EPIDEMIOLOGY, ANTIBIOGRAMS AND MULTILOCUS SEQUENCE TYPING OF Salmonella enterica AMONG Ayam Kampong (Gallus gallus domesticus Linnaeus) FROM SOUTH-CENTRAL PENINSULAR MALAYSIA

SALEH MOHAMMED JAJERE

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By

SALEH MOHAMMED JAJERE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

January 2020

DEDICATION

This work is dedicated to my late father, Alhaji Mohammed Ahmed Jajere of blessed memory, and to my mother, Hajiya Rukayyatu Abdullahi.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EPIDEMIOLOGY, ANTIBIOGRAMS AND MULTILOCUS SEQUENCE TYPING OF *Salmonella enterica* AMONG *Ayam Kampong* (*Gallus gallus domesticus* Linnaeus) FROM SOUTH-CENTRAL PENINSULAR MALAYSIA

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Chairman : Professor Latiffah binti Hassan, DVM PhD
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Salmonella enterica represents an important foodborne pathogen worldwide. In Malaysia, several large foodborne disease outbreaks involving human fatalities have been linked to eating chicken and its products contaminated with *Salmonella*. Village chickens are presumed to be raised in a more ‘organic’ environment and have not been studied for the prevalence of *Salmonella*. With increasing consumer awareness on food safety issues related to chemical residues and antibiotics used in producing commercial chickens, the demand for village chickens in Malaysia and other parts of the world has increased. Consumers are willing to pay higher price for products raised in organic setups. However, the epidemiology of *Salmonella* infection amongst village chickens in Malaysia remains largely unknown. Thus, the current study investigates the epidemiology, antibiograms and genetic diversity of *Salmonella enterica* amongst village chickens from the South-central Peninsular Malaysia.

Thirty-five village flocks were sampled from Selangor (*n*=19), Melaka (*n*=10), Johor (*n*=4), and Negeri Sembilan (*n*=2). In total, 1042 samples were collected; these included cloacal swabs (*n*=675), eggs (*n*=62), pooled drinking water (*n*=175), pooled feeds (*n*=70), and pooled flies (*n*=60). Isolation of *Salmonella* from these samples was carried out according to the protocols and recommendations of the World Organization for Animal Health (OIE) terrestrial manual. The prevalence of *Salmonella* at an individual bird-level was 2.5% (17/675, 95% CI: 1.6–4.0). All eggs screened were negative. For environmental samples, *Salmonella* was detected in 5.14% (9/175), 7.14% (5/70), and 5.0% (3/60) for water, feed, and flies, respectively. Thirty-four isolates and eight *Salmonella* serotypes were identified. *S.* Weltevreden (20.6%) was the most common, followed by *S.* Typhimurium and *S.* Agona (17.6%),

S. Albany and *S.* Enteritidis (8.8%), *S.* Molade (5.9%), *S.* Corvallis and *S.* Schleissheim (2.9%), and others grouped as *Salmonella* spp. (11.8%). Multivariable logistic regression models revealed that *Salmonella* positivity among flocks could be strongly predicted by storage of feeds (uncovered feeds; OR=10.38; 95% CI: 1.25−86.39; *p=0.030*) and uncovered water tanks (uncovered tank; OR=6.43; 95% CI: 1.02−40.60; *p=0.048*).

Among the isolates, 26.5% (n=9) were susceptible to all antibiotics, while 73.5% (n=25) were resistant to at least one antibiotic tested. Multidrug resistance was displayed by 8 isolates (23.5%). Ciprofloxacin (100%), gentamicin (97.1%), norfloxacin (97.1%), cefotaxime (97.1%) and ceftiofur (97.1%) were very effective against most isolates. The highest level of resistance was observed for tetracycline (35.3%) and streptomycin (35.3%). Eight isolates (23.5%) were MDR to two or more antibiotic agents belonging to ≥ 3 antimicrobial classes. Colistin resistance (Minimum inhibitory concentrations: 4 - 16 mg/L) was detected among 5 (14.7%) isolates comprising *S.* Weltevreden, *S.* Albany, *S.* Typhimurium and *Salmonella spp*. Representative *Salmonella* isolates (n=15) were examined by multilocus sequence typing (MLST) using seven housekeeping genes from the MLST online database. The *Salmonella enterica* serovars were resolved into 7 sequence types (STs). The ST13 (n=3) and ST11 (n=3) were the predominant STs respectively found in serovars Agona and Enteritidis, followed by ST34 (n=2) and ST36 (n=2), ST365 (n=2), ST1541 (n=2) and ST19 (n=1) respectively found in serovars Typhimurium, Weltevreden, Corvallis and Typhimurium. It was found that, with the exception of serotype Typhimurium, the MLST indicated a strong correlation between the STs and serovars. Each serovar of the Enteritidis, Agona, Weltevreden and Corvallis strains was represented unique STs.

