UNIVERSITI PUTRA MALAYSIA

THE COMPARISON OF DUCK EGG CONTAMINATION BETWEEN DIFFERENT EGG LAYING SPOTS

LAU JEE BIN

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A project paper submitted to the
Faculty of Veterinary Medicine, University Putra Malaysia
in partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
University Putra Malaysia
Serdang, Selangor Darul Ehsan

MARCH 2016

CERTIFICATION

It is hereby certified that I have read this project paper entitled "The comparison of duck egg contamination between different egg laying spots" by Lau Jee Bin and in my opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999.

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DEDICATIONS

This final year project is dedicated to my beloved family, my girlfriend, my supervisor, housemates, classmates, all lecturers and friends that were involve either directly or indirectly in this project

ACKNOWLEDGEMENTS

I would like to acknowledge my supervisor Dr. Lokman, co-supervisor Prof. Dr. Saleha Abdul Aziz for their guidance, time and support in this study. I wound also like to acknowledge Prof. Dr. Mohd Ariff Omar for his guidance in statistic. I will not have been able to get through this project without their help and guidance. Towards the staff at Public Health Laboratory, FPV, UPM, I send the greatest thanks for their excellent assistance throughout this study. I also thank Perak Duck Food Industry Sdn. Bhd. for their generous and kind heart in providing me the samples as well as a trip to visit their personnel's. I extend my thanks and appreciation to my co-final year project Mr. Chong Chiew Foong for his help.

For always believing in me and encouraging me to do the best I can do, I am ever thankful to my family, my girlfriend and friends whom although so far away were always behind me. Last but not least, thank you to all my friends class DVM 2016 who has helped me in a way or another I will be forever grateful.

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

THE COMPARISON OF DUCK EGG CONTAMINATION BETWEEN DIFFERENT EGG LAYING SPOTS

by

LAU JEE BIN

2016

Supervisor: Dr. Lokman Hakim Idris

This study compares on the bacteria contamination on duck eggshell between different egg laying spots. These egg laying spots are pond-side, floor, and sand. A total amount of 30 duck eggs from Khaki Campbell in two different layers farms which were using same type of commercial diet were subjected to this study. This egg contamination experiment was conducted using coliform count method, *Salmonella spp* isolation and identification test method, and temperature detection method. Coliform count method was an enumeration test for quantitative detection of coliform on the duck eggshell which includes coliforms such as *Escherichia spp*, *Enterobacter spp*, *Citrobacter spp*, and *Klebsiella spp*. The statistic result shows that the pond-side eggs are more coliform contaminated than the sand eggs but only applicable to certain management practice while the floor eggs have no significant difference in contamination to other eggs. Meanwhile, *Salmonella spp* isolation and identification method was a screening test for qualitative detection of *salmonella spp* on the eggshell and the egg content. The prevalence result shows negative for all samples either on eggshell or egg content except one positive from the egg content only. Temperature detection method was used to determine the temperature on each spots. The statistic result shows that there was neither statistically correlation nor support relationship between the temperatures of the spots with the egg coliforms count. Thus, the duck eggs from different egg laying spots contributed to the egg coliform contamination in little extend only.

Keyword: Khaki Campbell layer, Coliform, Egg laying spots, Salmonella, Temperature.

ABSTRAK

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

KAJIAN PERBANDINGAN PENCEMARAN TELUR ITIK DI ANTARA TEMPAT-TEMPAT PENELURAN YANG BERBEZA

oleh

LAU JEE BIN

2016

Penyelia: Dr. Lokman Hakim Idris

Kajian ini membandingkan pencemaran bakteria pada kulit telur itik di antara tempat-tempat peneluran yang berbeza. Tempat-tempat peneluran adalah di tepi kolam, lantai dan pasir. Sejumlah 30 telur itik Khaki Campbell dari dua ladang telur yang berbeza dengan diberi diet komercial yang sama adalah digunakan dalam kajian ini. Kajian eksperimentasi pencemaran telur itik ini dijalankan dengan menggunakan kaedah kiraan koliform, kaedah pengasingan dan identifikasi *Salmonella spp.* serta kaedah pengesanan suhu. Kaedah kiraan koliform adalah ujian penghitungan bagi pengesanan kuantitatif koliform pada kulit telur itik termasuk koliform seperti *Escherichia spp*, *Enterobacter spp*, *Citrobacter spp*, dan *Klebsiella spp*. Hasil statistik menunjukkan bahawa telur di tepi kolam lebih tercemar oleh koliform berbanding dengan telur di pasir tetapi hanya boleh digunakan dalam amalan pengurusan yang tertentu manakala telur di atas lantai tidak mempunyai perbezaan pencemaran yang ketara dengan telur yang lain. Manakala, kaedah pengasingan dan identifikasi *Salmonella spp.* adalah ujian saringan untuk pengesanan kualitatif *Salmonella spp.* pada kulit telur dan kandungan telur. Hasil kelaziman menunjukkan negatif untuk semua sampel sama ada pada kulit telur atau kandungan telur kecuali satu positif daripada kandungan telur sahaja. Kaedah pengesanan suhu digunakan untuk menentukan suhu di setiap tempat. Hasilan statistik menunjukkan bahawa tiada korelasi statistik mahupun hubungan sokongan antara suhu satu tempat dengan kiraan koliform telur. Oleh itu, telur itik dari tempat peneluran yang berbeza hanya menyumbang sedikit kepada pencemaran koliform pada telur.

