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OCCURENCE AND ANTIBIOTIC RESISTANCE OF *Salmonella* spp. ISOLATED FROM EGGS OF CHICKEN RAISED UNDER FREE-RANGE AND CONVENTIONAL CAGED FARMS

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CERTIFICATION

It is hereby certified that I have read this project paper entitled "Occurrence and Antibiotic Resistance of *Salmonella* spp. Isolated from Eggs of Chicken Raised Under Free-range and Conventional Caged Farms", by Siti Noor Fadhilah Azihi 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- Project

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ABSTRACT

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

OCCURENCE AND ANTIBIOTIC RESISTANCE OF *Salmonella* spp. ISOLATED FROM EGGS OF CHICKEN RAISED UNDER FREE-RANGE AND CONVENTIONAL CAGED FARMS

By

Siti Noor Fadhilah binti Azihi

2016

Supervisor: Prof. Dr. Saleha Abdul Aziz

Free-range and conventional caged farming systems implement different practices in raising their chicken; and therefore this could contribute to differences in the *Salmonella* and other bacterial contamination levels of eggs. The objective of this study was to determine the occurrence of *Salmonella* in eggs produced by free-range chickens and commercial layers. The isolates were then subjected to an antibiotic sensitivity test to determine the antibiotic resistance pattern. A total number of 36 free-range chicken eggs were purchased from three free-range chicken farms and another 36 commercial chicken eggs were purchased from three conventional farms raising chickens in battery cages.

Salmonella spp. occurred in 2.8% (1/36 shell swab sample) and 8.4% (1/36 shell swab and 2/36 egg content samples) of conventional caged and free-range chicken eggs, respectively. Chi-square test showed significant association between farming system and level of *Salmonella* contamination in shell swabs and egg contents ($p < 0.05$). The one isolate from conventional caged chicken eggs was resistant to ampicillin. Two isolates (66.7%) from free-range chicken eggs were resistant to nalidixic acid, and 33.3% resistant to tetracycline, streptomycin, and trimethoprim- sulphamethaxzole. The pattern of antibiotic resistance of isolates from eggs obtained from free-range chickens' eggs and those from conventional caged was different. This study suggested that eggs from both production systems may not be as wholesome because of the presence of *Salmonella*, although low in number, but it is of public health significance.

Keyword: *Salmonella* spp., chicken eggs, free-range, conventional cages, antibiotic resistance

ABSTRAK

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

KEJADIAN DAN KETAHANAN ANTIBIOTIK *Salmonella spp.* YANG DIASINGKAN DARIPADA TELUR AYAM YANG DIBESARKAN SECARA TERNAKAN BEBAS DAN KOMERSIL

Oleh

Siti Noor Fadhilah Azihi

2016

Penyelia: Prof. Dr. Saleha Abdul Aziz

Sistem penternakan ayam secara bebas dan konvensional melaksanakan amalan perladangan yang berbeza dalam penternakan ayam mereka; oleh yang demikian perkara ini boleh menyumbang kepada perbezaan tahap kontaminasi oleh *Salmonella* dan bakteria lain di dalam telur. Kajian ini bertujuan untuk menentukan kejadian *Salmonella* dalam telur yang dihasilkan oleh ayam ternakan secara bebas dan komersil. Ujian sensitiviti antibiotik telah dijalankan ke atas isolat *Salmonella* untuk menentukan corak ketahanan antibiotik. Sejumlah 36 biji telur ayam kampung telah dibeli daripada tiga ladang ternakan ayam secara bebas dan 36 biji telur ayam komersial telah dibeli daripada tiga ladang konvensional yang menternak ayam dalam sangkar bateri. Terdapat 2.8% *Salmonella* spp. (sampel calitan kulit 1/36) pada

telur ayam sangkar dan 8.4% (1/36 calitan kulit dan sampel kandungan telur 2/36) pada telur ayam yang diternak bebas. Ujian *Chi-Square* menunjukkan terdapat hubungkait bererti antara sistem penternakan dengan tahap kontaminasi oleh *Salmonella* pada calitan kulit dan kandungan telur. Satu isolat pada telur ayam komersil didapati tahan terhadap *ampicillin*. Dua isolat (66.7%) daripada ayam yang diternak bebas adalah tahan terhadap *nalidixic acid*, dan 33.3% tahan terhadap *tetracycline*, *streptomycin* serta *trimethoprim-sulphamethaxzole*. Corak ketahanan antibiotik yang diperolehi daripada telur ayam yang diternak bebas dan ayam komersil adalah berbeza. Kajian ini menenemukan bahawa telur daripada kedua-dua sistem pengeluaran mungkin tidak seberapa selamat kerana adanya kehadiran *Salmonella*, walaupun pada tahap yang rendah, tetapi ia mempunyai kepentingan kesihatan awam.

Kata kunci: *Salmonella*, telur ayam, sangkar ayam, konvensional, ketahanan antibiotik

1.0 INTRODUCTION

Foodborne disease is one of the most important public health problems, which contributed by large number of cases and associated with economic cost (Kaferstein *et al*., 1997). *Salmonella* are the most common microorganisms causing foodborne disease (Kaferstein, 2003; Mead *et al*., 1999). Several studies reported that human affected by salmonellosis are frequently due to the consumption of poultry and poultry products; other foods involved include consuming raw and unpasteurized milk, drinking untreated water, and handling of pests and infected animals (Wingstrand *et al*., 2006). Eggs can be contaminated by *Salmonella* either on outer shell or in the egg content. The contamination of egg content is due to the penetration of the organism through the egg shell or due to direct contamination of egg contents before oviposition, which originate from infection of the reproductive organs (Gantois *et al*., 2009).