In conclusion, it was found that the prevalence of *Salmonella* in village chickens in the study area was lower than that reported from commercial chickens in Malaysia. Uncovered feed and water storage were strongly associated with *Salmonella* positivity among the village chicken flocks. The present study also demonstrated the contamination of local village chickens with MDR non-typhoidal *Salmonella* strains, some of which were resistant to multiple classes of antimicrobials. Generally, the level of resistance was meaningfully lower than those reported from commercial chicken carcases and its products in Malaysia and elsewhere. However, despite the minimum application of antibiotics in the free-range chicken production and lack of antibiotic growth promotion use, the resistance to colistin was observed. The exposure to natural environment such as wild birds, proximity to commercial chicken farms as well as contaminated water and soils may play complex role in determining antibiotic resistance. Molecularly, all sequence types identified among the isolates tested in the present study has been documented in Malaysia previously, and were also reported in many Asian countries including China, Singapore, Indonesia, Thailand, Vietnam and Myanmar (ST11). ST365 was also reported in Singapore. This finding highlights that these village chicken *Salmonella* strains are not unique and have been circulating in the Asian region in multiple animal species and humans.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

EPIDEMIOLOGI, ANTIBIOGRAM DAN PENJENISAN MULTILOKUS JUJUKAN *Salmonella enterica* DI KALANGAN AYAM KAMPUNG (*Gallus gallus domesticus* Linnaeus) DARI BAHAGIAN TENGAH-SELATAN SEMENANJUNG MALAYSIA

Oleh

SALEH MOHAMMED JAJERE

Januari 2020

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Salmonella enterica merupakan patogen makanan yang penting di seluruh dunia. Di Malaysia, beberapa wabak penyakit yang besar mengakibatkan kematian manusia telah dikaitkan dengan daging ayam serta produknya yang telah dicemari dengan *Salmonella*. Ayam kampung dianggap dipelihara dan dibesarkan dalam persekitaran yang lebih 'organik' dan belum dikaji prevalens (kelaziman) terhadap *Salmonella*. Dengan meningkatknya kesedaran pengguna mengenai isu keselamatan makanan yang berkaitan dengan bahan kimia dan penggunaan antibiotik dalam penghasilan ayam komersil, permintaan ayam kampung di Malaysia dan negara-negara lain di dunia telah meningkat. Pengguna sanggup membayar dengan harga yang lebih tinggi untuk produk yang dikeluarkan dalam persekitaran organik. Walaubagaimanapun, epidemiologi jangkitan Salmonella di kalangan ayam kampung di Malaysia masih tidak di ketahui. Oleh itu, kajian ini mengkaji epidemiologi, antibiogram dan kepelbagaian genetik *Samonella enterica* di kalangan ayam kampung di selatan-tengah semenanjung Malaysia.

Tiga puluh lima kawanan (*flocks*) ayam kampung dari Selangor (n=19), Melaka (n=10), Johor (n=4), dan Negeri Sembilan (n=2) telah di ambil sampelnya. Secara keseluruhan, 1042 sampel telah dikumpulkan; ini termasuk swab kloaka (n=675), telur (n=62), air minuman yang di satukan *(pooled)* (n=175), makanan yang dikumpulkan (n=70) dan lalat yang dikumpulkan (n=60). Pemencilan *Salmonella* dari sampel-sampel ini dilakukan mengikut protokol dan cadangan dari manual Pertubuhan Kesihatan Haiwan Sedunia (OIE). Prevalens *Salmonella* pada setiap tahap-burung (bird-level) ialah 2.5% (17/675, 95% CI: 1.6-4.0). Semua telur yang telah diperiksa adalah negatif. Untuk sampel persekitaran, *Salmonella* dikesan sebanyak 5.14% (9/175), 7.14% (5/70), dan 5.0% (3/60) masing-masing pada air,

makanan, dan lalat. Tiga puluh empat pencilan dan lapan serotip *Salmonella* telah dikenalpasti. *S.* Weltevreden (20.6%) adalah yang paling banyak, diikuti oleh *S.* Typhimurium dan *S.* Agona (17.6%), *S.* Albany dan *S.* Enteritidis (8.8%), *S.* Molade (5.9%), *S.* Corvallis dan *S.* Schleissheim (2.9%) dan selebihnya sebagai *Salmonella* spp. (11.8%). Model regresi logistik multipemboleh ubah menunjukkan bahawa *Salmonella* adalah positif dalam kawanan dapat diramalkan dengan simpanan makanan (makanan yang terdedah; OR = 10.38; 95% CI: 1.25-86.39; p = 0.030) dan tangki air yang terbuka; OR = 6.43; 95% CI: 1.02-40.60; p = 0.048).

