transmission of *Salmonella*. Most *Salmonella* serotypes are pathogenic to humans, and clinical signs of the disease vary according to the serotype (Suelen, 2015). Many studies also revealed that *Salmonella typhimurium* is a major pathogenic *Salmonella* which is transmitted through food (Boonmar *et al*.,1998; Forshell and Wierup, 2006 ; Kanwal *et al*., 2015). It can affect humans and animals. Swiftlets, like other wild birds have an important role in the dissemination of this disease (Winfield *et al*.,2003). However, to determine either they show any clinical signs is quite questionable as no close monitoring has been done on these birds.

2.2 *E.coli*

Escherichia coli which is commonly known as *E.coli* belongs to Enterobacteriaceae family. It is of public health concern in most countries. *E.coli* resides in the intestinal tract of healthy animals, represents a source of direct and indirect infection to humans. This bacteria has adhesion fimbrae that allow them to bind at intestinal mucosa and cause damage to the absorptive surface of intestine, leading to diarrhea (Vincent *et al*., 2010 ; Kanwwal *et al*., 2015). Most of the clinical manifestation to human or animals infected with *E.coli* is diarrhea. The infection can be diagnosed based on isolation of pathogen from faeces, blood, and urine (Suelen *et al*., 2015). Besides, *E. coli* can cause mortality in birds, that eventually lead to significant economic losses all over the world (Schouler *et al*., 2012 ; Suelen *et al*., 2015), which commonly affects the poultry industry. However, even in a bird population with high number of animals positive for *E. coli*, there may be no sick animals, as many serotypes are commensals. Nevertheless, the possible pathogenicity of these strains to human is unavoidable.

According to a study done by Sien *et al*., (2013) , researchers have isolated *E.coli* from the air inside the swiftlet houses at Sarikei, Sarawak by using 16S rRNA gene sequence analysis. Another study revealed the isolation of *E.coli* positive from three out of ten swiftlet houses as shown in the Figure 1 (Sien *et al*., 2013). This study showed that it is normal to find *E.coli* in the swiftlets (Simpson, 2002; Sien *et al*., 2013) as their normal flora.

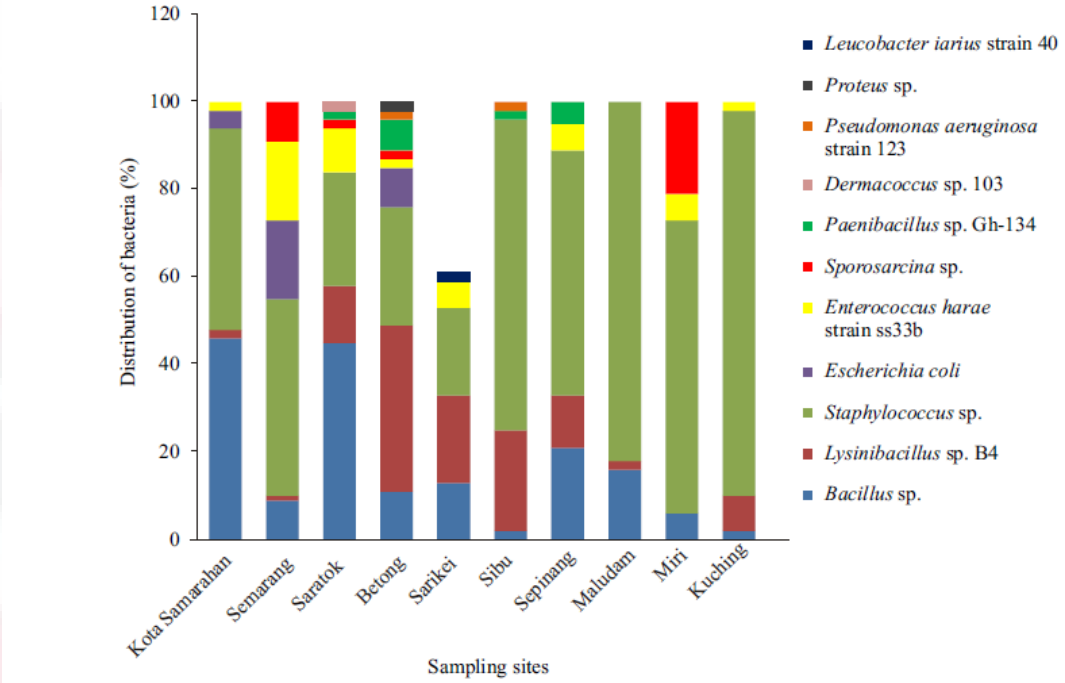

Figure 1. Distribution (%) of bacteria in swiftlet faeces, Sarawak, Malaysia

(Sien *et al*., 2013)