Kata kunci: Penelur Khaki Campbell, Koliform, Tempat peneluran, Salmonella, Suhu.

1.0 INTRODUCTION

1.1 Study background

Duck farm industry is part of poultry industry in Malaysia. Duck farm industry comprising broiler and layer duck farms. The breeds used in broiler duck farm were Pekin, Cherry Valley and Muscovy while the breeds used in layer duck farm was mainly khaki Campbell. The subject of this study is the eggs from khaki Campbell. The duck layer farms in Malaysia are mainly dominated by the duck-fish integrated farming system. The duck house is built beside a pond which allows the ducks fully access to water. Thus, the ducks are able to bath and express its natural behavior. Most of the integrated farms include areas such as pond, pond-side, sand, and the floor of the duck house. In certain farms, it may also include some areas such as nest, grass, mud, and palm oil trees. Eggs are found on all over the farm in every morning, due to ducks can lay egg on everywhere. Most of the eggs will be found on egg laying spots such as pond-side, sand, and the floor that is covered with feces. All eggs were collected and sold together.

The interest of this study is the presumption that the egg laid on pond-side was more prone to coliform contamination than the egg on floor followed by the egg laid on sand due to the contact with the contaminated ponds and feces respectively. The farms chosen for this study are located in Tanjung Tualang, Perak. Farm A has up to 5,000 ducks populations and farm B has up to 10,000 ducks population. Majority of the duck eggs production were sold to the salted egg, century egg, bakery, and food processing factories. There is no categorization of duck eggs according to the egg laying spots and there is lack of study done on the egg contamination related to the egg laying spots in Malaysia. Thus, this study will address this issue.

1.2 Justifications

i. The production of duck eggs is mainly for domestic consumption. Thus, it is important for us to closely monitor the quality of our egg product.

ii. Limited studies done on ducks in Malaysia especially on the duck eggs contamination.

iii. To reduce the food hazard from duck eggs, increase the food safety of duck eggs, and improve the public health.

iv. To categorized or priced the duck eggs according to the laying spots, thus provide consumers a better choice of eggs.

v. To provide an option or guideline on how to reduce the duck egg contamination.

1.3 Study Objectives

i. To determine the level of egg contamination (Coliform) between the egg laying spots in duck farms.

ii. To determine the present of *Salmonella spp* contamination between eggshell and egg content.

iii. To evaluate the effect of temperature of the egg laying spots on the egg coliform contamination.

1.4 Hypothesis

i. The coliform contamination of the duck egg is highest on the pond-side, followed by floor and on the sand.

ii. The eggshell is more contaminated with *Salmonella spp* than the egg content.

iii. The higher the temperature of the egg laying spots will have a lower degree of egg contamination.

2.0 LITERATURE REVIEW

2.1 The duck industry in Malaysia

Malaysia has achieved self-sufficiency for duck eggs production since the nineties. Duck meat and eggs production accounts for approximately 4.8 percent of the total supply of country's meat and eggs production (JPV, 2010). In 2014, the combination of chicken and duck eggs have a self-sufficiency Ratio, SSR of 113.8%, Import dependency Ratio, IDR of 0%, and 19.1kg of egg per year per capital consumption, PCC (JPM, 2015). In 2014, the total numbers of layer and broiler duck showed the highest increase of 12.4 per cent as compared to 2013 followed by chicken (6.4%), goat (5.0%) and cattle (1.3%) (JPM D. O., 2015). Although chicken dominate the world poultry egg and meat industry, in parts of Asia duck egg and meat are commercially more important than chicken (Unklesbay, 1992). Total duck eggs production in year 2010 was

773,245,214 eggs from 3,623,801 duck layers of 314 layer farms among the whole Malaysia (IPPV, 2012).

2.2 The duck rearing

Egg of hen and duck are mainly used for human consumption. Egg is a complete and perfect food by itself. It is an important source of protein and an ideal protective food owing to presence of important essential amino acids (Sujata, 2014). Rearing ducks is not as difficult as rearing chickens. In fact the ducks possessed a higher survival rate and are not prone to disease attacks. The method of rearing and management of layer ducks are easier and varies slightly from those of chickens (Zulkifli, 2011).

Khaki Campbell is a best known duck laying breed. In many countries, Khaki Campbell ducks are allowed access to open water during the day and are housed only at nights. This system is kept primarily for egg production. In some tropical areas, the system has been developed into a fully integrated farming system with fish harvested commercially. This is because the fertilization of the aquatic flora by the duck droppings allows obtaining a higher yield from fish (Cherry & Morris, 2008).

Khaki Campbell rearing was divided into 3 stages which were starting stage from 0 – 4 weeks of age, growing stage from 4 to 18 weeks of age (Thongwittaya, 1992), and laying age from 18 weeks and older.