Di antara pencilan, 26.5% (n=9) adalah rentan terhadap semua antibiotik, manakala 73.5% (n=25) adalah rintang terhadap sekurang-kurangnya satu antibiotik yang diuji. Kerintangan dadah-pelbagai (MDR) ditunjukkan oleh 8 pencilan (23.5%). Kebanyakan pencilan rentan terhadap *ciprofloxacin* (100%), *gentamicin* (97.1%), *norfloxacin* (97.1%), *cefotaxime* (97.1%) dan *ceftiofur* (97.1%). Tahap rintangan tertinggi dilihat pada *tetracycline* (35.3%) dan *streptomycin* (35.3%). Lapan pencilan (23.5%) adalah MDR kepada dua atau lebih agen antibiotik yang tergolong dalam kelas antimikrob ≥ 3. Rintangan kolistin (kepekatan perencatan minimum: 4-16 mg / L) dikesan dari 5 pencilan (14.7%) yang terdiri dari *S.* Weltevreden, *S.* Albany, *S.* Typhimurium dan *Salmonella* spp.

Perwakilan pencilan *Salmonella* (n=15) telah diperiksa menggunakan penjenisan jujukan multilokus (MLST) dengan menggunakan tujuh gen jaga selia (*housekeeping gene*) dari pangkalan data dalam talian MLST. *Salmonella enterica* serovar telah dirumuskan menjadi 7 jenis jujukan (ST). ST13 (n=3) dan ST11 (n=3) adalah ST yang pradominan masing-masing ditemui dalam serovar Agona dan Enteritidis, dikuti oleh ST34 (n=2) dan ST36 (n=2), ST365 (n=2), ST1541 (n=2) dan ST19 (n=1) masing-masing ditemui dalam serovar Typhimurium, Weltevreden, Corvallis dan Typhimurium. Didapati bahawa, kecuali serotype Typhimurium, MLST menunjukkan korelasi yang kuat antara STs dan serovars. Setiap strain serovar daripada Enteritidis, Agona, Weltevreden dan Corvallis telah diwakili STs yang unik.

Sebagai kesimpulan, didapati bahawa prevalens *Salmonella* dalam kalangan ayam kampung di kawasan kajian lebih rendah berbanding yang dilaporkan dalam kalangan ayam komersil di Malaysia. Makanan dan air takungan yang tidak ditutup paling berkait dengan positifnya Salmonella dalam kawanan ayam kampung. Kajian ini juga menunjukkan bahawa kontaminasi (pencemaran) ayam kampung tempatan dengan strain MDR bukan tifoid, sebahagiannya adalah rintang terhadap pelbagai kelas antimikrobia. Secara amnya, tahap kerintanganan adalah lebih rendah berbanding yang dilaporkan daripada bangkai (*carcases*) ayam komersil serta produknya yang terdapat di Malaysia dan di tempat lain. Walaubagaimanapun, aplikasi minimum antibiotik terhadap pengeluaran ayam *free-range* dan kurangnya penggunaan promosi pertumbuhan antibiotik, kerintangan terhadap kolistin diperhatikan. Pendedahan terhadap persekitaran semulajadi seperti burung liar, berdekatan dengan ladang ayam komersil serta air dan tanah yang tercemar mungkin memainkan peranan yang kompleks dalam menentukan rintangan antibiotik. Secara molekular, semua jenis jujukan yang dikenal pasti daripada pencilan yang diuji dalam kajian ini telah di dokumentasikan di Malaysia sebelum ini, dan juga dilaporkan di banyak negara Asia termasuk China, Singapura, Indonesia, Thailand, Vietnam dan Myanmar (ST11). ST365 juga dilaporkan di Singapura. Penemuan ini memperlihatkan bahawa strain *Salmonella* ayam kampung ini tidak unik dan telah beredar (*circulating*) ke rantau Asia dan melibatkan pelbagai spesies haiwan dan manusia.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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ACSSuT	Ampicillin, Chloramphenicol, Streptomycin, Sulfamethoxazole, Tetracycline
AFLP	Amplified fragment length polymorphism
ASEAN	Association of Southeast Asian Nations
ATCC	American type culture collection
Amc	Amoxicillin-clavulanate
Amp	Ampicillin
BAM	Bacteriological analytical manual
BGA	Brilliant green agar
BPW	Buffered peptone water
CDC	Centres for disease control and prevention
cgMLST	Core genome multilocus sequence typing
CI	Confidence interval
CLSI	Clinical and laboratory standards institute
Cn	Gentamicin
Ct	Chloramphenicol
Ctx	Cefotaxime
DNA	Deoxyribonucleic acid
DVS	Department of veterinary services
ELISA	Enzyme linked immunosorbent assay
ESBLs	Extended-β-lactamases
EUCAST	European committee on antimicrobial susceptibility testing
F	Nitrofurantoin
FAO	Food and agriculture organization of the United Nations
FDA	Food and drug administration
g	Gram
gDNA	Genomic deoxyribonucleic acid

