ISOLATION AND IDENTIFICATION OF COMMON ENTERIC BACTERIAL PATHOGENS IN QUAIL EGGS IN SELECTED MARKETS IN KOTA BHARU KELANTAN

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CERTIFICATION

This is to certify that we have read this research paper entitled **'Isolation and Identification of Common Enteric Pathogens in Quail Eggs in Kota Bharu, Kelantan'** by Dayangku Umi Aqilah Binti Pengiran Isa, and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course DVT 5436 – Research Project.

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Thank You

DEDICATIONS

I dedicate my dissertation work to my family and many friends. I express my gratitude to my amazing parents, Hajah Maslena and Pengiran Haji Isa, whose always been supportive of me and very loving. My beloved younger siblings, Balqis, Syifrah, Waez, Dana, Maya and Sara, also my dearest Zeyena, who are my sources of strength to never quit.

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ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 5436 – Research Project.

Enteric pathogens may present in the quail eggs and foodborne disease outbreaks may occur when the food contaminated with pathogenic strain is consumed by people which leads to a public health threat. This is a study to determine the presence of common enteric pathogens in quail eggs from selected markets in Kota Bharu, Kelantan. Thus, a total of 136 quail eggs were sampled from eight different markets. Isolation and identification of the bacteria using the bacterial culture method and biochemical tests were done and the results showed the presence of several bacteria which are *Shigella* spp., *Proteus vulgaris, Proteus mirabilis, Escherichia coli, Klebsiella* spp. *Enterobacter* spp., and *Staphylococcus aureus*. The outcome from the research showed that quail eggs are fairly contaminated with important enteric pathogens, therefore, raises a public health concern towards human consumer.

Keywords: *Bacterial isolation, Enteric pathogens, Foodborne diseases, Quail eggs*

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 5436 – Projek Penyelidikan.

Telur puyuh berpotensi untuk mengandungi patogen enterik dan wabak penyakit bawaan makanan boleh berlaku apabila makanan yang tercemar dengan strain patogen dimakan oleh orang ramai dan ini adalah satu ancaman kesihatan awam. Kajian ini adalah untuk menentukan patogen enterik yang lazim dalam telur puyuh dari pasar-pasar yang terpilih di Kota Bharu, Kelantan. Oleh itu, sebanyak 136 telur puyuh telah disampel dari lapan pasar berbeza. Pengasingan dan pengecaman bakteria telah dilakukan menggunakan kultur bakteria dan ujian biokimia menunjukkan keputusan adanya beberapa bakteria iaitu *Shigella* spp., *Proteus vulgaris, Proteus mirabilis, Escherichia coli, Klebsiella* spp. *Enterobacter* spp., dan *Staphylococcus aureus.* Penyelidikan ini menunjukkan bahawa telur puyuh cukup tercemar dengan patogen enteric yang menimbulkan kebimbangan kesihatan awam terhadap pengguna.

Kata kunci: *Pengasingan bakteria, Patogen enterik, Penyakit bawaan makanan, Telur puyuh*

1.0 Introduction

Foodborne diseases have multiple aetiologies such as bacteria, fungi, viruses and parasites. The most common pathogens that cause outbreaks are bacteria such as *Salmonella* Typhi, *Escherichia coli, Staphylococcus aureus, Vibrio cholera, Campylobacter jejuni, and Listeria monocytogenes* (Abdul-Mutalib et. al, 2015 Diarrhoeal diseases causes 3% of two million deaths every year (Abdullah & Ismail, 2021). World Health Organisation (2015) estimated that foodborne diseases cause almost 1 in 10 people to show signs of illness from eating contaminated food and as a result, 420,000 die. Food poisoning is a long-standing public health problem in Malaysia, with an incidence rate of 44.18 per 100,000 people in 2010, 50.42 per 100,000 people in 2014, and 47.2/100,000 people in 2016, and a fatality rate of 0.041 per 100,000 people in 2016 (Abdullah & Ismail, 2021).