Duck producers provide outdoor runs for their ducks often with access to water so that the ducks can bath. This allows the ducks to choose between being indoors and outdoors, according to the prevailing weather. The access to bathing water is a valuable means of keeping ducks cool during hot weather (Cherry & Morris, 2008). This duck-fish system gives better profit because of higher fish yield. Growth rate of the ducks in the integrated system was found to be comparable to mono-cultural systems. The water quality of the integrated ponds was also found to be normal (Mukherjee, Geeta, Rohani, & Phang, 1992).

Rearing of ducks gives maximum return with minimum cost. Ducks are efficient converter of agricultural by-products; kitchen wastes, seeds, grains, garden left over, insects, green grasses and all other human refusal that would be otherwise wasted. Ducks occupy second place in comparison with chicken in producing meat and egg in the country. Ducks are traditionally raised under scavenging (Salahuddin, Barua, Rashid, & Howlider, 1991).

2.3 Coliform Count on duck eggshell

The shell and egg contents at the time of oviposition are generally sterile or harbor very few microorganisms, contamination of the shell occurs from nest material, floor litter, avian fecal matter, collector's hands, packing materials and improper washing (Moats, 1980). The shell can already be infected when passing through the vent, but many researchers suggest that the main bacterial contamination occurs within a short period after laying due to contact with dirty surfaces (Gentry & Quarles, 1972). Messens, Grijspeerdt, & Herman reported in 2005 that increasing numbers of micro-organisms on the eggshell consequently increase the risk of microbial eggshell penetration and egg content contamination.

The surfaces of eggs laid in farms are obviously not sterile. An eggshell surface monitoring is consequently a very good help for checking and used whenever a contamination problem has been identified by the egg breakout program. Eggs were classified according to the bacteria per eggshell such as freshly laid eggs with 300+ colonies, clean eggs with 3,000+ colonies, soiled eggs with 25,000+ colonies, and dirty eggs with 400,000+ colonies (Turblin, 2010). Nest should be provided to encourage ducks to lay their eggs in clean well-littered nests. Floor eggs, even when apparently clean, are invariably heavily contaminated with microorganisms. Dirty eggs and floor eggs should be identified with a waterproof mark, washed and then incubated separately from nest eggs because they suffer substantially higher levels of infection, even after washing, and will adversely affect the hatchability of clean egg (Morris, 2008). Duck eggs collected from nest boxes had lower surface contamination than floor eggs (Joyce & Chaplin, 1978). The porosity of the cell increases making possible the infiltration of bacteria and moulds when an egg age (Sujata, 2014). Washing the egg with a chlorine sanitizer was highly effective in reducing surface bacterial counts on eggshells and prolonged storage reduced bacterial counts on clean eggs (Baker & Qureshi, 1984).

Bacterial loads on washed and clean unwashed duck eggs were low, ranging from too few to count to 10^2/shell. A higher proportion of dirty eggs were heavily contaminated with counts ranging from 10s to 10^6/shell. In another trial, bacterial loads on washed and nest-clean eggs ranged between 10^2 to 10^3/shell and 10^2 to 10^4 /shell, respectively (Baker & Qureshi, 1984).

According to the study that was carried out in Beni-Suef city, it evaluate the microbiological quality of poultry farms eggs, the coliform counts was $1.5x10^3 \pm 8.4\times10^2$ cfu / shell for the shell. Statistical analytical results of the examined samples of eggshells of poultry farms based on their coliform counts by using (MPN/ shell). Eggshells Minimum : <3 ; Maximum : $2.1x10^4$; Mean ± SEM : $1.5\times10^3\pm8.4\times10^2$ Counts per shell (El-kholy, Hassan, & Dalia, 2014).

Recently more research focused on the bacterial contamination of table eggs, e.g. eggshell and egg content contamination. The micro-flora of the eggshell is dominated by Gram-positive bacteria, whereas Gram-negative bacteria, coliforms, *Enterococcus* and aerobic bacteria are best equipped to overcome the antimicrobial defenses of the egg content (Reu, et al., 2009). Therefore, I will be focus on gram-negative bacteria which is the coliforms in my study.

2.4 Salmonella on duck eggshell and content

Salmonella human infection resulting from the consumption of contaminated eggs is still a major public health problem (Reu, Grijspeerdit, Heyndrickx, Debevene, Uyttendaele, & Herman, 2006).

No salmonellae could be detected on shells or in the content of all washed or clean unwashed duck eggs examined. However, *Salmonella enteritidis* was detected on dirty eggshells in four of six farms. We conclude that proper egg washing and confinement of duck breeders should minimize the problem of salmonellosis in ducklings (Baker & Qureshi, 1984).

Recent research indicates that beside horizontal route of bacterial infection of eggs, egg contamination also occurs through the vertical or transovarian route. In the transovarian route (vertical transmission), the yolk (very infrequently the yolk itself), the albumen and/or the membranes are directly contaminated as a result of bacterial infection of the reproductive organs. (Messens, Grijspeerdt, & Herman, 2005). On the other hand, egg may become contaminated with salmonella organism through ovarian infection before it is laid or after laying through entry of microorganisms into the whole eggs and constitute a public health hazard (Board & Fuller, 1994).

Much of the research on eggshell and egg content contamination focuses on *Salmonella,* since infection with *Salmonella enteritidis*, resulting from the consumption of contaminated eggs or egg products, is still a major health problem. Observed *Salmonella* prevalence on the eggshell and in the egg content vary, depending on the fact whether investigations were based on randomly sampled table eggs or on eggs from naturally infected hens (Reu, et al., 2009).