2.3 The trends of EBN ranched in housed-system

Traditionally, EBN is harvested from limestone caves in Borneo Island, such as Niah Cave in Sarawak , Mandai and Guamantong Caves in Sabah (Ismail 1999 and Lim and Cranbrook, 2002). In compliance with the increase demand for EBN, Ministry of Agriculture and Agro-based Industries emphasis the swiftlet ranching activity is conducted in man-made house system which is also known as swiftlet houses which imitate cave-like environment (Koon, 2011; Vaiappuri *et al*., 2012) such as the temperature, humidity, light intensity are controlled by human. Lately, the swiftlet houses are found in the residential and urban areas (Azizon *et al*., 2013). In this type of farming, the rancher does not control the movement, breeding and diets of the

birds (Vaiappuri *et al*., 2012). Instead, the swiftlets are totally free to do their own activities without any interference from humans.

The main difference between the natural habitat and the man made swiftlet houses is easier the harvesting process of the bird nest. A succcessful swiftlet ranched in house system is able to increase the production of bird nest and it is the best way to gain profit with low operating cost. As a result, there are many swiftlet houses available in Malaysia from the past few years.

3.0 MATERIALS AND METHODS

3.1 Sample collection

A total of 64 swabs of unharvested EBN and fresh guano droppings were collected from three different bird houses (Birdhouse A,B and C) in the state of Terengganu (Figure 2a, 2b and 2c). All the samples were collected using simple random sampling technique.

Clean food wrapping paper were placed at few spots in each bird house . The spot was chosen randomly where there were presence of the bird nest attached to the nest board (papan sarang) on the ceiling (Figure 3). The papers were left overnight and the guano samples were collected on the next morning (Figure 4). Each of the

bird nest swabs was kept in a sterile bottle (Figure 5) and the dropping/guano was kept in the sterile plastic bag (Figure 6). Twelve raw unclean white EBN was collected at random from Farm A.

All samples were immediately stored in an ice box and transported to the Veterinary Public Health Laboratory for microbiological works. The raw EBN was kept at room temperature (Figure 7). The laboratory works on samples were conducted within less than 24 hours after sampling.