Bacteria belonging to the Enterobacteriaceae family is the most common cause of gastrointestinal illnesses (Qazilbash, 2006). More than half of foodborne disease causes diarrhoea and causes infection to 550 million people and with an annual mortality of more than 230,000. The source of the diarrhoeal cases includes eating raw or undercooked meat, eggs, fresh produce and dairy products; these may be contaminated by pathogenic bacteria (World Health Organization, 2015).

Japanese quail, or its scientific name *Coturnix japonica*, is available in Kelantan, where the people consume the meat and eggs as a delicacy dish. Since quail eggs can be contaminated with various enteric bacterial pathogens, an investigation is made to find out the presence of these organisms in quail eggs in Kota Bharu, Kelantan by sampling the quail eggs in wet markets.

2.0 Research problem

Poultry meats and its products are one of the primary sources of transmission of enteric bacterial pathogens including *Enterobacteriaceae* to human consumers. No studies have been conducted to determine the presence of Enterobacteriaceae in quail eggs and its products in Kota Bharu, Kelantan.

3.0 Research questions

3.1 Are the quail eggs in Kota Bharu free from foodborne enteric bacterial pathogens?

3.2 What are the common bacteria present in quail eggs in Kota Bharu?

4.0 Research hypothesis

4.1 Majority of the quail eggs are contaminated with foodborne pathogens.

4.2 *Enterobacteriaceae* are commonly found in quail eggs.

5.0 Objectives

5.1 To isolate the enteric bacterial pathogens from quail eggs from selected wet markets in Kota Bharu, Kelantan.

5.2 To identify the enteric bacterial pathogens from quail eggs from selected wet markets in Kota Bharu, Kelantan.

6.0 Literature review

6.1 Japanese Quails in Kelantan

In the early 1990s, quail farming was introduced to Kelantan, Malaysia, to produce eggs and meat. So, it is a relatively newer poultry industry in Kelantan. Demand for quail meat and eggs has increased in recent years, making quail production a viable alternative for the Malaysian poultry industry. Because of their inexpensive initial capital investment, small housing requirements, easy management, and rapid financial returns, quail production in Malaysia has increased (Palanisamy & Bamaiyi, 2015).

6.2 Quail eggs

Animals, birds, mice, livestock animals, and eggs can all be infected by the pathogen. Still, eggs are an excellent source of chlorine and selenium and a great source of riboflavin. Eggs are a great source of protein and vitamins A, D, E, and K, folic acid, pantothenic acid, and Zinc; the yolk also includes choline (Réhault-Godbert et al., 2019).

Egg consumption has risen over the world due to its nutritional value to humans. Eggs are consumed increasingly often as a source of protein because of their nutritious value, which has led to an increase in chicken rearing. It is consumed as a substitute for beef meat since it is limited and costly. Research shows that eggs have a high cholesterol level, but people still eat them raw or undercooked and use them in recipes for everything from mayonnaise to cake to salad dressing. Although eggs are a great source of nutrients for humans, they are also an excellent nutrient source for other organisms. When bacteria breach eggshells and membranes, the yolk serves as a source of nourishment

for them as well. Eggs and egg-based products containing raw eggs have been classified by the Scientific Committee on Veterinary Measures relevant to public health as a food category that poses a public health danger. Eggs and egg-based products containing raw eggs have been classified by the Scientific Committee on Veterinary Measures relevant to public health as a food category that poses a public health danger (Bose et al., 2020).

6.3 Common bacteria in quail eggs

A study by Pondit et al. (2018) found that on the quail eggshells, the prevalence of *Staphylococcus spp.* and *S. aureus* was 16.25% and 5%, respectively. *S. aureus* was also isolated during a food poisoning outbreak in Terengganu in 2016 and it accounted for 23.5% of the isolation. The food may be contaminated by *S. aureus* due to poor food handling practices, e.g. not wearing gloves, as S. aureus is the normal flora of the human skin (Abdullah & Ismail, 2021b; Atere et. al; Ruby et al., 2019).