There is perception by many consumers that chicken reared in commercial battery cages were under overcrowded housing condition and overwhelming used of antibiotic to make the birds grow faster. Therefore, it was perceived that this type of birds are more prone to be infected with *Salmonella* and other bacterial pathogens than the free-range or organic birds, which are grown under more ‘‘natural’’ conditions (Bailey *et al*., 2005). Previous studies have also provided evidence indicating higher risks of bacterial contamination that occurred in eggs originated from non-housed chickens due to a lesser degree of contact of the caged animals with fecal material (Fossum *et al*., 2009, Kaufmann-Bart and Hoop, 2009). Therefore this study aimed to identify if such trends are also prevalent in the eggs obtained from free-range and conventionally-raised chicken farms in Malaysia.

Conventional poultry farms are also perceived by the public to have higher usage of antibiotics compared to the free-range farms, with the latter also perceived to be associated with an organic, antibiotic-free farming system (Bailey and Cosby, 2005, Harper and Makatouni, 2002). This however remains as presumption requiring more evidence. This study therefore tests the sensitivity levels of *Salmonella* spp. isolated from eggs.

Thus, the objectives of this study were:

1. to determine the prevalence of *Salmonella* in eggs obtained from free-range and conventional battery cage-raised chickens.

2. to compare the *Salmonella* contamination rates on eggs obtained from free-range and conventional caged layers and if such difference correlates with the housing type and farming practices implemented between the two types of layer farms

3. to determine the antibiotic sensitivity levels of *Salmonella* species isolated from eggs obtained from free-range and conventional caged-raised chickens.

2.0 LITERATURE REVIEW

2.1 *Salmonella* spp.

Salmonella genus is a member of the Enterobacteriaceae family, comprising Gram-negative, facultative anaerobes, rod-shaped (1-2μm), nonspore-forming bacteria, which their main reservoir is the intestinal tract of humans and animals (Bhunia, 2007). There are more than 2500 serotypes, and most of the serotypes are motile by using peritrichous flagella for example *S. gallinarum* and *S. pullorum* (Kerry *et al*., 2011). Infective dose for *Salmonella spp*. is different according to the serotypes of the bacteria, food vehicles, and the health of the host. The lowest infective dose can be as low as 20 cells and as many as 10^6 cells (Adams and Moss, 2000) to cause infection. The growth of *Salmonella* has been recorded from temperature just above 5 ˚C and up to 47 ˚C with the optimum temperature of 37 ˚C. *Salmonella* is heat sensitive and is readily destroyed by pasteurization temperature. *Salmonella* which is primarily inhabitant of gastrointestinal tract can be disseminated via faeces to soil, water, foods, and feeds and to other animals including human (Adam and Moss, 2000).

2.2 Prevalence of *Salmonella* in Poultry

According to a study done by Rusul *et al* (1996) on the prevalence of *Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia, a total of 158 out of 445 (35.5%) and 52 out of 104 (50.0%) broiler carcasses obtained from wet-markets and processing plants were contaminated with *Salmonella*, respectively. *Salmonella* was also isolated from 14 out of 98 (14.3%) samples of intestinal contents. Litter samples from broilers

and breeders farms were positive for *Salmonella*, 8 out of 40 (20%) and 2 out of 10 (20%) respectively. This study also stated that the predominant serovars were S. enteritidis, *S. Muenchen*, *S. Kentucky*, and *S. Blockley*.

Another study done by Saleha *et al* (2013) on isolation of *Salmonella* from duck eggs and cloacal swabs reported 12 out 75 (16%) samples of cloacal swabs were positive for *Salmonella*, and none of the eggs were positive for *Salmonella*. From those positive samples obtained, three *Salmonella* serovars were identified which were *S. Agona* (16.7%), *S. Braenderup* (50.0%), and *S. Corvallis* (33.3%). Study done by Loong *et al*., 2015 stated that 3.7% of *Salmonella* was isolated from the surface of 162 conventional broiler eggs, and another 3.7% was isolated from the surface of 162 "Kampung" chicken eggs. However, there was no *Salmonella* isolated from the carrying trays. In addition, a study done by Hassan *et al* (2005) revealed *Salmonella* spp. was isolated from eight samples that were tested, of which three were isolated from commercial layers eggs (7.5%) and five from free-range chickens' eggs (12.8%).

2.3 *Salmonella* Contamination of Eggs

Salmonella can contaminated the eggs on the both of outer shells surface and internally. Surface contamination may be due to fecal contamination or infection of the vagina. Outer shell membrane contamination occur following oviposition. Any contaminated environment in the area of the laid eggs, such as the nest box, the hatchery environment or the hatchery truck, can lead to outer shell contamination. Survival and growth of *Salmonella* are facilitated by the presence of chicken manures and other moist organic material, by

providing the required nutrients and a degree of physical protection to the bacteria (Gantois *et al*., 2009). According Schoeni *et al* (1995), stated that when eggs are artificially contaminated on the shell with faeces containing *Salmonella* and subsequently stored at 25 °C, the numbers of bacteria increased by 1–2 logs by day 1 and 4–5 logs by day 3.