Bisgaard in 1981 observed a high incidence of *S. typhimurium* in the intestinal tract of ducks reared in open houses and suggested that these holding and housing practices contributed to the spread of infection by enhancing feco-oral cycling. Additional sources of infection include feed and contamination of open houses from free-flying birds or wild fauna. Eggs have gained a degree of notoriety as sources of salmonella in large foodborne outbreaks of salmonellosis.

Baker & Qureshi studied the prevalence of salmonella in 1984, only 0.21% of the eggshells yielded salmonellae, all three of which were identified as *S. typhimurium.* No salmonellae were found in the content of these eggs. In another experiment done by Baker *et al.,* Oral administration of the *S. typhimurium* did not result in contamination of eggshells or contents despite the presence of organisms in the feces. Intravenous injection did not lead to fecal shedding of salmonellae nor to contamination of shell or egg contents.

According to Trongpanich & Dawson in 1974, they did a research on bacterial counts of duck eggs from one commercial farm but did not find salmonella.

The usual pasteurization practice to kill all Salmonella organisms involves heat treatment at $60^{O}C$ for not less than 3 ½ minutes. Increasing the acidity of the egg whites before pasteurization seems to protect the proteins from damage to heat (Sujata, 2014). Little research is done on the influence of egg laying spots on eggshell and egg content contamination with *Salmonella spp.*

3.0 MATERIALS AND METHODS

3.1 Duck Eggs Sampling

The sampling was done at Chong Lee Poultry farm located at Tanjung, Tualang, Perak. A total number of 30 duck egg samples were collected from two different farms of the same company, the farm A and farm B. There were three egg laying spots of collection from each farm and the spots were pond-side, floor, and sand. Five eggs were collected from each spots. All 30 duck eggs were laid by Khaki Campbell. Random sampling technique was done at the duck farm whereby the size of the duck egg and the age of the layer were not included in selection criteria. All of the duck eggs were fed with the same commercial duck layer feed. Each egg was place in an individual small plastic bag to avoid cross contamination. Then the bags from each spot were place into a larger plastic bag and transported into an ice box to the laboratory on the same day. The samples were cultured at the next day after arrival at the lab at night.

Figure 3.1a: Pond-side

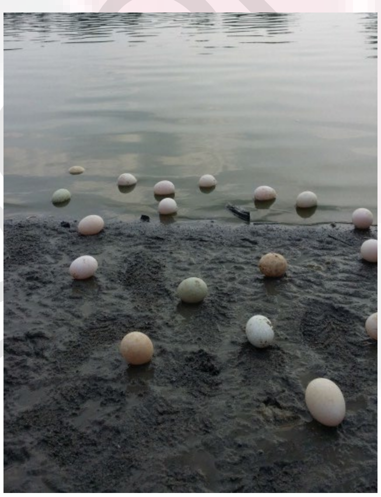


Figure 3.1b: Floor

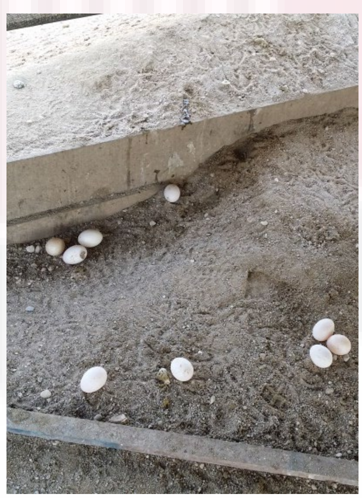


Figure 3.1c: Sand

3.2 Experimental Design

The egg samples from the farms were used for the experiment to be conducted in Public Health Laboratory. Prior to the study, the duck eggs were stored at 4°C for 10 hours before the experiment was conducted. The chilled samples of the duck egg, the eggshell was swabbed for both the coliform plate count and *salmonella spp* isolation and identification test while the egg content was used for *salmonella spp* isolation and identification test only.

Figure 3.2a: Eggshell swab

Figure 3.2b: Egg Content

3.3 Coliform Plate Count Method

This is a quantitative test use for enumerate the number of coliform bacteria per duck eggshell. Coliform bacteria are a commonly used as bacterial indicator of sanitary quality of foods and water. Coliform bacteria are defined as Gram-negative, non-spore-forming, oxidase-negative, rod-shaped facultative anaerobic bacteria that ferment lactose (with β-galactosidase) to acid and gas within 24–48h at 36±2°C (Ashbolt, Willie, Grabow,

& Mario , 2001). Common coliforms are *Escherichia spp*, *Enterobacter spp*, *Citrobacter spp*, and *Klebsiella spp*

3.3.1 Isolation of Coliform from Eggshell

A sterile cotton swab moistened with Peptone water (PW) was used to swab the egg surface randomly and placed in 10 ml Peptone water (PW) and shaken vigorously which formed the dilution 10^{-1}. Serial dilutions were done from 10^{-1} – 10^{-9} with 9ml peptone water . After dilution was done, 1ml aliquot of each dilution (10^{-1} – 10^{-9}) was transferred onto petri-film. The petri-film was incubated at 37ºC for 24 hours.