IBM	International business machines
IMR	Institute of medical research
K	Kanamycin
mm	Millimetres
µM	Micromolar
µL	Microliters
mg	Milligram
mL	Millilitres
MAR	Multiple antibiotic resistance
MCR-1	Plasmid-mediated colistin resistance gene
MDR	Multidrug resistant
MEGA	Molecular evolutionary genetics analysis
MIC	Minimum inhibitory concentration
MLEE	Multilocus enzyme electrophoresis
MLST	Multilocus sequence typing
MLVA	Multilocus variable number of tandem repeats analysis
MLVF	Multilocus variable number of tandem repeats fingerprinting
MS	Microsoft
Na	Nalidixic acid
NA	Nutrient agar
NJ	Neighbour joining
NTS	Non-typhoidal *Salmonella*
OIE	World Organization for animal health
OR	Odds ratio
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
RAPD	Randomly amplified polymorphic deoxyribonucleic acid
rpm	Revolutions per minutes

R/I/S	Resistant, Intermediate, Susceptible
RNA	Ribonucleic acid
RVB	Rappaport vassiliadis broth
S	Streptomycin
S3	Sulfonamides
SE	*Salmonella* Enteritidis
SEAs	Southeast Asian countries
SG	*Salmonella* Gallinarum
SLVs	Single locus variants
SNP	Single nucleotide polymorphism
SP	*Salmonella* Pullorum
SPI	*Salmonella* Pathogenicity Island
SPSS	Statistical package for social sciences
ST	*Salmonella* Typhimurium
ST	Sequence Type
TBE	Tris-Borate ethylenediaminetetraacetic acid
Te	Tetracycline
TLVs	Triple locus variants
tRNA	Transfer ribonucleic acid
TSB	Tryptic soy broth
UPM	Universiti Putra Malaysia
VNTR	Variable number of tandem repeats
VRI	Veterinary research institute
W	Trimethoprim
WGS	Whole genome sequencing
XLD	Xylose lysine desoxycholate

CHAPTER 1

INTRODUCTION

1.1 Study background

Village chickens or "*Ayam Kampung*" are hybrids from the natural crossbreeding between the Malay fowl, jungle fowl and mixed exotic breeds brought in by the Europeans (Azahan & Zahari, 1983; Azahan, 1994). They are common in the Southeast Asia particularly Malaysia and Indonesia, and are traditionally raised as backyard chickens where they are let free to roam and scavange for food and sometimes fed with household leftovers or scraps to supplement their dietary requirements (Azahan et al., 1980; Azahan & Zahari, 1983; Azahan, 1994; Padhi, 2016). Recently, the emerging food safety and animal welfare issues such as antibiotic and chemical drug residues in food animals as well as increased development of antibiotic resistance by foodborne pathogens particularly common in commercial poultry production, has resulted in increased demand for organically grown food animals such as village chickens because consumers believed they are drug - or antibiotic - free, wholesome and medicinal (Hassan et al., 2005; Miao et al., 2005; Rahman & Haziqah, 2015; Suhaila et al., 2015).