Escherichia coli is a normal intestinal microflora of humans and animals. Gita et al. (2021) found positive results of *E. coli* in quail eggs by 2 out of 30 quail eggs which is 6.7%. Thus, *E. coli* can be present in quail eggs and it is possible that *E. coli* from the intestine causes this positive result.

Salmonella spp. is a common finding from quail eggs. There are two classifications of Salmonellosis; Typhoidal *Salmonella* and Nontyphoidal *Salmonella*. Typhoidal *Salmonella* is caused by *S. enterica* subsp. Enterica, which results in a typhoid-like illness in humans, is adapted to only human

transmission, i.e. no animal reservoir. Nontyphoidal salmonellae are the zoonotic serovars (CIDRAP, 2013). In 2016, 33 food poisoning cases were reported in Terengganu and 4.8% were caused by eggs while 52.4% of the micrbial agents detected was *Salmonella* spp. (Abdullah & Ismail, 2021b).

6.4 Effects of common bacteria on humans

When ingested, *S. aureus* may cause food poisoning. In fact, staphylococcal food poisoning ranks among the top three foodborne diseases occurring in humans, and one of the possible origins is from animal and animal products (Pondit et al. 2018). Toxins secreted by *S. aureus* are one of the virulence factors to cause food poisoning. One of the toxins is Superantigens (SAgs), and there are more than 23 staphylococcal SAgs are found. Staphylococcal enterotoxins (SEs) are the ones that cause food poisoning and symptoms such as vomiting and diarrhoea can be observed in the infected person, and SEA is the most common SE that causes food poisoning. By directly cross-linking some T cell receptor Vβ domains with recognition sites on major histocompatibility complex class II (MHC II) molecules, SAgs activate a significant percentage of T cells at the same time. The ability of macrophages to control T cell responses to SAgs appears to be influenced by MHC II molecules. The released SAgs have a systemic effect, prompting a large number of T-cells to release vast quantities of pro-inflammatory cytokines (IL-2, IFN-, and TNF), resulting in symptom manifestation such as high fever, diarrhoea, vomiting, hypotension, and may result in multiple organ failure (Oliveira et al., 2018; Pinchuk et al., 2010).

E. coli can cause food poisoning when humans consume contaminated food such as vegetables, raw milk and its products, undercooked eggs and meats (Gita et al. 2021). Enterogenic *E. coli* (ETEC) adheres to the small intestine mucosa by its fimbriae and produces heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST). Depending on the strain, either one of these enterotoxins may be produced, or both can be present at the same time. Both of these enterotoxins generate net secretion of ions and water, resulting in watery diarrhoea by the activation of the cystic fibrosis transmembrane regulator (CFTR) chloride channel due to the increase of cyclic AMP (cAMP) or cyclic guanosine monophosphate (cGMP) levels. However, LT is found to cause severe dehydrating diarrhoea in infected patients (Singh et al., 2019).

The most common ones that infect humans worldwide are *Salmonella* Typhimurium (ST) and *Salmonella* Enteritidis (SE). Newport, Javiana, I 4,[5],12:i:-, Muenchen, Bareilly, Montevideo, Heidelberg, Saintpaul and Infantis are among the serovars that have been isolated from unwell individuals (CFSPH, 2013).

6.5 Factors leading to egg contamination

There are several factors that lead to egg contamination with microorganisms on eggs. The shell may have oil, water or faeces that stuck to the shells. Farm hygiene and biosecurity play a role in microbial contamination. For instance, eggs can be contaminated with *Salmonella* Enteriditis through vertical transmission and humans get food poisoning if the contaminated egg is consumed raw or undercooked. Another example is *Salmonella* Typhimurium

enters the external layer of the egg and penetrates in, i.e. horizontal transmission (Martelli & Davies, 2012; Teixeira et al., 2013). Egg handling in the market and on the farm may cause contamination of the egg. *Staphylococcus aureus* is a normal microflora in the skin of humans and it can be transmitted to the quail eggs when the worker does not wear gloves when handling the eggs.