3.3.2 Enumeration of Coliform

All petri-films were counted at the same time by using the colony counter. The dark red colonies on the petri-film with less than 400 colonies were counted. The results were compute into excel and the data was selected according to the rules for selecting plates and counting colonies accredited by American Public Health Association(APHA) and USFDA Bacteriological Analytical Manual (BAM).

Figure 3.3a: Petrifilm

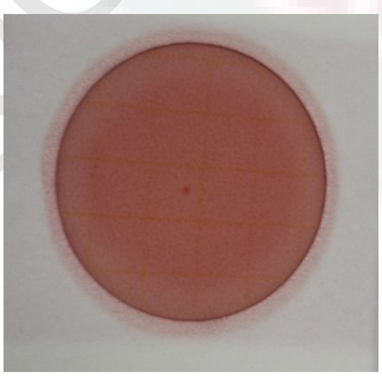


Figure 3.3b : Colony counter

3.4 *Salmonella spp* Isolation and Identification Test

3.4.1 Isolation of Salmonella

3.4.1.1 Eggshell

A sterile cotton swab moistened with Buffered Peptone water (BPW) was used to swab the egg surface randomly and placed in 10 ml Buffered Peptone water (BPW). The bottles were incubated at 37 °C for 24 hours. After incubation 0.1 ml aliquot of the BPW which is the pre-enrichment culture was pipetted into 10ml Rappaport-Vassiliadis (RV) enrichment broth and incubated at 42 °C for 24 hours. A Loopful of RV broth was plated by streaking on the surface of Xylose lysine desoxycholate (XLD) agar and Brilliant Green agar (BGA). The plates were incubated for at 37 °C for 24 hours.

3.4.1.2 Egg content

Eggshell was cleaned aseptically with alcohol. Each egg was cracked using a sterile forceps and the content was placed into a sterile plastic bag. The egg content was thoroughly homogenized with Bag Mixer and 1ml of the egg content mixture was transferred into 9ml BPW. The bottles were incubated at 37 °C for 24 hours. After incubation 0.1 ml aliquot of the BPW which is the pre-enrichment culture was pipetted into 10ml Rappaport-Vassiliadis (RV) enrichment broth and incubated at 42 °C for 24 hours. A Loopful of RV broth was plated by streaking on the surface Xylose lysine desoxycholate (XLD) agar and Brilliant Green agar (BGA). The plates were incubated at 37 °C for 24 hours.

3.4.2 Identification of Salmonella

Colonies from the selective plate agar which resembled Salmonella were noted as in Table. Three typical colonies were picked and purified by streaking on the Nutrient agar and incubated at 37ºC for 24 hours.

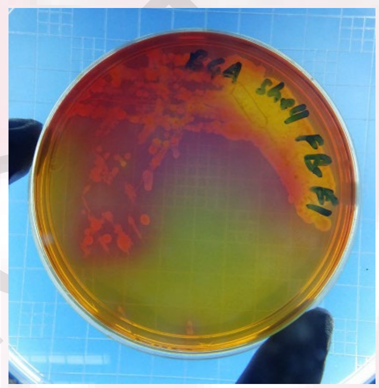
Selective agar	Appearance of colonies
Brilliant green agar(BGA)	Colonies are red or pink  **Figure 3.4a: BGA with red colonies**
Xylose lysine desoxycholate (XLD) agar	Clear colonies with black centres  **Figure 3.4a: XLD with black colonies**

Table 1: The *Salmonella* colonies on the selective agar.

Then the suspected colonies were subjected to the confirmatory biochemical test Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), SIM, citrate and urease test. After that, serological test was done by using slide agglutination test with polyvalent O Salmonella antisera. The confirmed Salmonella species was inoculated into a slanted nutrient agar and sent to Veterinary Research Institute, Ipoh for serotyping.

3.5 Temperature Detection Method

Temperature detection method was used to determine the temperature on each spots. Thermometer was used to detect the temperature under the egg at each spot upon egg collection. Temperature was recorded at each spot to correlate the effect of temperature toward the coliform count at each spot.

3.6 Statistic

Results were collected and recorded during experiments. The result was first computed into a table by using excel. Then follow by compute the data into Statistical Package for the Social Sciences (SPSS). For the coliform plate count, data was analyzed by using descriptive statistic to check for the mean value of the coliform count at each spot. Then follow by Test of Normality for normal distribution and Levene's Test for equal variances assumption. After that, independent sample T-Test was used to analyses the significant difference of the coliform plate count between farms. Then, univariate test from general linear model was used to analyses the significant difference of the coliform plate count between the egg laying spots. For the *Salmonella spp* isolation and identification, result was compute into a table; prevalence was calculated according to the spots, eggshell and egg content. For the temperature detection, the temperature recorded at each spot was computed into SPSS, and then bivariate correlation and linear regression were done to correlate the temperature and the coliform plate count.

4.0 RESULTS

4.1 Coliform Plate Count Method

The total of 30 samples, 15 from the farm A and another 15 from farm B, all the results were recorded according to the egg laying spots and dilutions.