Besides that, according to Gantois *et al* (2009), two possible routes of internal contamination of eggs may be due to penetration of *Salmonella* through the eggshell and shell membranes after outer shell contamination and direct contamination of the yolk, yolk membranes, albumen, shell membranes and egg shell originating from infection of the ovary, infundibulum, magnum, isthmus and shell gland, respectively. In addition, *Salmonella* deposited in the albumen and on the vitelline membrane are able to survive and grow in the antibacterial environment. They are also capable of migrating to and penetrating the vitelline membrane in order to reach the yolk. After reaching this rich environment, they can grow extensively.

2.4 Public Health Significance

Salmonellosis is one of the most frequent food-borne disease, being an important public health problem in almost all industrialized countries (Antunnes *et al*., 2003). Salmonellosis is an important zoonotic infection and human salmonellosis causes widespread morbidity and economic loss (Wray and Davies, 2003). Human food-borne salmonellosis usually manifests clinically as acute gastroentritis which can be serois and may lead to hospitalization and even death (Fegan *et al*., 2004). Salmonellosis is the second leading cause

of bacterial gastroentritis after *Campylobacter* and is responsible for most of the food-borne disease outbreaks particularly in developed country (Fegan *et al*., 2004).

Most of human infection may be due to the consumption of foods of animal origin such as beef, pork, poultry, eggs, or dairy products that may be contaminated with *Salmonella* (Gebreyes *et al*., 2000; Rajashekara *et al*., 2000). According to Lee *et al* (2013), contaminated poultry-derived products, which were commonly chicken eggs became an important vehicles of *Salmonella* infections, especially with the pathogen presence in the egg contents. Futhermore, this issue was recently highlighted in the *Salmonella* outbreak in shell eggs that occurred in the United States in May 2010 (US Food and Drug Administration, 2010).

In Malaysia, from 1978 to 1997, a prevalence of 57% of diarrheal cases among children due to *Salmonella* was reported (Berger, 2010). Cases of foodborne illness in Malaysia is lower compared to other developed countries such as United States and United Kingdom because most of many cases go unreported and a chain of events need to be addressed first before it is brought to the authority (Soon *et al*., 2011).

The symptoms of salmonellosis in human comprise diarrhea, fever, headache, nause, abdominal pain, vomiting, and bloody stools (Chung *et al*., 2003). This *Salmonella* food borne infection is commonly caused by the ingestion of foods that contain significant numbers of non-host-specific serotypes of *Salmonella*. From the time of ingestion of food, the symptoms usually develop in 12-24 hours, and the symptoms may persist for 2-3 days. The average mortality rate is 4.1% which varies from 5-8% during the first year of life, to 2% between the first and 50 years old, and 15% in persons over 50 years of age. *S.*

choleraesuis has been reported to produce the highest mortality rate which is about 21% (Jay, 2000).

2.5 Antibiotic Resistance

Antimicrobial agents started to be introduced in the early 20th century, and become one of the greatest achievement of scientific medicine. However, the use of these antimicrobial agents for clinical and growth promotion purposes (Gunal *et al*., 2006) had increased the number of organisms that are able to resist these agents. From the previous study that was carried out, resistance bacteria were found within three to five years from the time of introduction of the antimicrobial agents into clinical use (Schwarz and Chaslus-Dancla, 2001; Swartz, 2002). Antibiotic resistance also contributed to problem in food safety where the use of antibiotic in food animals for treatment, disease prevention, and growth promotant allow resistant bacteria and resistance genes to spread from food animals to the human through the food-chain (WHO, 2011).

In Malaysia, *S. Agona* and *S. Weltevreden* became the second and third most prevalent *Salmonella* serovars isolated from animal and livestock products over a 5-year period, which was 1996-2001 (Maria *et al*.,2002). In England and Wales, penta-resistant (ACSSuT) S. Typhimurium DT104 become the second most prevalent *Salmonella* serovars in humans, from fewer than 250 isolates in 1990 to 2973 in 1994 and 3837 in 1995 (Threlfall *et al*.,1996). *S. Typimurium* definitive type 104 (DT104) is characterized by its resistance to five antimicrobials, comprised ampicillin (AMP), chloramphenicol (C), streptomycin (S), sulfa

drugs (SXT), and tetracycline (TE). In addition, DT104 has acquired resistance to trimethoprim and the fluoroquinolones (Jay, 2000).

Furthermore, in the UK, the multi-resistance of *S. Typhimurium* to AMP, C and SXT has increased from 1% to 25%, 1.5% to 25% and 0% to 25%, respectively, from 1986 to 1993 (Threlfall *et al*.,1996). In the USA, resistance to TE in *Salmonella* species increased from 9% to 24% between 1980 and 1990 (Lee *et al*. 1994), resistance to AMP increased from 10% to 14%, C from 1% to 8% and SXT from 0% to 3%, between 1980 and 1995 (D'Aoust., 2001).

Other researchers have found higher levels of resistance to AMP and C among human clinical isolates (Cohen and Tauxe 1986; Lee *et al*., 1994; Hsu *et al*., 2006; Livermore *et al*., 2007). Although AMP is still therapeutically administered in food-producing animals, C has been banned in food-producing animals since the 1970s. In addition, TE has been one of the most commonly used antimicrobial agents for production animal, so the very frequent occurrence of resistance is probably a consequence of this (Threlfall *et al*., 2003).