7.0 Materials and methods

7.1 Sample collection

A total of 136 quail eggs were collected from eight selected markets denoted by Market 1 until 8 in Kota Bharu. The eggs were sampled randomly and carefully kept in sterile ziplock bags with stuffed cotton to prevent shell damage. A total of 10-20 eggs were collected from each market and labelled properly. Upon collection, the eggs were stored in the refrigerator at 4°C before processing.

7.2 Isolation and identification of enteric bacteria

Initially, this study targeted the isolation and identification of *Salmonella* spp. 0.5 cm of eggshell was taken and put in peptone water. A sterile swab was dipped in sterile buffered peptone water and swabbed on the shell. The eggshell was wiped with 70% alcohol prior to cracking to minimise outer contamination. A total of 7 eggs-each representing the market were cracked open and pooled together in the sterile ziplock bag and mixed thoroughly. Next, 5 ml of the pooled sample was pipetted into 10 ml of peptone water and

incubated at 37˚C for 24 hours. After 24 hours, 100µl of the sample was pipetted onto the Xylose Lysine Deoxycholate agar (XLD agar, Oxoid, England) surface and dispersed using a streaking method. For each pooled sample, a total of three XLD agar were used. The agar plate containing egg samples was incubated at 42°C for 24-48 hours. Secondary cultures were performed on the XLD agar with similar incubation conditions to obtain individual colonies. Negative samples on XLD agar were discarded after no growth was observed after 48 hours. Biochemical tests such as citrate, Urease, TSI (Triple Sugar Iron), SIM (Sulphide, Indole, Motility) and MRVP (Methyl Red and Voges-Proskauer) were used to identify the bacteria until the genus level.

7.3 Data analysis

Data were recorded and statistically analysed manually by using manual record and tabulation by Microsoft Office on the identification of Enterobacteriaceae.

8.0 Results

8.1 Isolation and identification of enteric bacteria

Table 8.1 shows the colony morphology of the XLD agar. The colony morphology and Gram staining results were based on the pure culture growth observed on the secondary culture. Out of all eight shops, there were two shops that were negative for any bacterial growth.

No growth was observed from Shop 1. The agars also had no change of colour. For Shop 2, the swab/shell culture revealed red, transparent colonies and Gram staining

revealed pink baccili. Meanwhile, yellow, opaque colonies were observed from the culture of the egg from Shop 2 and purple cocci bacteria was observed when stained with Gram stain. Shop 3 had two different growth on the primary culture from swab/shell sample; colonies with the morphology of yellow with black centres were isolated from the sides of the agar plate grew on the XLD agar with the same morphology as the primary culture. The Gram staining revealed pink baccili. Another type of colony was observed within the streak lines of the primary culture, it had the morphology of yellow with black centres. The secondary culture morphology is the same as the primary culture and the Gram staining revealed pink baccili. Whereas for the egg culture of Shop 3, two types of colonies were observed on the primary culture. The smaller colonies were yellow and opaque and grew at the same morphology for the secondary culture. Gram staining reveals pink bacilli for this colony. The second colony was also yellow and opaque but it the size was larger. Gram staining of the secondary culture revealed pink bacilli.

All cultures from Shop 4 had large, yellow, opaque colonies in both primary and secondary cultures. The Gram staining revealed pink bacilli. For shop 5, the egg/shell culture was contaminated therefore no valid results can be obtained. However, yellow, opaque colonies were observed from the egg culture from this shop and the Gram staining revealed pink bacilli. The swab/shell culture from Shop 6 shows red, transparent colonies on the XLD agar and the Gram staining shows appearance of pink bacilli. However, there was no growth observed for the culture of the egg sample.