	FARM A														
	Pond-Side					Floor					Sand				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
10^{-1}	2	41	37	66	8	0	10	0	89	80	14	10	73	29	5
10^{-2}	0	9	4	16	3	0	0	0	11	8	0	1	2	2	0
10^{-3}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-4}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-6}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-8}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-9}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2a: Coliform count of the petrifilm for farm A

	FARM B														
	Pond-Side					Floor					Sand				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
10^{-1}	TNTC	12	367	TNTC	TNTC	65	18	118	173	141	23	13	21	8	14
10^{-2}	19	0	23	40	24	13	0	12	28	24	2	1	2	0	5
10^{-3}	2	0	0	2	1	0	0	0	0	0	0	0	0	0	0
10^{-4}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

10^{-6}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-8}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-9}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2b: Coliform count of the petrifilm for farm B

	FARM A														
	Pond-Side					Floor					Sand				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
CFU/egg	20	410	370	660	80	0	100	0	890	800	140	100	730	290	50
	FARM B														
CFU/egg	1900	120	3670	4000	2400	650	180	1180	1827	1410	230	130	210	80	140

*CFU/egg: Colony forming unit per eggshell surface.

Table 2c: Selected and adjusted data according to the rules for selecting plates and counting colonies accredited by American Public Health Association (APHA) and USFDA Bacteriological Analytical Manual (BAM)

The results from Table 2c was compute into Statistical Package for the Social Sciences (SPSS), the descriptive analysis result show that the mean for Pond-side, Floor, and Sand are 1363, 704, and 210 respectively. If the data was split according to the farm, the mean for Pond-side, Floor, and Sand in Farm A are 308, 358, and 262 respectively ; Pond-side, Floor, and Sand in Farm B are 2418, 1049, and 158 respectively.

After that, Levene's Test for Equality of Variances was done and the result shows that the equal variance was not assumed due to $P<0.05$. The test was followed by independent sample T-Test and the result shows that there was a significant difference ($P<0.05$) of the coliform plate count between farms. Thus, the coliform plate count between the egg laying spots was analyzed according to the each farm differently.

Independent Samples Test

	t-test for Equality of Means		
	T	df	Sig. (2-tailed)
Number of coliforms on eggshell (Equal variances not assumed)	-2.572	15.614	.021

Table 2d: Independent sample T-Test for the coliform plate count between farms

Then, univariate test from general linear model was used to analyses the significant difference of the coliform plate count between the egg laying spots according to each farm. For Farm A, the result (p-value>0.05) shows that there was no significant difference between the three egg laying spots, thus no Tukey test was done.

Tests of Between-Subjects Effects[a]

Dependent Variable: Number of coliforms on eggshell

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Egg_Laying_Spots	23053.333	2	11526.667	.100	.905

a. Which farm were the samples taken = Farm A

Table 2e: Univariate test for coliform plate count between egg laying spots in Farm A.

For Farm B, the result (p-value<0.05) shows that there is a significant difference between the egg laying spots, thus Tukey HSD test was done for further comparison.

Tests of Between-Subjects Effects[a]

Dependent Variable: Number of coliforms on eggshell

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Egg_Laying_Spots	12958766.533	2	6479383.267	6.880	.010

a. Which farm were the samples taken = Farm B

Table 2f: Univariate test for coliform plate count between egg laying spots in Farm B.

The Tukey HSD test result shows that there was no significant difference between floor eggs compare to sand eggs and pond-side eggs. However, there was significant difference between sand egg compare to pond-side egg. From the subset, we can conclude that pond-side egg (μ=2418) is more contaminated with coliform compare to sand egg (μ=158) in farm B.

Number of coliforms on eggshell[a]

Tukey HSD

Spot where the duck egg was laid	N	Subset	
		1	2
Sand	5	158.00	
Floor	5	1049.40	1049.40
PondSide	5		2418.00
Sig.		.347	.106

Means for groups in homogeneous subsets are displayed. Based on observed means.
The error term is Mean Square(Error) = 941823.933.

a. Which farm were the samples taken = Farm B

b. Uses Harmonic Mean Sample Size = 5.000.

c. Alpha = 0.05.

Table 2g: Tukey HSD test for coliform plate count between egg laying spots in Farm B.

4.2 *Salmonella spp* Isolation and Identification Test

The total of 60 samples, 30 from the eggshell and another 30 from egg content, all the results were recorded according to the egg laying spots. All the samples from eggshell were tested negative against the *salmonella spp*. However, for the samples from egg content, one of the samples is tested positive toward *salmonella spp* which is confirm to be *salmonella serovar non-typhi* from the biochemical test result and the other 29samples were tested negative toward *salmonella spp.* The only positive sample was from the content of the egg laid on floor in farm B.

The result was compute into a table; prevalence was calculated according to the spots, eggshell and egg content.

		Present of salmonella on shell		Present of salmonella on content		Prevalence
		Positive	Negative	Positive	Negative	
		Count	Count	Count	Count	%
Egg laying Spots	Pond-Side	0	10	0	10	0
	Floor	0	10	1	9	5
	Sand	0	10	0	10	0
Prevalence	%	0	100	3.33	96.67	

Table 3: Prevalence studies according to the eggshell, egg content, and egg laying spots.