3.0 MATERIALS AND METHODS

3.1 Study Design

A prevalence study was conducted from 11th January 2016 to 29th January 2016. In this study design, 36 eggs from commercial battery cages chickens and another 36 free-range chicken eggs were randomly selected and sampled for the presence of *Salmonella* spp. on eggs shells and in eggs content.

3.2 Sources of Eggs

The target population was the commercial battery cages chicken eggs and free-range chicken eggs. The source of commercial battery cages chicken eggs were from the three private farms in several areas in Selangor and Melaka, while free-range chicken eggs were collected from another three private farms in Selangor and Negeri Sembilan. Twelve eggs were obtained from each farm. A total of 72 eggs were sampled from the six farms. The eggs were brought to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia in icebox to preserve the microorganism. The eggs were processed on the same day of sampling.

3.3 Isolation and Identification

The isolation of *Salmonella* from the both type of chickens' eggs followed the method described in Laboratory Protocol of Isolation of *Salmonella* spp. from Food and Animal Feces (World Health Organization, 2010). The shell of each egg was swabbed by using a sterile swab moistened with Buffer Peptone Water (BPW) (Oxoid), and then the swab was placed in a bottle containing 9 ml BPW as pre-enrichment medium, and incubated at 37˚ C for 24 hours. The shell of the entire egg was cleaned and then immersed in the 70% alcohol

solution for one minute before it was cracked open aseptically using a sterile forceps. The whole content of an egg was placed in the sterile stomacher bag and being homogenized. One ml of the egg homogenate was pipetted out and placed in a bottle containing 9 ml BPW as pre-enrichment medium, and incubated at 37˚ C for 24 hours.

After 24 hours, 0.1 ml of the broth was transferred into 9 ml Rappaport Vassiliadis Soy Broth (Oxoid) as selective enrichment medium and further incubated at 42˚ C for 24 hours. After the incubation period had completed, two to three loopful of each enriched broth culture was streaked onto the surface of Xylose-Lysine Desoxycholate agar (XLD) (Oxoid) and Brilliant Green agar (BGA) (Oxoid), and incubated at 37˚ C for 24 hours.

Typical colonies of *Salmonella* appeared as pink to red with black centers on XLD agar (Plate 1), and red to pink colonies on the BGA agar due to the bacteria does not ferment lactose or sucrose (Plate 2). Two to three presumptive colonies were then cultured on nutrient agar, and incubated at 37˚ C for 24 hours. Pure cultures obtained were subjected to biochemical tests which included Triple Sugar Iron Agar (TSI) (Oxoid), Urea broth (Oxoid), Lysine Iron Agar (LIA) (Oxoid), Sulphide-Indole-Motility (SIM) (Oxoid), and Citrate (Oxoid) to identify *Salmonella*. Positive reactions were showing in Plate 3.

The isolates were then further confirmed as *Salmonella* spp. by using the serological test. A small amount of culture from nutrient agar that was tested positive by biochemical test was mixed with a loopful of 0.85% normal saline on a clean glass slide. A drop of *Salmonella* O Polyvalent Antiserum (Remel Europe Ltd) was added. The mixture was then

tilted back and forth for approximately one minute and observed against a black background. A positive reaction was indicated by a rapid, strong agglutination (Plate 4).

3.4 Antibiotic Sensitivity Tests

The antibiotic susceptibility test was conducted based on standard disk diffusion method of the Clinical Laboratory Standards Institute (CLSI) guidelines. Nine common antibiotics were used including chloramphenicol (30 µg), ampicillin (10 µg), streptomycin (10 µg), nalidixic acid (30 µg), gentamicin (10 µg), trimethoprim-sulphamethoxazole (25 µg), and tetracycline (30 µg), ceftriaxone (30 µg), and ciprofloxacin (5 µg). Pure cultures were grown in Tryptic Soy Broth (TSB) at 37 °C, for 2 to 6 hours. The concentration of the culture was adjusted using sterile TSB until 0.5 McFarland turbidity was attained. One hundred microliters of the culture was then swabbed onto Mueller Hinton agar (Oxoid, UK) using a sterile cotton swab. Antimicrobial disks were then placed on the surface of the agar plate at a distance to avoid overlapping of inhibition zones. The plates were then incubated at 37 °C for 16 to 18 hours and the results interpreted. The zone around an antibiotic disk that has no growth is referred to as the zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. This zone is then measured in millimetre and compared to a standard interpretation chart to categorize the isolate as susceptible, intermediately susceptible or resistant.

3.5 Species Identification

The confirmed isolates were then sent to the Veterinary Research Institute (VRI), Ipoh for serotyping.

3.6 Data Analysis

The percentages of *Salmonella* spp. in both type of rearing system were calculated using the formula below:

$$No.\ of\ positive\ samples \div Total\ no.\ of\ samples \times 100$$

Chi-square test was done to determine the association between farming system and the level of *Salmonella* contamination in shell swabs and egg contents, at $\alpha = 0.05$.