For Shop 7, yellow colonies with an egg-like appearance were observed on the agar of the swab/shell sample. The Gram stain revealed purple cocci formed in grape-like

clusters. There was two growth on the agar of the egg sample; one was yellow colonies with an egg-like appearance, and the Gram stain was the same as of the swab/shell sample; another growth was large, yellow, opaque colonies, and it was a pink bacilli when stained with Gram stain.

Lastly, for shop 8, there was no growth of any bacteria observed on all agars of samples cultured. The agars also had no change of colour.

Table 8.1 Colony morphology on XLD agar and Gram staining results

Shop ID	Sample	Colony morphology on the secondary culture	Gram staining results
Market 1	Swab/shell	No growth	-
	Egg	No growth	-
Market 2	Swab/shell	Red, transparent colonies	Pink bacilli
	Egg	Yellow, opaque colonies	Purple cocci
Market 3	Swab/shell	Yellow colonies with black centre (isolated from the side of the agar)	Pink bacilli
		Yellow colonies with black centres (isolated from the of centre agar)	Pink bacilli
	Egg	Yellow, opaque colony	Pink bacilli
		Large, yellow, opaque colony	Pink bacilli
Market 4	Swab/shell	Large, yellow, opaque colonies	Pink bacilli
	Egg	Large, yellow, opaque colonies	Pink bacilli
	Swab/shell	Contaminated	-

Market 5	Egg	Yellow, opaque colonies	Pink bacilli
Market 6	Swab/shell	Red, transparent colonies	Pink bacilli
	Egg	No growth	-
Market 7	Swab/shell	Yellow colonies, egg-like appearance	Purple cocci
	Egg	Small, yellow colonies, egg-like appearance	Purple cocci
		Large, yellow, opaque colonies	Pink bacilli
Market 8	Swab/shell	No growth	-
	Egg	No growth	-

After performing Gram staining of the secondary cultures, biochemical tests were done and the results were then interpreted to what bacteria grew on the agar. Table 8.2 shows the biochemical tests and the result interpretation of bacteria presence in quail eggs.

Shop 1 is negative for any growth. The swab/shell sample from Shop 2 was suggested to be *Shigella* spp. For shop 3, both *P. vulgaris* and *P. mirabilis* were found from the swab/shell sample, and *Escherichia coli* and *Klebsiella* spp. were isolated from the egg sample. Additionally, *Klebsiella* spp. was found in both swab/shell and egg samples. The swab/shell sample from Shop 5 was contaminated thus no biochemical results are available. However, the egg sample revealed *Enterobacter* spp. *Shigella* spp. was found in the swab/shell sample from Shop 6 and there was no growth found from the culture of the egg sample. From Shop 7, *S. aureus* and *Klebsiella* spp. was identified in both/shell and eggs samples. Lastly, no bacteria was isolated from Shop 8.

Table 8.2 Result interpretation of bacteria presence in quail eggs

Market ID	Sample	TSI (Butt/ Slant)	Citrate	Urease	Sulfur	Indole	Motility	MR	VP	Coagulase	Interpretation/suspected bacteria
Market 1	Swab/shell	ND	ND	ND	ND	ND	ND	ND	ND	ND	No growth
	Egg	ND	ND	ND	ND	ND	ND	ND	ND	ND	No growth
Market 2	Swab/shell	A/K, Gas, No H_2S	-	-	-	-	-	+	-	ND	*Shigella* spp.
	Egg	A/K, No Gas, No H_2S	-	-	-	-	-	-	+	+	*Enterococcus spp.*
Market 3	Swab/shell	A/A, Gas, No H_2S	-	+	-	+	+	+	-	ND	*Proteus vulgaris*
	Swab/shell	K/A, Gas, H_2S	+	+	+	-	+	+	-	ND	*Proteus mirabilis*
	Egg	A/A, Gas, No H_2S	-	-	-	+	+	+	+	ND	*Escherichia coli*
	Egg	A/K, Gas, No H_2S	+	+	-	-	-	-	+	ND	*Klebsiella* spp.