From this table, we can interpret it as there is only 3.33% egg content yielded *salmonella spp* and none of the eggshell yielded *salmonella spp.* Besides, only 5% of the sample from the floor yielded *salmonella spp* and none of the egg from sand and pond-side yielded *salmonella spp.*

Figure 4: Biochemical tested positive toward *salmonella serovar non-typhi.*

4.3 Temperature Detection Method

For the temperature detection, the mean temperature for pond-side, floor and sand are 35.9 ºC, 34.2 ºC, and 41.4ºC respectively. Then the temperature recorded at each spot was computed into SPSS, and then bivariate correlation and linear regression were done to correlate the temperature and the coliform plate count.

For the Correlation of the temperature with the coliforms plate count, the P-value is more than 0.05, thus we fail to reject H_0 which means that there was no statistically correlations between the temperatures with the coliforms plate count.

Correlations

		Number of coliforms on eggshell	Temperature of the spot where the egg was taken
Number of coliforms on eggshell	Pearson Correlation	1	-.307
	Sig. (2-tailed)		.099
	N	30	30
Temperature of the spot where the egg was taken	Pearson Correlation	-.307	1
	Sig. (2-tailed)	.099	
	N	30	30

Table 4a:.Correlation of the temperature with the coliforms plate count

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	4628.014	2276.367		2.033	.052
	Temperature of the spot where the egg was taken	-104.195	61.100	-.307	-1.705	.099

a. Dependent Variable: Number of coliforms on eggshell

Table 4b:.Regression of the temperature with the coliforms plate count

For the Regression of the temperature with the coliforms plate count, the equation will be Y= -104.2X+4628, however, the p value is more than 0.05, thus we fail to reject H_0 which means that there is no support relationship between temperature and coliform plate count.

5.0 DISCUSSION

5.1 Coliform Plate Count

The result shows that there was a significant difference of the coliform plate count between farms. Thus, the coliform plate count between the egg laying spots was analyzed according to the each farm differently and the results for both farm are different. For farm A, there was no statistically significant difference of the egg coliforms count between the egg laying spots.

However, there was significant difference of the egg coliforms count between the egg laying spots in farm B. In farm B, we can only statistically conclude that the pond-side eggs are more contaminated with coliform than the sand eggs. The farm B result does agreed my hypothesis to some extend which is pond-side eggs are more contaminated with coliform followed by the sand eggs. However, this experiment does not agree that the floor eggs have any difference in term of contamination compare to pond-side eggs and sand eggs.

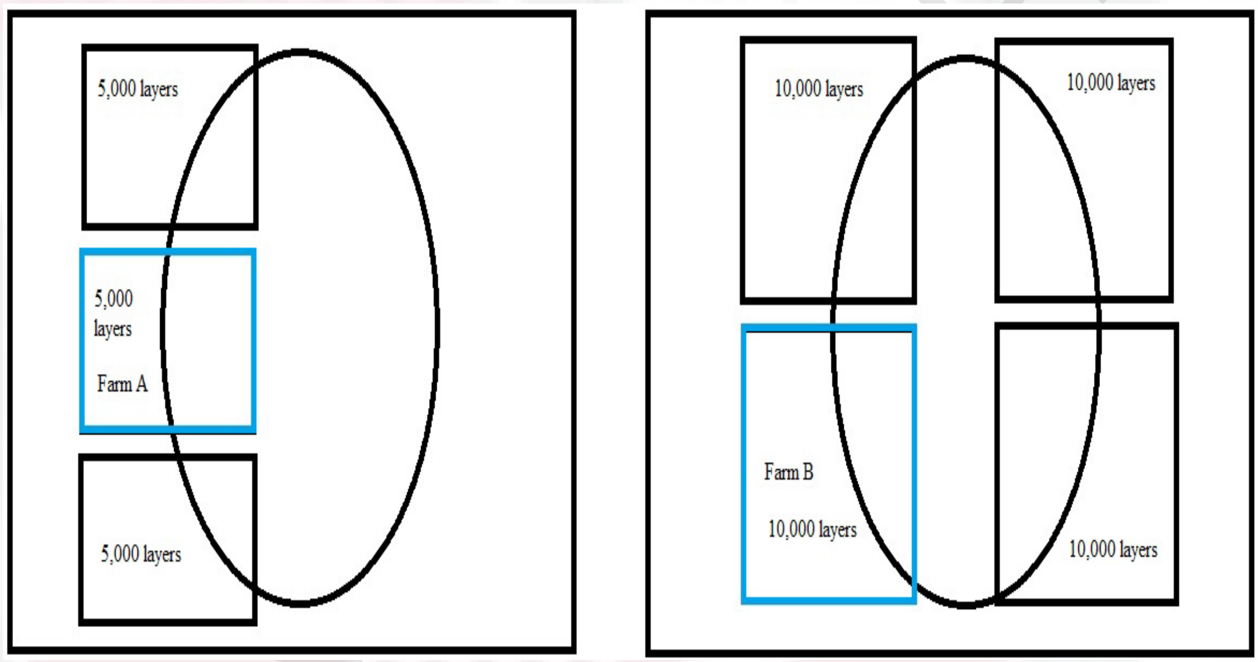
There are few possibilities that explain the different results yield from different farm. The first possibility is that the farm A just treated its pond-side area with limestone powder about 1 month ago before the layers are allow to access to the pond, said farmer. While the pond-side from farm B was not treated for more a year due to the layers are quite old. Limestone powder is directly used to neutralize the acid soils and lakes, which also able to kill bacteria due to the heating from neutralization (Harwood & Lodge, 2014).