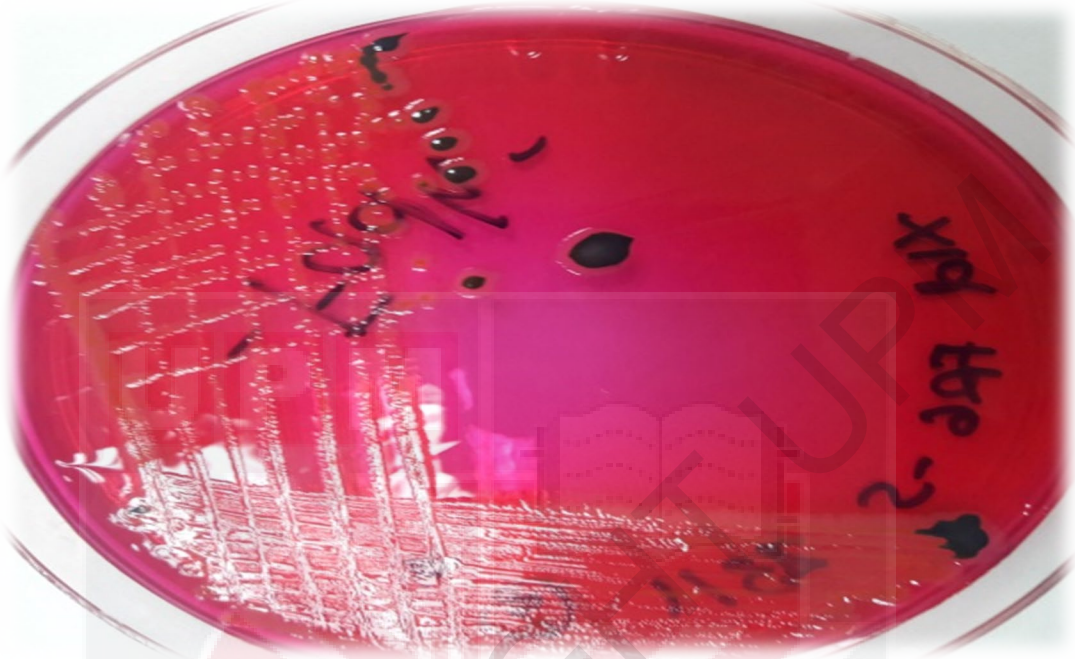

Plate 1: Presumptive *Salmonella* colony on XLD agar

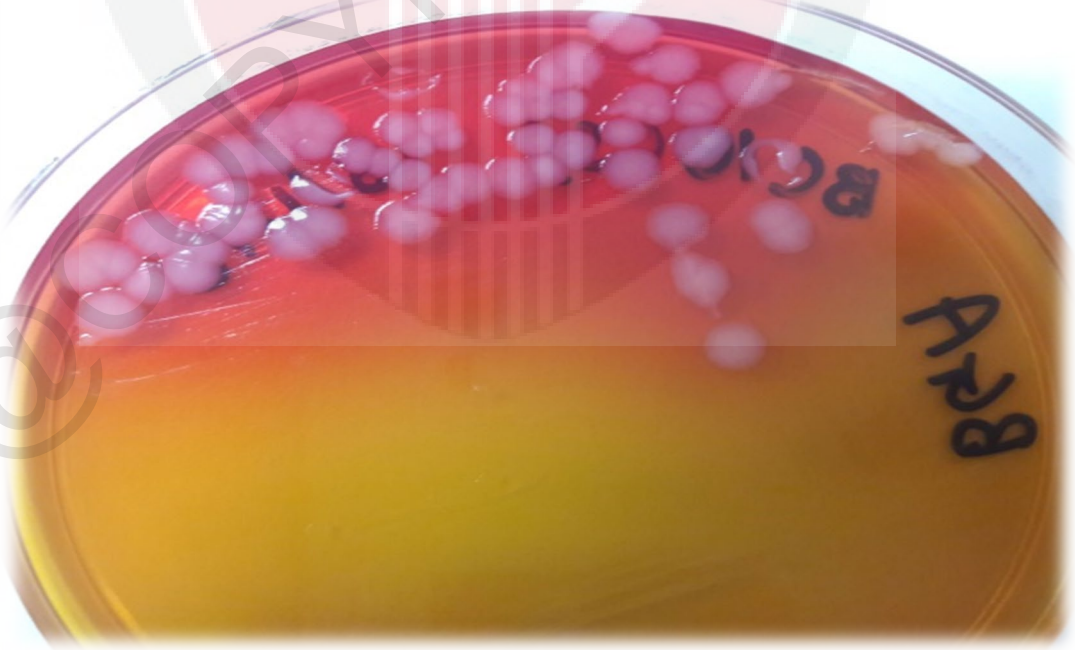

Plate 2: Presumptive *Salmonella* colony on BGA

Plate 3: Positive biochemical reaction for *Salmonella* spp. Note (1) Triple Sugar Iron (TSI), (2) Urea Test, (3) Citrate Test, (4) Sulphur Indole Motility (SIM), (5) Lysine Sugar Iron (LIA)