Figure 2a. Birdhouse A

Figure 2b. Birdhouse B

Figure 2c. Birdhouse C

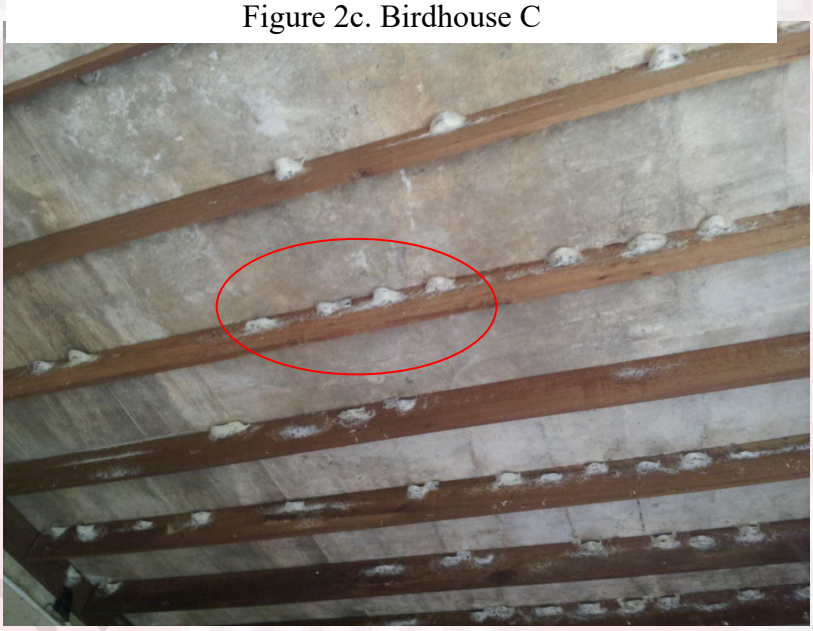

Figure 3. EBN attached to the nest board on the ceiling

Figure 4. Food wrapping paper left overnight in the birdhouse

Figure 5. EBN swabs were kept in sterile bottles

Figure 6. Guano samples were kept in sterile plastic bags

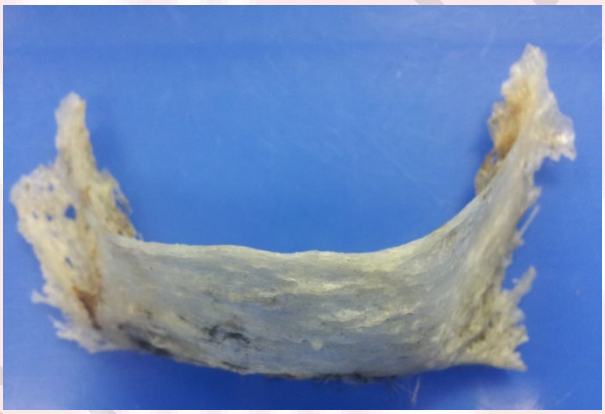

Figure 7. Raw uncleaned EBN harvested from Birdhouse A

3.2. Isolatio

At the site of sampling, the swab from each of the nest was placed in a sterile bottle whereas the pooled guano samples were placed in a sterile plastic bag. Upon arrival at the laboratory, each swab was transferred into 9 ml of Buffer Peptone Water (BPW) . The guano samples were weighed to 1 gram per site of guano collection before transferred into the 9 ml of BPW . The BPW containing the sample was incubated for 18 to 24 hours at 37^{o}C for pre- enrichment. Then, 1 ml of each pre-enriched mixture was transferred into 10 ml of Rappaport-Vassiliadis (RV) Enrichment Broth (Oxoid), incubated for 18 to 24 hours at 42 ^{O}C for enrichment. The enriched mixture was then

inoculated onto Xylose Lysine Deoxycholate(XLD) agar (Oxoid) and Brilliant Green Agar (BGA) and then the plates were incubated for 24 to 48 hours at 37 ^{O}C. The colonies of the *Salmonella* appeared on BGA as pink to red colonies and the *Salmonella* colonies on XLD agar appeared as pink to red colonies with blackish center. The suspected positive *Salmonella* colonies were then inoculated onto Nutrient agar, incubated for 18 to 24 hours for 37 ^{O}C to obtain the pure culture.The pure cultures obtained were subjected to confirmatory biochemical tests which were Triple Sugar Iron (TSI) agar, Lysine Iron Agar (LIA), Sulphide Indole Motility (SIM), Citrate and Urease tests. The *Salmonella* isolates were confirmed by the slide agglutination test using the commercial *Salmonella* polyvalent O anti-sera (Refer to Appendix 9.1).

3.3. Isolation and identification methods for *E.coli*

1 g sample was added to 9 ml of Buffered Peptone Water (BPW) at ambient temperature, incubated for 16–20 hours at 37°C. Then, inoculated the samples onto the Eosin Methylene Blue Agar (EMBA), incubated aerobically at 37 ^{0}C for 24-48 hours. The colonies with green metallic sheen on EMBA agar was presumptively for *E.coli*. The suspected colonies were subcultured on Nutrient agar to get pure cultures and subsequently were identified based on biochemical reactions. It was tested with Kovacs reagent as confirmatory test in which the reagent change to pink color if there was presence of *E.coli* (Refer to Appendix 9.2).

3.4 Microbial content of raw unclean EBN

Each EBN was pre-cleaned by using sterile forceps to remove feathers and dirt. The number of microorganisms in the raw pre-clean EBN sample was quantified using Standard Plate count method whereas the number of coliform by using 3M® pertrifilm. After weighing the EBN, it was fragmented into smaller pieces using sterile forceps. The sample was then added into BPW using 1 part of the sample to 9 part of BPW. Then, the sample was homogenized using a stomacher for about 2 minutes before preparing the dilution. The dilution of sample was carried out by adding 1 part sample to 9 part BPW from 10^{-1} to 10^{-9} dilutions. After that, plating of the dilutions was carried ou using surface plate method. In this method, 0.1ml aliqout from each dilution was pipetted onto a Standard Plate Count agar and the inoculum was then spread out over the entire surface of the agar using a L-shaped disposable spreader as quickly as possible (Refer to Appendix 9.3).

3.5 Statistical analysis

The bacteriology results were analysed and compared using SPSS version 20.

4.0 RESULTS

4.1 Isolation of *Salmonella* and *E.coli* in EBNs and guano samples

In this study, a total of 64 samples of EBN's swab and guano samples were obtained from Birdhouse A, B and C. Out of 32 samples of EBN's swab tested, no *Salmonella was* isolated and only 1 (3.13%) sample was positive for *E.coli*. From a total of 32 guano samples, 4 (12.5 %) were positive for *Salmonella* and 22 (68.75%) were positive for *E.coli* (Figure 8).