The another possibility is the layer density of the pond, the pond in farm A is less contaminated may due the pond was shared with 15,000 layers only while the pond in farm B is share by 40,000 layers. The lay-out of the farm is shown in figure 5. The higher the layer densities around the pond, it may induce more contaminated pond water that causes egg contamination. Risk factors for developing duck layer colisepticemia include close proximity to other poultry farms or marked pond water contamination due to high stocking density (Swayne, 2013).

Eggs were classified according to the bacteria per eggshell such as freshly laid eggs with 300+ colonies, clean eggs with 3,000+ colonies, soiled eggs with 25,000+ colonies, and dirty eggs with 400,000+ colonies (Turblin, 2010). If according to Vincent Turblin, the duck eggs from all spots in both farms of this study are classified as clean eggs despite that there was significant difference between pond-side egg and sand egg in farm B.

Bacterial loads on washed and clean unwashed duck eggs were low, ranging from too few to count to 10^2/shell. In another trial, bacterial loads on washed and nest-clean eggs ranged between 10^2 to 10^3/shell and 10^2 to 10^4 /shell, respectively (Baker & Qureshi, 1984). If according to Baker and Qureshi, all the duck eggs from this study are consider as clean as washed or nest-clean eggs.

Figure 5: Farm lay-out that show the layer densities of the pond that shared with the farm where eggs were collected.



Therefore, the main finding is the pond-side eggs are more contaminated than the sand eggs only applicable to certain management practice. This study only agreed the hypothesis to certain extend.

5.2 *Salmonella spp* Isolation and Identification

From the results, none of the eggshell yielded *salmonella spp* and there is only 3.33% egg content yielded *salmonella spp.* Besides, only 5% of the sample from the floor yielded *salmonella spp* and none of the egg from sand and pond-side yielded *salmonella spp.*

This result is similar with the research did by Trongpanich on bacterial counts of duck eggs from one commercial farm but did not find salmonella (Trongpanich & Dawson, 1974).

However, this result is conflict with the study did by Baker and Qureshi about the studied of the prevalence of salmonella, only 0.21% of the eggshells yielded salmonellae. No salmonellae were found in the content of these eggs (Baker & Qureshi, 1984), which in my study only egg content yielded salmonellae not eggshell.

This result can be explain by Board, egg may become contaminated with salmonella organism through ovarian infection before it is laid or after laying through entry of microorganisms into the whole eggs and constitute a public health hazard (Board & Fuller, 1994). In my study, only one sample from the egg content was tested positive while none of the eggshell samples were positive, thus the *salmonella spp* contamination is most probably through ovarian infection.

None of the eggshell yield *salmonella spp* contamination. Therefore, it does not make any sense if we make judgment of the egg *salmonella spp* contamination by comparing the egg laying spots. Besides, the hypothesis that the eggshell is more contaminated with *Salmonella spp* than the egg content is not valid in this study.

5.3 Temperature Detection

There was no statistically correlation between the temperatures with the coliforms plate count and there was no support relationship between temperature and coliform plate count. This is because the mean temperature for pond-side, floor and sand are 35.9 ºC, 34.2 ºC, and 41.4ºC respectively which is fall in the temperature range that is optimize for coliform to growth. According to Roy Cullimore, the right temperature for total coliform to growth is 35 - 37ºC, however fecal coliform can growth well in 44.5ºC (Cullimore, 2000). Therefore, the hypothesis of the higher the temperature around the egg will have a lower egg contamination is not valid in this study.

5.4 Limitation

Although this research was carefully prepared and able to answer the objective of this study, I am still aware of its limitations and shortcomings. Here are the limitations that possibly impacted my results. First limitation was the sample size, the number of the eggs and number of farms in this study should be increase. Besides, sample should be collected randomly from different companies and the farms from whole Malaysia, so that the results can be imply to all duck farms in Malaysia.

Second limitation was the lack of available data, data that is related to duck egg contamination that related to egg laying spots were extremely insufficiency especially in Malaysia. Thus, more studies should be done in future. For example, studies related to the confounding factors such as egg size, management practice, age of the layer, layer densities, environment bacteria contamination, and time of exposure of the eggs to the environment. Besides, further studies can be more focus on the pathogenic agent or topics such as egg contaminations between raw duck eggs with processed duck eggs such as salted eggs and century eggs.

Third limitation was the measure and method used to collect the data, method such as total plate count method, *Escherichia coli* isolation and identification, and antibiotic sensitivity test can be used for more detail understanding of the contamination.

Therefore, further studies should be done to address these limitations.

6.0 CONCLUSION

In conclusion, the pond-side eggs are more coliform contaminated than the sand eggs but only applicable to certain management practice while the floor eggs have no significant difference in contamination than other eggs. It is not valid to say that the eggshell is more contaminated with *Salmonella spp* than the egg content. There was no statistically correlation and support relationship between the temperatures with the coliforms plate count.

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