Salmonella enterica with more than 2,500 different serotypes identified, has been recognised as a major bacterial foodborne pathogen for humans, and until today has remained a leading cause of foodborne illnesses worldwide (Eguale, 2018; Nair et al., 2018; Shang et al., 2018; Zhang et al., 2018). For instance, in the United States, it is responsible for high mortality rates and increasing cost associated with the treatment (Batz et al., 2012). The World Health Organization (WHO) and the Food Agriculture Organization (FAO) have declared *Salmonella* since 1950s, as the most common and important zoonosis; and this has led to its subsequent inclusion in the terrestrial animal health code of the WHO (Mouttotou et al., 2017). Salmonellosis is caused by two species of *Salmonella* namely *S. enterica* and *S. bongori*, the former being the most important aetiological agent for human systemic and diarrheal infections (Saravanan et al., 2015). The *S. enterica* serovars, *S.* Typhimurium (ST), *S.* Enteritidis (SE) and *S.* Heidelberg are the epidemiologically important serotypes among the non – typhoidal *Salmonella* (NTS) that can be found in both domestic and wild animals including birds worldwide (Najwa et al., 2015; Prestinaci et al., 2015; Saravanan et al., 2015). Human infections with NTS is frequently associated with consumption of *Salmonella* contaminated foods or food-producing animals. Although, non-typhoidal *Salmonella* has been isolated from different food-producing animals and sources (Heredia & García, 2018), poultry and poultry products have particularly served as major reservoirs with contaminated poultry products as major vehicles of human infections along the *farm-to-fork* continuum (Andino & Hanning, 2015; Andoh et al., 2016; Foley et al., 2008).

Recently, the similarity of the *Salmonella* isolates including multidrug resistant ST recovered from food-producing animals, with those responsible for human salmonellosis suggests a possible transmission from food animals to humans (Hong et al., 2018; Kagambèga et al., 2018; Li et al., 2013). Poultry and poultry products such as eggs, food products containing eggs, undercooked poultry meat are the most commonly implicated sources and primary vehicles of infection caused by ST and SE (Abdullah et al., 2010; Afshari et al., 2018; Ong et al., 2014; Saravanan et al., 2015; Thung et al., 2018, 2016) . While *Salmonella* and other related foodborne infections in poultry is often asymptomatic, its colonization in chickens may present a significant health risk to humans as *Salmonella* may transmit to humans from the infected birds through ingestion of undercooked retail meat contaminated with *Salmonella* (Thakur et al., 2013).

In chicken farms, several factors play critical role in the spread and perpetuation of *Salmonella*. Of these factors, feed and water contaminated with *Salmonella* remain important sources of infection (Frederick & Huda, 2011). Additionally, critical factors commonly identified as sources of *Salmonella* contamination in farms included the contaminated drinkers, feeders, litters, feedstuffs, air inside chickens houses, soil, bedding and faecal matter (Hoelzer et al., 2011; Hoover et al., 1997; Rodriguez et al., 2006). Transmissions of *Salmonella* among birds are horizontal or vertical. Horizontal transmission occurs through the intermittent shedding of the *Salmonella* by asymptomatic birds thereby contaminating the dust, feathers of other birds, soil, water, litters etc. In contrast, the vertical involves systemic *Salmonella* infection of the parent laying birds in the hatchery and this is passed onto chicks through the infected reproductive tissues of the parent birds (Mouttotou et al., 2017).

Antimicrobial resistance by bacterial foodborne pathogens like *Salmonella* has become a major public health threat worldwide due to the potential of these resistant agents to transmit to humans via the food chain (Brown et al., 2017; Nair et al., 2018; Xiong et al., 2018). The emergence of this resistance has been linked with the routine use of antibiotics in animals for prevention and treatment of diseases as well as a growth promoter (Nair et al., 2018; Tang et al., 2017). Furthermore, studies from different countries have demonstrated antibiotic resistance including multidrug resistant (MDR) profiles of *Salmonella* isolates recovered from humans and food-producing animals (Cameron-Veas et al., 2018; Chatham-Stephens et al., 2019; Dang-Xuan et al., 2019; Sharma et al., 2019; Yang et al., 2019). In Malaysia, MDR *Salmonella* has been reported from retail chicken carcasses, beef, vegetables and other animal derived food products (Salleh et al., 2003; Khoo et al., 2015; Kuan et al., 2017; Thung et al., 2018; Yoke-Kqueen et al., 2008). The reported prevalence of *Salmonella* in Malaysia ranged from 10.5% to 44.4% as reported by different authors from retail markets, processing plants, wet markets and other ready-to-eat-foods. These studies highlighted *Salmonella* contamination rates in raw fresh fruits and vegetables (Salleh et al., 2003), poultry litters (Rusul et al., 1996), poultry farms (Rusul et al., 1996), chicken meat portions (Arumugaswamy et al., 1995), liver and gizzard (Arumugaswamy et al., 1995), ready-to-eat cooked meat, prawn and oriental shrimps (Arumugaswamy et al., 1995). Because village chickens are often allowed to roam freely in the house compound, they are at high risks of coming in contact with pathogens in the environment. However, no research or information could be found regarding the microbiological quality of *Ayam Kampung* and its products in Malaysia. Therefore, exploring the on-farm transmission dynamics of *Salmonella* serotypes and their antibiotic resistance profiles in village chickens will allow the relevant Malaysian government agencies to better implement appropriate control measures and strategies in order to mitigate its prevalence and the threat it posed to human health. Information on antibiotic resistance phenotypes and genotypes of *Salmonella* from food animals and their products is necessary to combat the spread of resistance and public health threat posed by antibiotic resistant *Salmonella*. This information will aid in exploring the epidemiology of antibiotic resistance, identify or trace new emerging resistant pathogens and assist in prudent use of clinically available (last resort) antimicrobials in both humans and animals.