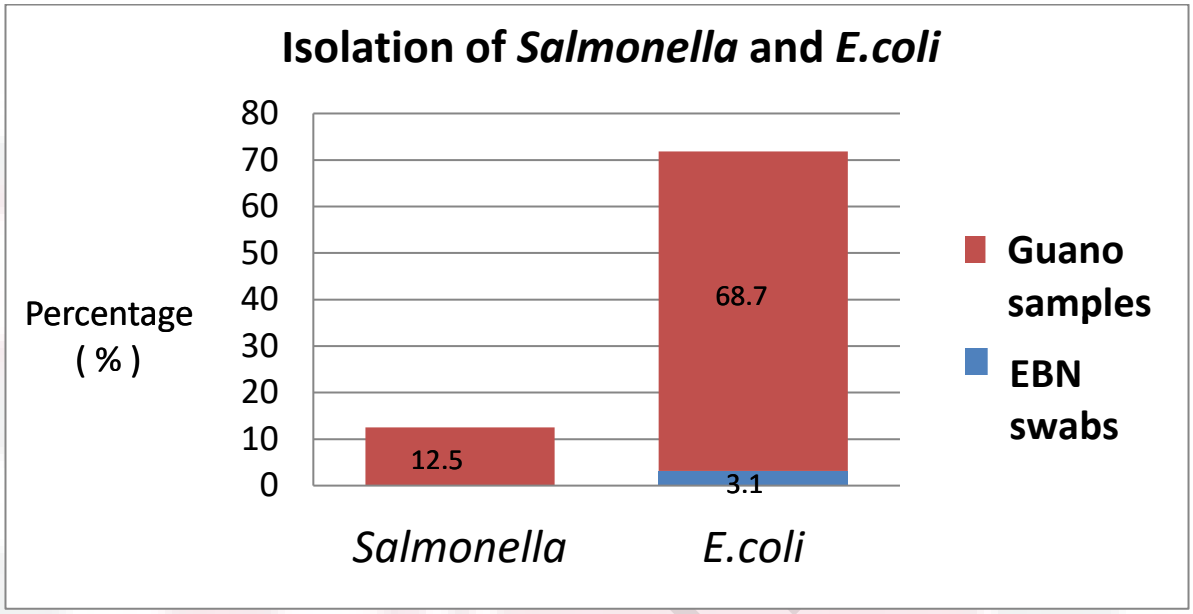

Figure 8. Isolation of *Salmonella* and *E.coli* in EBNs and guano samples

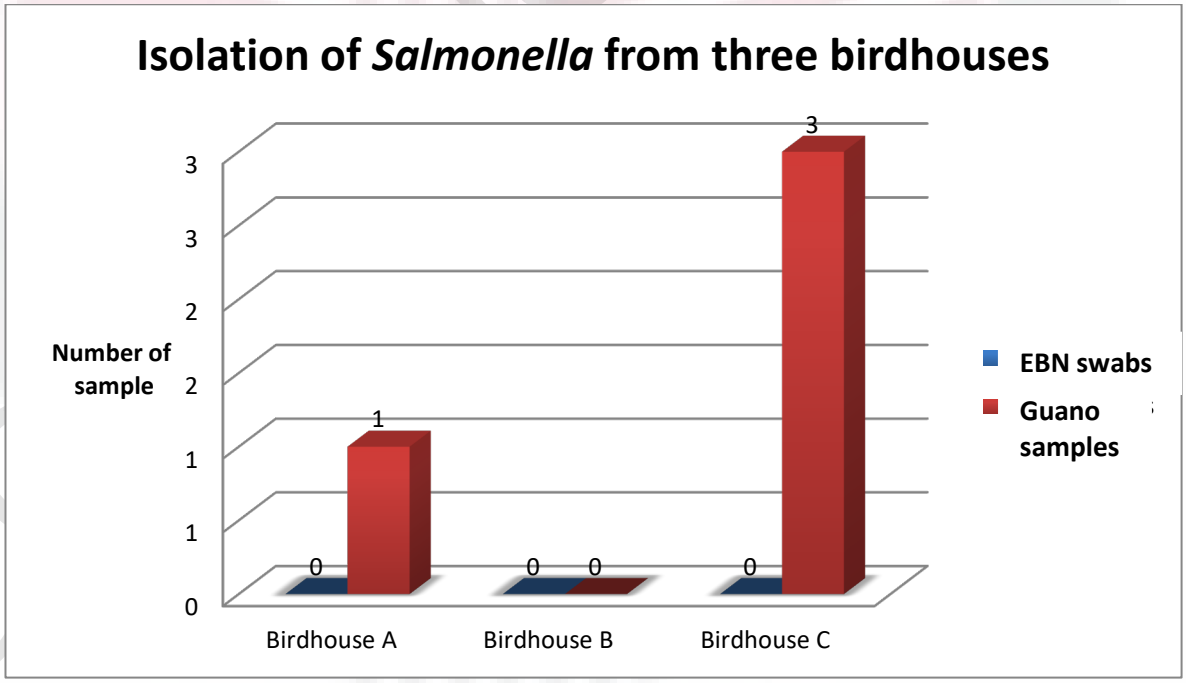

Figure 9. Occurence of *Salmonella* in Birdhouses A, B and C

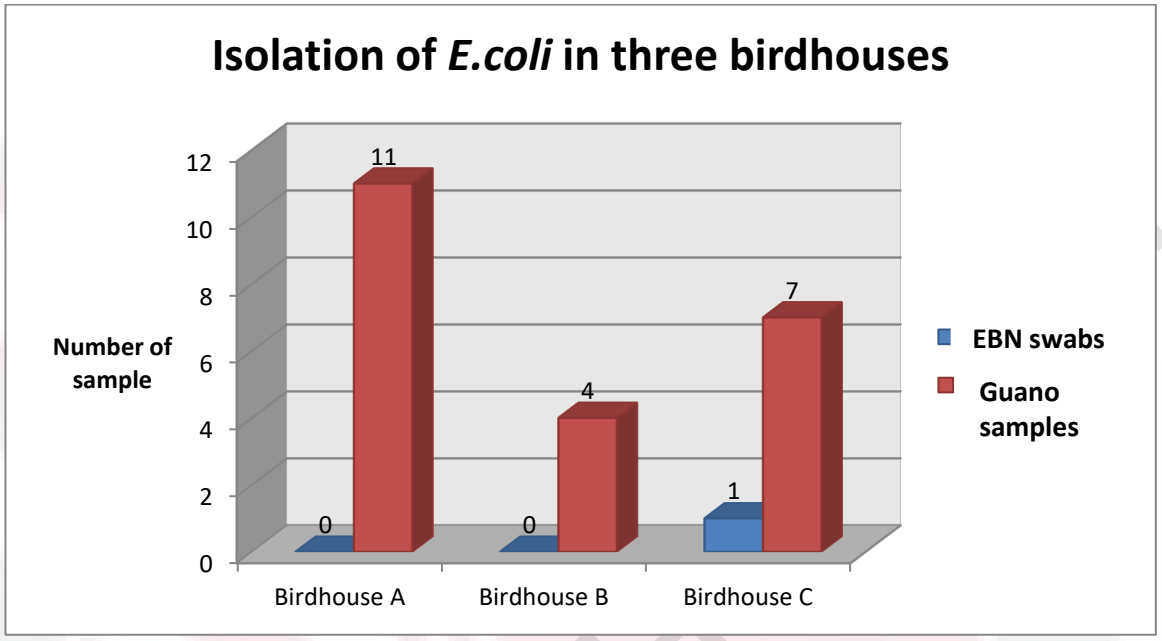

Figure 10. Occurence of *E.coli* in Birdhouses A, B and C

4.2. Microbial content of raw unclean EBNs

The colony counts were performed after 24 hours of incubation at 37 ^{O}C. The results for 12 raw unclean EBN after pre-cleaned were recorded as shown in Table 2 below.

Table 2 : The microbiology result of raw unclean EBN after pre-cleaning

Sample	Standard Plate Count (CFU/g)	Coliform Count (CFU/g)	Tolerance limit
EBN 1	4.5×10^6	$\leq 1.0 \times 10^2$	Exceed
EBN 2	8.6×10^5	$\leq 1.0 \times 10^2$	Not exceed
EBN 3	4.1×10^5	$\leq 1.0 \times 10^2$	Not exceed
EBN 4	$< 25 \times 10^4$	$\leq 1.0 \times 10^2$	Not exceed
EBN 5	$< 25 \times 10^4$	$\leq 1.0 \times 10^2$	Not exceed