Market 4	Swab/shell	A/K, Gas, No H_2S	+	+	-	-	-	-	+	ND	*Klebsiella* spp.
	Egg	A/K, Gas, No H_2S	+	+	-	-	-	-	+	ND	*Klebsiella* spp.
Market 5	Swab/shell	ND	ND	ND	ND	ND	ND	ND	ND	ND	Contaminated
	Egg	A/K, Gas, No H_2S	+	+	-	-	+	-	+	-	*Enterobacter* spp.
Market 6	Swab/shell	A/K, Gas, No H_2S	-	-	-	+	-	+	-	ND	*Shigella* spp.
	Egg	ND	ND	ND	ND	ND	ND	ND		ND	No growth
Market 7	Swab/shell	A/A, No Gas, No H_2S	+	+	-	-	-	+	+	+	*Staphylococcus aureus*
	Egg	A/A, No Gas, No H_2S	+	+	-	-	-	+	+	+	*Staphylococcus aureus*
	Egg	A/K, Gas, No H_2S	+	+	-	-	-	-	+	ND	*Klebsiella* spp.
Market 8	Swab/shell	ND	ND	ND	ND	ND	ND	ND	ND	ND	No growth
	Egg	ND	ND	ND	ND	ND	ND	ND	ND	ND	No growth

ND = Not Determined , A = Acidic, K = Alkaline

9.0 Discussion

The initial purpose of this study was to isolate and identify *Salmonella* species that are commonly found in eggs. However, no *Salmonella* colony was observed; other enteric pathogens were found instead.

In this study, several enteric bacteria were identified including *S. aureus*. Out of 8 markets, only one market was identified to be contaminated with *S. aureus* both on the swab/shell and egg samples. A study by Pondit et al. (2018) found that on the quail eggshells, the prevalence of *S. aureus* was 5% and it was identified by Gram stain and biochemical tests such as coagulase, sugar fermentation test, Methyl Red Voges Proskauer test, indole, catalase, and motility test. *S. aureus* is able to ferment mannitol sugar to produce acid in the end product. So inoculating on Mannitol Salt Agar (MSA), results in the change of colour from red to yellow due to the change of the phenol red indicator. *S. aureus* can cause foodborne illness in humans consuming poultry products and can cause problems with resistance (Abebe et al., 2020).

Next, we managed to isolate and identify *Klebsiella* spp. The organism is commonly found in eggs, which indicates direct contamination from faecal materials to the surface of the egg (Mounam et. al, 2019; Yoon et al., 2020). Similar to *S. aureus*, *Klebsiella* spp. also possesses an increasing instance of antimicrobial resistance. Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria are a type of developing Gram-negative bacteria that cause infections that are associated with substantial morbidity and mortality (Arnold et al., 2011; Bassetti et al., 2018).

E. coli is a normal intestinal microflora of many animals, including humans, and it is a common bacteria that was found in this study. There are two types of pathogenic *E.*

coli strains: those that cause intestinal problems and those that cause extraintestinal problems (Lorenz et al., 2020). A study conducted by M El Malt (2013) found *that E. coli* in eggshells was the most prevalent one isolated in a percentage of 7%; however *E. coli* could not be detected in egg contents. Avian pathogenic *E. coli* infects many bird species and causes septicemia, polyserositis, and airsacculitis. Healthy birds' intestinal microbiota contains avian pathogenic *E. coli*, and the majority of the diseases they cause are secondary to host and environmental risk factors. They further stated that ventilation and humidity control are essential for the prevention and management of these illnesses.