1.2 Problem statement

Malaysia is considered as one of the countries with highest chicken consumers, and according to a recent report by the department of statistics, Malaysia, among the livestocks and products, chicken meat had the highest per capita consumption (52kg) per year followed by chicken/duck eggs (22.2kg/year) and pork (16.3kg/year) (Department of Statistics Malaysia, 2017). Chicken meat and eggs are the most popular and cheapest sources of protein in the country, mainly because there exist no restrictions or any religious prohibition of its consumption. Malaysia is self sufficient in terms of egg and even exports appreciable amounts to a number of neighbouring countries (Department of Veterinary Services, 2012). Chicken's meat and eggs are considered the major vehicles harbouring and transmitting foodborne *Salmonella* to humans via the food chain. In Malaysia, several large foodborne disease outbreaks involving human fatalities have been reported in recent time. A substantial proportion of the outbreaks have been linked to eating foods, fruits and vegetables, chickens or eggs contaminated with *Salmonella* (Salleh et al., 2017; Soon et al., 2011).

The increasing concern about the overuse of drugs and chemical in livestock production and improving awareness of food safety and animal welfare resulted in expanding demand for free-range livestock products including chickens. Consumers are willing to pay more to obtain products they consider safer and more wholesome. Therefore, *Ayam Kampung* has gained popularity and demand over the past few years. However, there have been no reports on the microbiological quality of *Ayam Kampung* and their products in Malaysia to substantiate the assumptions of safety. This study will address that gap of knowledge.

1.3 Justification of the study

Majority of published work on chickens has focused on processed chicken meat, gizzards, liver, and other organs of chicken carcasses, most of which were at retail outlets and wet markets. None of these studies explored on the microbiological quality of village chickens and their products despite getting more attention and increased preference among consumers compared to the commercial and other exotic chicken and chicken products in Malaysia. Consumers are paying more for something that is believed to be safer and more wholesome. This study will provide information on the safety aspect of the meat, by focusing on the most common foodborne pathogen *Salmonella* and determine the antibiotic resistance patterns of the isolates as well as their genetic diversity.

1.4 Research Questions

i. What is the prevalence of *Salmonella* infection among *Ayam Kampung* in the central and southern Peninsular Malaysia?
ii. What are the epidemiological risk factors that contribute to the occurrence of salmonellae among *Ayam Kampung* in the central and southern Peninsular Malaysia?
iii. What are the antibiotic resistance profiles/susceptibility patterns of the *Salmonella* isolates?
iv. What are the genotypes of the *Salmonella* isolates?

1.5 Research Hypothesis

i. The prevalence of *Salmonella* among *Ayam kampung* in the South-central Peninsular Malaysia is high.
ii. Certain farm management factors play a vital role in the occurrence and epidemiology of salmonellosis among *Ayam Kampung*
iii. *Salmonella* isolates from village chickens are multidrug resistant
iv. *Salmonella* isolates recovered from *Ayam Kampung* are genetically diverse.

1.6 General Objective

To determine the epidemiology, antibiotic resistance level, associated risk factors and genetic diversity of *Salmonella* isolates among *Ayam kampung* in the south-central Peninsular Malaysia.

1.7 Specific Objectives

i. To determine the prevalence of salmonellosis and epidemiological risk factors associated with its occurrence among *Ayam kampung* in the South-central Peninsular Malaysia.
ii. To determine the antimicrobial resistance susceptibility patterns of the *Salmonella* isolates recovered.
iii. To determine the genetic diversity of the *Salmonella* isolates by multilocus sequence typing.

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