EBN 6	$< 25 \times 10^4$	$\leq 1.0 \times 10^2$	Not exceed
EBN 7	$< 25 \times 10^4$	$\leq 1.0 \times 10^2$	Not exceed
EBN 8	3.6×10^5	$\leq 1.0 \times 10^2$	Not exceed
EBN 9	2.8×10^4	$\leq 1.0 \times 10^2$	Not exceed
EBN 10	2.5×10^3	$\leq 1.0 \times 10^2$	Not exceed
EBN 11	5.9×10^4	$\leq 1.0 \times 10^2$	Not exceed
EBN 12	1.0×10^4	$\leq 1.0 \times 10^2$	Not exceed
Average	**3.2×10^5**	**$\leq 1.0 \times 10^2$**	**Not exceed**

5.0 DISCUSSION

The data above showed no *Salmonella* was detected in swabs on EBN. This finding is in agreement with another studies that no *Salmonella* was isolated in EBN . Following procedure of isolation and identification of *Salmonella,* it is growing well in BGA and XLD agar. However, it was explained by OIE (2010) that the culture techniques and media that may work best in a particular diagnostic situation depend on a variety of factors, including the *Salmonella* serovar, source and type of specimens, animal species of origin, experience of the microbiologist, and availability of selective enrichment and selective plating media (OIE,2010).

In this study, out of 32 guano samples that had been examined, four samples are positive for *Salmonella* . The occurence of *Salmonella* in guano samples of

swiftlets was about 12.5%. This results indicates that even though some of the swiftlest hosts may be the carrier or reservoir for *Salmonella,* it does not relate to the spread of pathogens that may lead to the contamination of the nest. Instead, the absence of *Salmonella* in their nests may be related to the behaviour of the swiftlet themselves where they are unable to perch (Idris *et al*., 2014) on the tree or ground as other wild birds do, that increase the possibilities of swiflet to be infected with *Salmonella* either from the other animals or food. Instead, they only perch vertically on the nest that they built. In addition, they also do not mix and interact with other species of birds even with their close relative, the black-nest swiftlet, *Aerodramus maximus* (Lim and Cranbrook, 2002; Aini, 2005). Hence, at the same time, this behavioural factor may prevent the transmission of pathogen to the swiftlets.

The occurence of *E. coli* in EBNs and guano samples were 3.13% and 68.75% respectively. Generally, the percentage of *E.coli* is higher in the guano samples as compared to EBNs. According to Simpson (2002), it is normal to find *E.coli* in swiftlet guano because almost all wild birds have *E.coli* as their normal flora. Meanwhile, the coliform count are ≤ 100 CFU/g .These findings indicate that failure in *E.coli* isolation and no growth on the petrifilm does not mean that the EBNs were free from the bacteria.

The occurence rate for *Salmonella* in guano samples is highest from Birdhouse C with 3(30%) followed by Birdhouse A with only 1(8.3%). From this study, no *Salmonella* was detected in guano and also EBN from Birdhouse B. Meanwhile, the occurence for *E.coli* is highest in guano samples from Birdhouse A, followed by Birdhouse C and Birdhouse B with 11 , 7 and 4 samples respectively.

The bacterial enumeration was done based on colony forming unit (CFU) per gram of sample. Based on result in Table 2 , eight samples out of 12 EBN samples had counts between 2.8 x 10^4 CFU per gram to 1.1 x 10^6 CFU per gram whereas four EBN samples had 2.5 x 10^5 CFU per gram . All samples did not exceed the limit and thus concluded that the EBN that are ranched in houses system is considered of high quality and meet the microbiological criteria. Apart from the microbiological analysis, physical aspect and grading of EBN is important to make sure the that EBN produced from the housing system is of high quality and safe for human consumption.

6.0 CONCLUSION

No *Salmonella* and low number of *E.coli (3.1%)* was isolated from EBN. *Salmonella (12.5%) and E.coli (68.8%)* were isolated from guano samples. The presence of *Salmonella* and *E.coli* in the droppings did not influence the presence of both bacteria in the EBN. Besides, low counts of total bacteria and coliform in EBN is conclusive that EBN is clean and of good microbiological quality from the aspect of food borne pathogens. The overall counts were below the tolerance level as stated in Code of Veterinary Practice set by SIRIM. Hence, the absence of *Salmonella* and low number of *E.coli* in EBN is a good news for the growing swiftlet industry in Malaysia. In addition, good animal husbandry as suggested by DVS should be followed by the swiftlet operators to reduce the risk of colibacillosis and salmonellosis in the birds and also to prevent the microorganisms from entering the food chain.