Plate 4: *Salmonella* O Polyvalent Antiserum Test

4.0 RESULT

4.1 Isolation and identification of *Salmonella* spp. in chicken eggs

Sampling of chicken eggs were illustrated in Figure 1

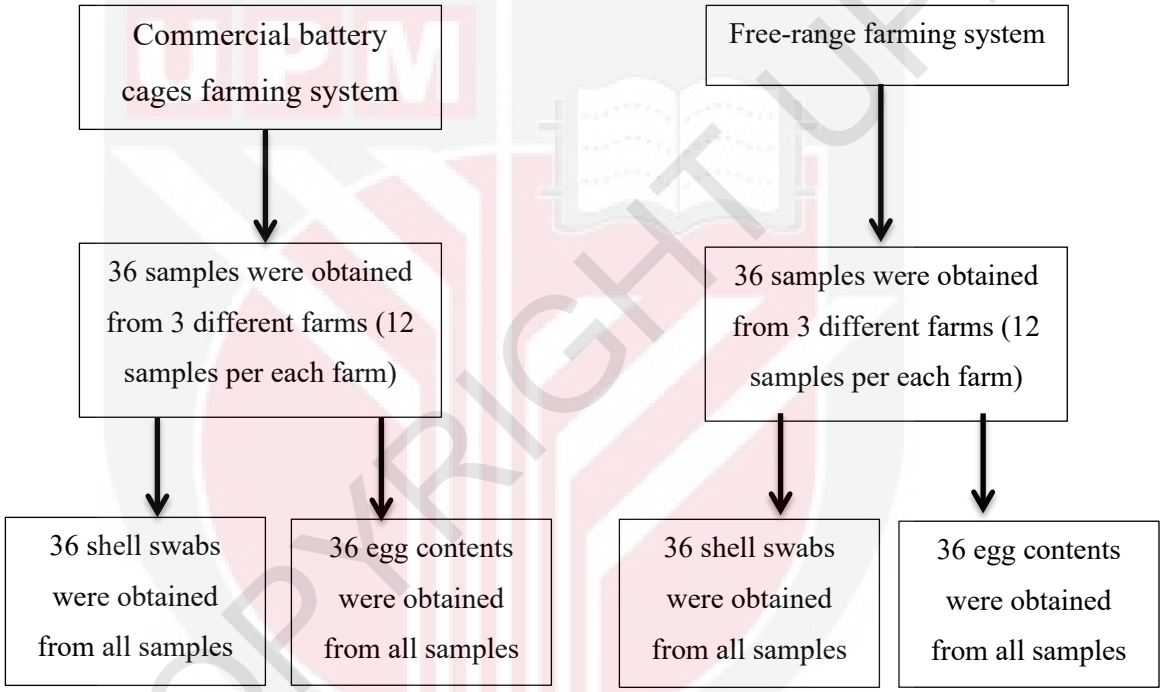

Figure 1: Samples collected for isolation of *Salmonella* spp.

For the commercial battery cages' chicken eggs, only 1 (2.8%) of shell swab out of 36 samples was positive for *Salmonella* spp., and none of samples from eggs content was tested positive for *Salmonella* spp. For the free-range chicken eggs, 2 (5.6%) samples of shell swab out of 36 samples were tested positive for *Salmonella* spp. and only 1 (2.8%) sample

from egg content were tested positive for *Salmonella* spp. In total, 2.8% *Salmonella* was isolated from commercial layer eggs, and 8.4% from the free-range chickens eggs (Figure 2).

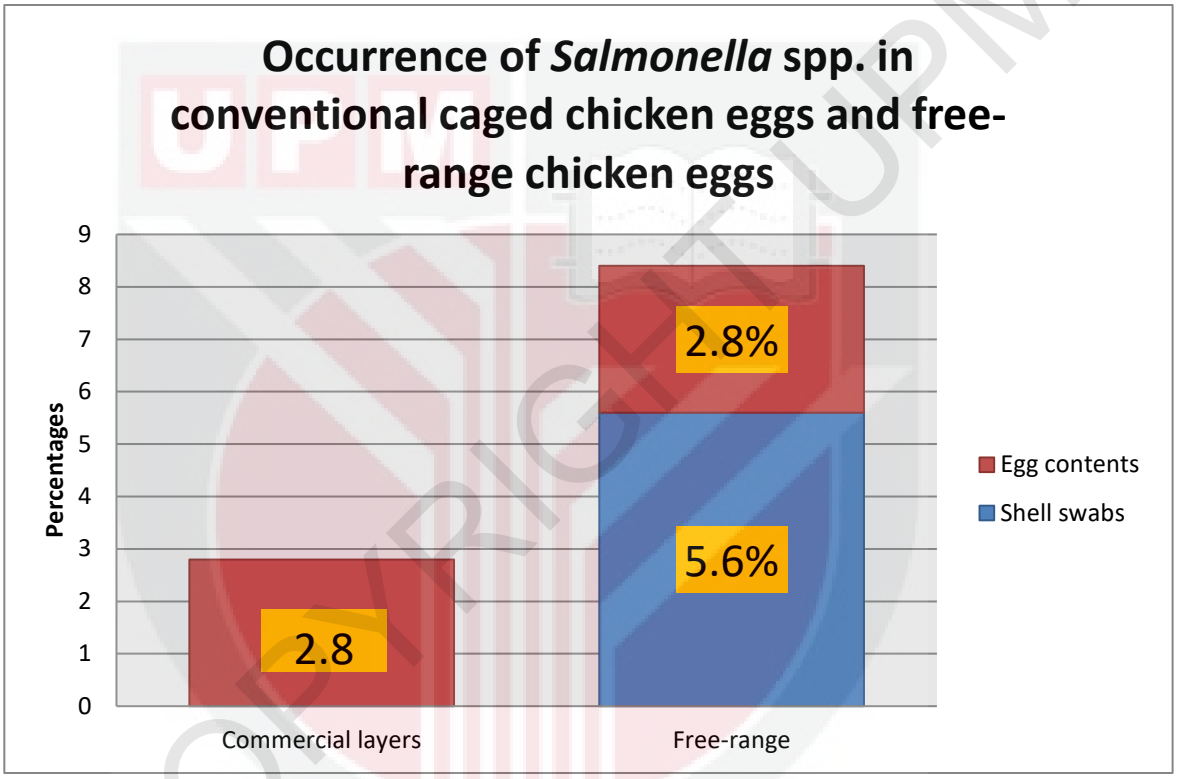

Figure 2: Occurrence of *Salmonella* in commercial layers chicken eggs and free-range

Chi-Square test showed association between farming system and level of *Salmonella* contamination in shell swabs and egg contents ($p < 0.05$)

4.2 Antibiotic Sensitivity Test

Antibiotic sensitivity test was carried out on *Salmonella* isolation and results of the antimicrobial test are listed in Table 5.