Lastly, we found *Proteus* spp. in the eggs. Typically, *Proteus* spp. is found in water, soil and the intestines of animals and humans. Many food poisoning outbreaks have been linked to *Proteus* group organisms. With the increased prevalence of *P. mirabilis*-related foodborne diseases, there is an urgent need for control and prophylactic for food poisoning outbreaks linked to meat products. To guarantee food safety and safeguard public health from microbial contamination, it is critical to investigate and eliminate such agents in foods. Recently, a few cases of food poisoning caused by *P. mirabilis* have been documented. They've been found in chicken, beef, pig, and poultry waste. *Proteus* spp. are responsible for a wide range of nosocomial diseases, including respiratory system infections, ear, nose, skin, burns, and wounds. They are significant urinary tract pathogens and the main pathogenic agents in individuals with indwelling urinary catheters. 90% of *Proteus* infections are caused by *P. mirabilis*, typically seen in immunocompromised persons (Ram et al., 2021). A study by Owoseni et al. (2021) sampled swabs from eggshells, feed, and drinking water from farms and found that 34.26% of samples are positive for *Proteus* spp., and the prevalence of *Proteus* spp. for swabs of eggshell is 28.38%.

The low contamination of quail eggs may be due to several reasons, for instance, egg washing after collection. It is a general practice for farm in Malaysia, especially in small farms. Washing the eggs after collection not only reduces the contamination of bacterial pathogens, but also improves the egg's attractiveness and increases the chance for the consumers to purchase it.

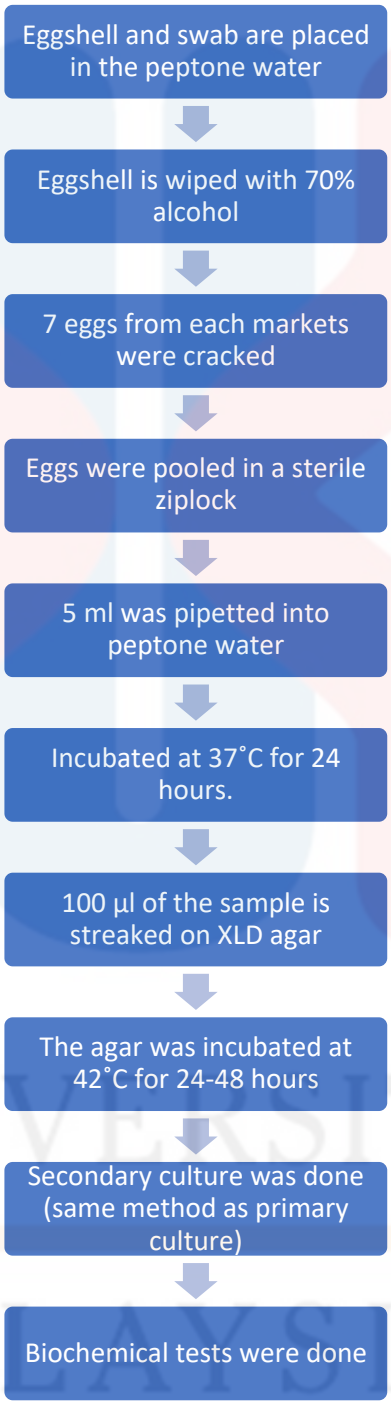
10.0 Conclusion

In conclusion, there is moderate contamination of bacterial enteric pathogens in quail eggs in Kota Bharu, Kelantan. However, the isolated bacteria in this study may potentially cause foodborne illnesses, which require proper handling and cooking to prevent potential transmission to consumers.

11.0 Recommendations and future work

Several limitations were noted in this study. For future study, it is wise to do an immediate culture of the quail egg samples. The sampling should be done on the farm instead of the market so there is no contamination caused by humans touching the eggs. An antibiotic sensitivity test is suggested to be done for the bacteria isolated so know what bacteria is resistant to the antimicrobials chosen. An advanced biochemical test and PCR are recommended to be done to identify the species of the bacteria as in this study, some bacteria were identified up to their genus. In addition, serotyping, for instance, for *E. coli*, can be done to differentiate between serotypes so the pathogenic serotypes can be found. Further study involving quail meat is recommended to be done; the findings in this study serve as a preliminary basis for further screening of enteric pathogens in other food types as well as notifying health authorities and educating the public with regards to food safety code to ensure enteric pathogens causing food-borne pathogens can be prevented in the community.