7.0 RECOMMENDATIONS

I would like to recommend to improve the future study by using larger sample size and diversify the site of sample collection to get more reliable data. Besides, further study and different methodology approach should be carried out to determine the presence of other food borne pathogens in EBNs. I also would like to recommend the future research to identify other contaminants in EBNs, such as, virus, fungi, or heavy metal that can reduce the quality of EBNs.

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9.0 APPENDICES

9.1. Isolation and identification methods for *Salmonella* sp.

1. Bird nest's swab/1g of guano sample + 9ml of Buffer Peptone Water (BPW). [Incubated for 18 to 24 hours at 37°C]

⇩

2. Transfer 1ml from the culture into 10 ml of Rappaport-Vassiliadis (RV) Enrichment Broth (Oxoid) [incubated for 18 to 24 hours at 42 °C]

⇩

3. Transfer loopful from RV into 2 selective agars

a. Xylose Lysine Deoxycholate(XLD) agar (Oxoid)

b. Brilliant Green Agar (BGA)

[Incubated for 24 to 48 hours at 37 °C]

⇩

4. Inoculate into Nutrient agar

[incubated for 18 to 24 hours for 37 °C]

⇩

5. Carry out biochemical test ; urea,citrate, TSI,LIA,SIM,

[lncubated for 24 to 48 hours at 37 ^{O}C]

6. Serology test by the slide agglutination test using the commercial polyvalent O.

7. Sent to VRI for serotyping

9.2 Isolation and identification methods for *E.coli*

1. Bird nest's swab/1g of guano sample + 9ml of Buffer Peptone Water (BPW).
[Incubated for 18 to 24 hours at 37ºC]

2. Inoculate the samples onto the Eosin Methylene Blue Agar (EMBA)

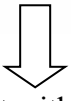
[Incubated aerobically at 37 ^{0}C for 24-48 hours]

3. Inoculate into Nutrient agar,

[Incubated for 18 to 24 hours for 37 ^{O}C]

4. Carry out biochemical test ; SIM

[Incubated for 24 to 48 hours at 37 ^{O}C]



5. Further test with Kovacs reagent.

9.3 Microbial content analysis

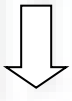
1. Pre-clean the edible bird's nest using sterile forceps to remove feathers.

2. Fragmented into smaller pieces and weighed.

3. Add BPW into sample by maintaining using 1 part of the sample plus 9 part of BPW

4. Homogenize using a stomacher for about 2 minutes.

5. Prepare the dilution by adding 1sample to 9 diluent of ratio.



6. Plating out of the dilution.

a. 0.1ml aliqout from each appropriate dilution is pipetted into a Standard Plate Count agar and the inoculum is then spread out over the entire surface of the agar by using a L-shaped disposable spreader as quickly as possible.

b. 1ml aliqout from each appropriate dilution is pipetted into the Petrifilm.

9.4 Summary for isolation of *Salmonella* and *E.coli* in EBNs and guano samples

Table 3 . Occurence of S*almonella* in EBNs and guano samples

Sample * *Salmonella* Cross tabulation

Count

		Salmonella		Total
		Negative	Positive	
Sample	EBN	32	0	32
	Guano	28	4	32
Total		60	4	64

Table 4 . Occurence of *E.coli* in EBNs and guano samples

Sample * *E.coli* Cross tabulation

Count

		E.coli		Total
		Negative	Positive	
Sample	EBN	31	1	32
	Guano	10	22	32
Total		41	23	64

9.5 List of figures

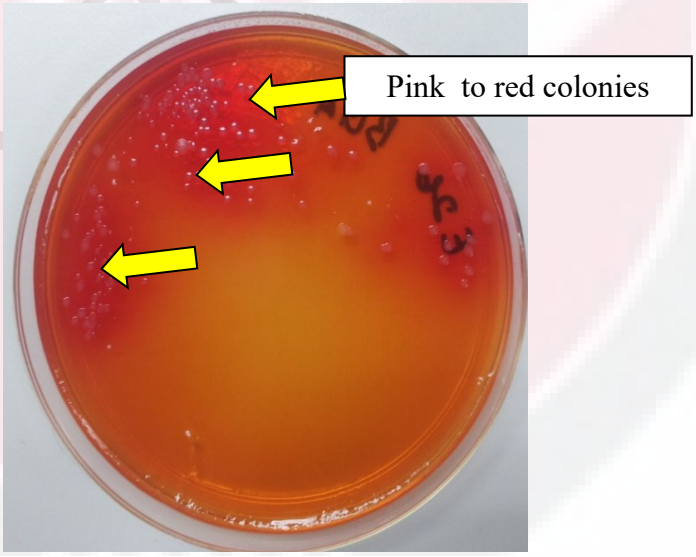


Figure 11. The colonies of *Salmonella* appear on BGA.