Table 1: Antibiotic sensitivity test for *Salmonella* against nine antimicrobial agents for both systems

Antimicrobial agents	Percentages Susceptible (%)		Percentages Intermediate (%)		Percentages Resistance (%)	
	CC	FR	CC	FR	CC	FR
TE (30 µg)	100%	66.7%	-	-	-	33.3%
AMP (10 µg)	-	66.7%	-	33.3%	100%	-
CRO (30 µg)	100%	100%	-	-	-	-
CIP (5 µg)	-	-	100%	100%	-	-
S (25 µg)	100%	66.7%	-	-	-	33.3%
SXT (25 µg)	100%	66.7%	-	-	-	33.3%
CN (10 µg)	100%	-	-	100%	-	-
C (30 µg)	100%	66.7%	-	33.3%	-	-
NA (30 µg)	-	33.3%	100%	25%	-	66.7%

Note: (1) Tetracycline (TE); Ampicillin (AMP); Ceftriaxone (CRO); Ciprofloxacin (CIP); Streptomycin (S); Trimethoprim/ Sulpha-methaxzole (SXT); Gentamicin (CN); Chloramphenicol (C); Nalidixic acid (NA). (2) Conventional caged (CC); Free-range (FR).

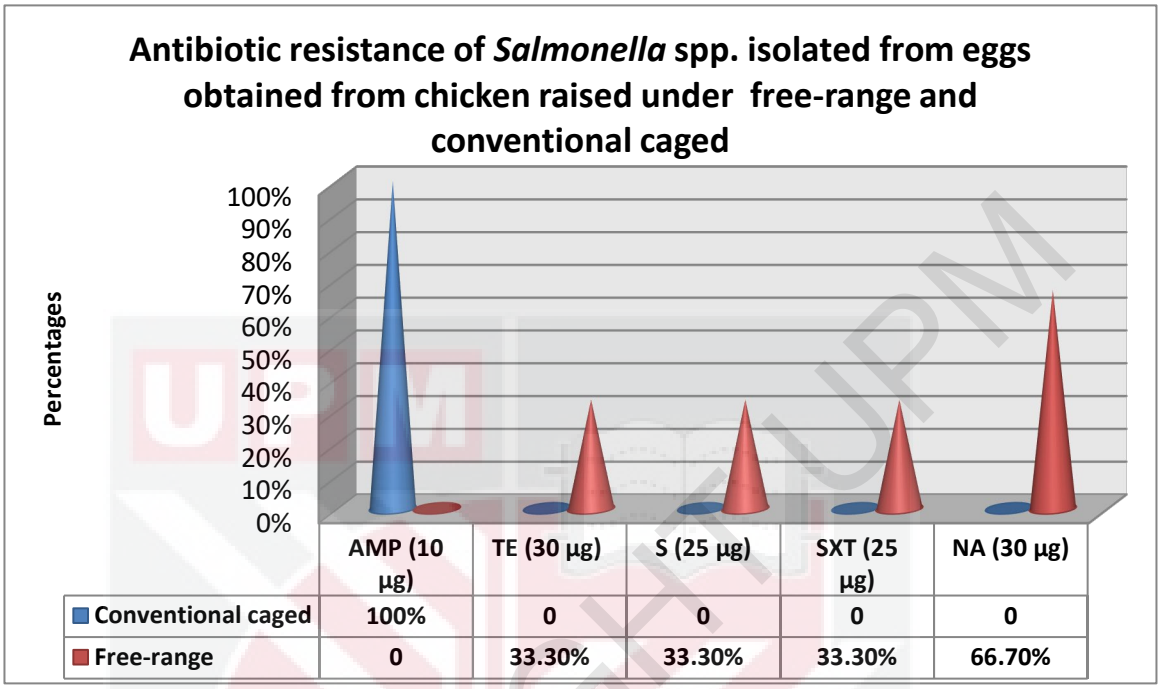

Figure 3: Antibiotic resistance of *Salmonella* spp. isolated form eggs obtained from chicken raised under free-range and conventional caged

Table 2: Antibiotic resistance pattern of *Salmonella* isolates

Antibiotics	No. of samples
AMP	1
NA	2
TE-S-SXT	1

Note: Tetracycline (TE); Ampicillin (AMP); Streptomycin (S); Trimethoprim/ Sulpha-methoxazole (SXT); Nalidixic acid (NA).

5.0 DISCUSSION

In this study, *Salmonella* spp. occurred in 2.8% (1/36 shell swab sample) and 8.4% (1/36 shell swab and 2/36 egg content samples) of conventional caged and free-range chicken eggs, respectively. In a study done by Hassan *et al* (2005) it reported that *Salmonella* spp. occurred in 7.5% commercial layers and 12.8% in free-range chicken eggs. In addition, another study done by Parisi *et al* (2014) showed *Salmonella* spp. was detected in 2.4% of eggs shells from free-range eggs and no isolation from battery cages.