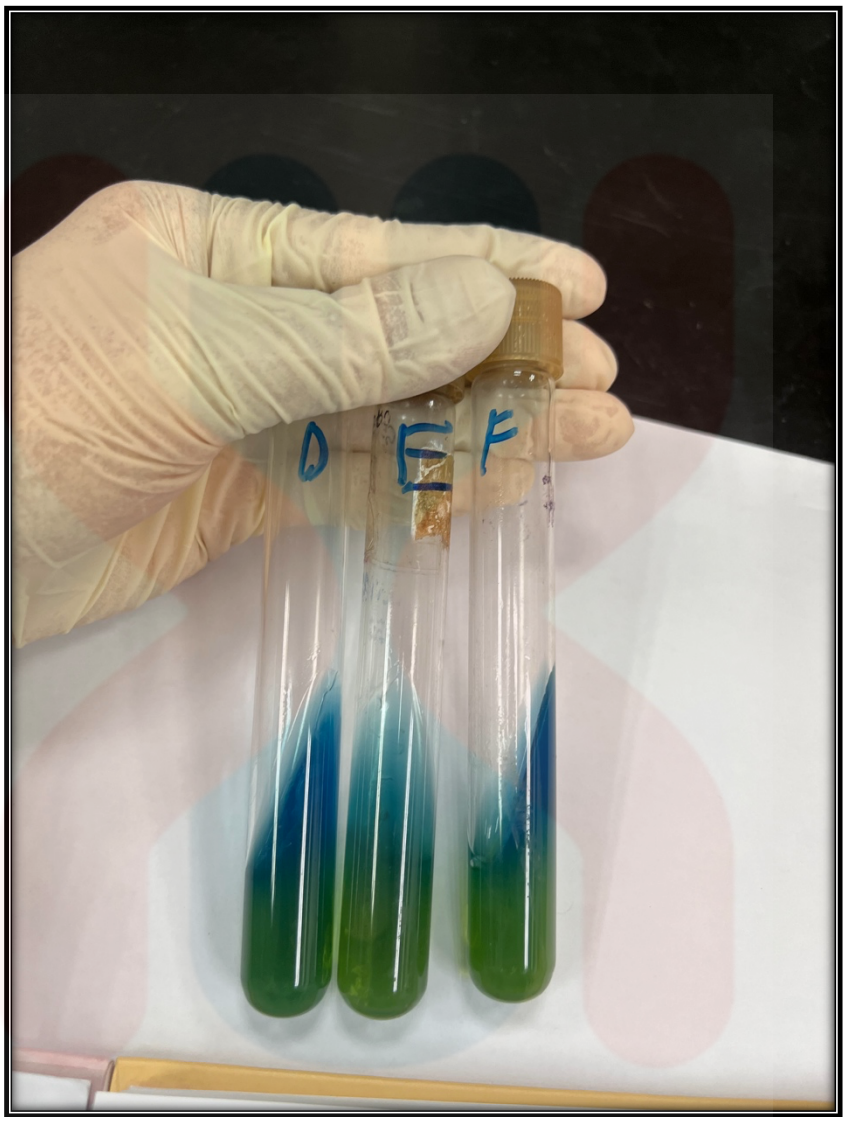
12.0 Appendix



Appendix 1: The flowchart of the method of isolation and identification of the bacterial pathogens

Appendix 2: Samples in peptone water incubated at 37°C

Appendix 3: Bacterial colony isolated



Appendix 4: An example of the biochemical test was done (citrate test)

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