Figure 12. The colonies of the *Salmonella* appear on XLD agar.

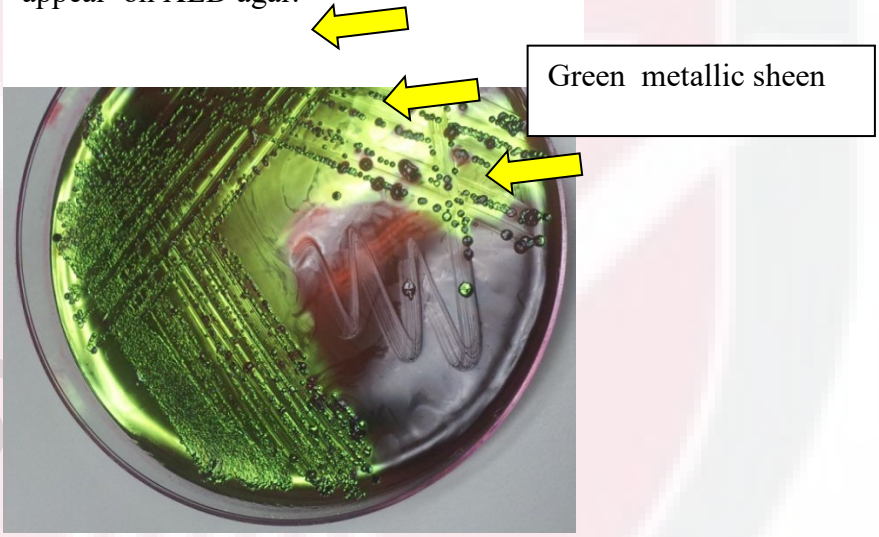


Figure 13. *E.coli* colonies on EMBA plate

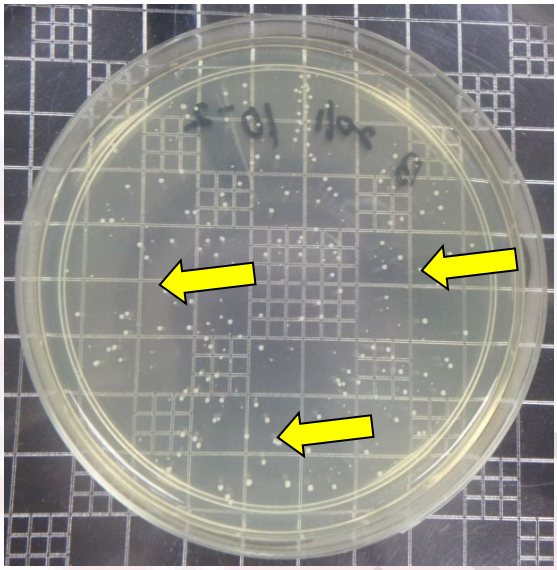


Figure 14. Bacteria colonies on Standard Plate Count agar

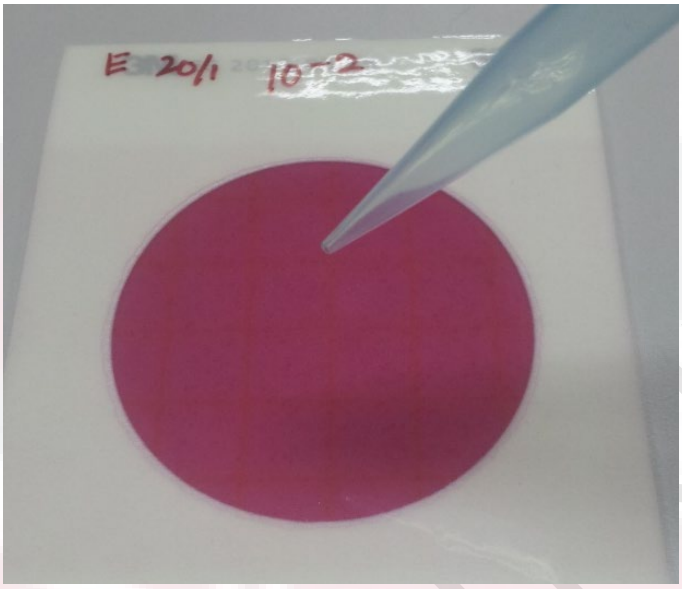


Figure 15. Coliform Count by using Petrifilm