The occurrence of *Salmonella* in free-range eggs was higher than in the conventional caged eggs. As *Salmonella* become one of the important pathogens in the poultry industry, trans-ovarian transmission of the pathogen to the eggs has been markedly reduced through testing and culling of affected layers (Coutts, 1981). Therefore, occurrence of *Salmonella* in the conventional caged chicken eggs was expected to be lower. Furthermore, most of the conventional caged farming system was practicing vaccination programmed as a routine management practice. According to the Gantois *et al* (2006), vaccination helps to reduce both the shedding and colonization of the reproductive tract which leads to a decrease of the number of internally infected eggs.

In free range chicken, there was 5.6% of *Salmonella* spp. was obtained from egg shells. However there was no isolation obtained from conventional caged chicken eggs. In conventional caged systems, such as the one used in this study, the cages are angled slightly to allow eggs to roll to a collection trough after oviposition and eggs are physically separated from the hens. Furthermore, hens which reared in battery cages are standing on wire slats that allow feces to fall to a manure collection system beneath the hens. This can prevent the contact of eggs with contaminated feces.

Conversely, free-range hens laid their eggs in nest boxes on shavings and the eggs remained in contact with hens, shavings and fecal material until they are collected. The longer contact time with free-range hens, shavings and feces would explain the higher Enterobacteriaceae counts on free-range eggs as compared to battery cages eggs (Hannah *et al*., 2011). The greater occurrence of *Salmonella* in free-range chickens also should not be surprising because free-range chickens have access to the outside, where there is sufficient opportunity for exposure to wild birds, insects, rodent droppings, and other potential carriers of *Salmonella* (Bailey *et al*., 2011).

Upon antibiotics sensitivity test, the isolates was resistant toward five antibiotics which comprised tetracycline (TE), ampicillin (AMP), streptomycin (S), trimethoprim/ sulpha-methaxzole (SXT), and nalidixic acid (NA). Two isolates (66.7%) from free-range chicken eggs were resistant to nalidixic acid, and 33.3% resistant to tetracycline, streptomycin, and trimethoprim- sulphamethaxzole. In addition, one isolate from free-range chicken eggs was multi-drug- resistant (TE-S-SXT). Study done by Hassan *et al* (2005) also revealed there was two isolates (100%) from commercial layers chicken eggs were resistant toward ampicillin, four isolates (100%) from free range chicken eggs were resistant towards both nalidixic acid and streptomycin.

Multi-drug resistant *Salmonella* in free-range chicken eggs in this study can occurred due to several factors. According to a study carried on by Mamber and Katz, (1985), they concluded that multiple antibiotic resistances could occur in almost any enteric bacilli, even though the animals were not given antibiotic supplements or medication, as long as they were exposed to an environment where other antimicrobial resistant strains were presence.

Therefore, as free-range chickens generally have a longer life span than most commercial layers; it is possible that antibiotic resistance was acquired over time through genetic material transfer from other resistant organisms. In addition, the resistance could have also been acquired from prolonged exposure to food with antibiotic residues, for example from leftovers of human food tainted with antibiotics

6.0 CONCLUSION AND RECOMMENDATION

A greater occurrence of *Salmonella* spp. was obtained from free-range chicken eggs compared to the conventional caged chicken eggs. However, this study suggested that eggs from both systems may not be as wholesome because of the presence of antibiotic resistant *Salmonella*, even though low in number, but it is of public health significance.

The difference in the occurrence of the organism for both systems may be influenced by the different rearing practice in the farms. These bacteria can lead to salmonellosis in human upon consumption of the eggs and the resistant bacteria can be transfer to the human through the food chain.

In future study, samples size should be increase to determine the actual condition and widen the localities for sampling to obtain actual occurrence of this bacteria in Malaysia

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APPENDIX 1

Type of farming system	Type of farms	Type of samples	No. of samples	No. of positive samples	Percentage of positive samples
Commercial	A	Shell swab	36	1	2.8%
battery cages		Egg content	36	0	0%
	B	Shell swab	36	0	0%
		Egg content	36	0	0%
	C	Shell swab	36	0	0%
		Egg content	36	0	0%
Free-range	A	Shell swab	36	2	5.6%
		Egg content	36	1	2.8%
	B	Shell swab	36	0	0%
		Egg content	36	0	0%
	C	Shell swab	36	0	0%
		Egg content	36	0	0%

APPENDIX 2

Inhibition Zone in Diameter (mm)				
Antimicrobial agents	Battery cages	Free-range		
	Isolate 1	Isolate 1	Isolate 2	Isolate 3
TE (30 µg)	22 (S)	27 (S)	0 (R)	23 (S)
AMP (10 µg)	13 (R)	23 (S)	20 (S)	16 (I)
CRO (30 µg)	31 (S)	32 (S)	31 (S)	30 (S)
CIP (5 µg)	26 (I)	28 (S)	26 (S)	28 (I)
S (25 µg)	18 (S)	17 (S)	0 (R)	19 (S)
SXT (25 µg)	28 (S)	26 (S)	0 (R)	20 (S)
CN (10 µg)	15 (S)	13 (I)	14 (I)	14 (I)
C (30 µg)	19 (S)	18 (S)	17 (I)	19 (S)
NA (30 µg)	15 (I)	12 (R)	19 (S)	12 (R)