UNIVERSITI PUTRA MALAYSIA

PRESENCE OF SHIGA-TOXIN PRODUCING Escherichia coli AND Escherichia coli O157: H7 IN LOCAL BEEF AND INDIAN BUFFALO MEAT

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PRESENCE OF SHIGA-TOXIN PRODUCING *Escherichia coli* **AND** *Escherichia coli* **O157: H7 IN LOCAL BEEF AND INDIAN BUFFALO MEAT**

By

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October 2018

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Shiga-like toxin producing *Escherichia coli* bacteria are well known to be the pathogenic bacteria that cause traveller diarrhea. *E. coli* O157: H7 from the group of Shiga-like toxin producing *E. coli* cause even severe infection which can lead to fatal in human. In this study, local beef and India buffalo were selected to determine the presence of Shiga-like toxin producing *E. coli* and *E. coli* O157:H7 using Most Probable Number-Polymerase Chain Reaction (MPN-PCR) method. Among one hundred and eight samples, eight samples from local beef and India buffalo were detected a positive on *E. coli* O157: H7 while thirteen samples were detected positive for Shiga-like toxin producing *E. coli* gene. Out of 108 samples (n=108), 11 isolates of *E. coli* O157: H7 were successfully isolated in order to carry out the antibiotic susceptibility test. Shiga-like toxin producing *E. coli* isolates were found susceptible to ceftazidime (100%), moxifloxacin (83.33%), sulphamethoxazole (66.67%), ampicillin (50%), amoxycillin (50%), ciprofloxacin (50%), erythromycin (33.33%) and penicillin G (33.33%). *E. coli* O157: H7 isolates were susceptible toward erythromycin (100%), ceftazime (100%), ciprofloxacin (100%) and moxifloxacin (100%), sulphamethoxazole (60%), ampicillin (20%), amoxycillin (20%), and penicillin G (0%).

In conclusion, both Shiga-like toxin producing *E. coli* and *E. coli* O157: H7 were detected from both local beef an India buffalo. In this research, effect of holding time and washing practice on growth pattern of *E. coli* O157:H7 on beef was studied. Beef samples that were washed and remain un-washed were placed in room temperature. Washed samples present a lower bacteria count. However, the longer the beef was placed under room temperature, the more the bacteria count detected on beef sample. It is recommended to keep raw meat immediately in fridge or freezer after purchase or thawing raw meat overnight in fridge (4 °C).

Keywords: *Escherichia coli*, Shiga-like toxin, Most Probable Number, Polymerase Chain Reaction, Antibiotics

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PRESENSI *Escherichia coli* YANG MENGHASILKAN SHIGA-TOKSIN DAN *Escherichia coli* O157: H7 DALAM DAGING LEMBU TEMPATAN DAN KERBAU INDIA

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Bakteria *Escherichia coli* yang menghasilkan Shiga-seperti toksin dikenali sebagai bakteria patogen yang menyebabkan cirit-birit pengembara. *E. coli* O157: H7 dari kumpulan *E. coli* yang menghasilkan Shiga-seperti toksin menyebabkan jangkitan yang teruk yang boleh membawa maut kepada manusia. Dalam kajian ini, daging tempatan dan kerbau India telah dipilih untuk menentukan kehadiran bakteria *E. coli* yang menghasilkan Shiga-seperti toksin dan *E. coli* O157: H7 menggunakan kaedah Bilangan Paling Mungkin-tindak balas rantaian polimerase (MPN-PCR). Antara seratus lapan sampel, lapan sampel dari daging lembu tempatan dan kerbau India dikesan positif pada *E. coli* O157: H7 manakala tiga belas sampel dikesan positif untuk bakteria *E. coli* yang menghasilkan Shiga-seperti toksin.

Daripada 108 sampel (n=108), 11 isolat *E. coli* O157: H7 berjaya diasingkan untuk menjalankan ujian kerentanan antibiotik. Isolat *E. coli* yang menghasilkan Shiga-seperti toksin didapati terdedah kepada ceftazidime (100%), moxifloxacin (83.33%), sulphamethoxazole (66.67%), ampicillin (50%), amoxycillin (50%), ciprofloxacin (50%), eritromisin (33.33%) dan penisilin G (33.33%). E. coli O157: H7 isolat terdedah kepada erythromycin (100%), ceftazime (100%), ciprofloxacin (100%) dan moxifloxacin (100%), sulphamethoxazole (60%), ampicillin (20%), amoxycillin (20%), dan penisilin G (0%).

Secara rumusnya, *E. coli* yang menghasilkan Shoga-seperti toksin dan *E. coli* O157: H7 telah dijumpai dalam daging lembu tempatan dan kerbau India. Dalam kajian ini, kesan masa dan cara pencucian atas penumbuhan *E. coli* O157: H7 dalam daging lembu tempatan telah dikaji. Sampel daging lembu yang dibasuh dan tidak dibasuh telah diletakkan dalam suhu bilik. Sampel yang dibasuh menunjukkan kiraan bakteria

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yang lebih rendah. Walau bagaimanapun, semakin lama daging lembu diletakkan di bawah suhu bilik, lebih banyak kiraan bakteria yang dikesan pada sampel daging lembu. Adalah disyorkan untuk menyimpan daging mentah dengan serta-merta dalam peti sejuk selepas membeli atau mencairkan daging mentah dalam peti sejuk (4 ° C).

Kata kunci: *Escherichia coli*, Shiga-seperti toksin, Bilangan paling mungkin, Tindak bala rantaian polimerase, Antibiotik

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LIST OF ABBREVIATIONS

bp	base pair
CFU	Colony Forming Unit
DNA	Deoxyribonucleic Acid
g	gram
MAR	Multiple Antibiotic Resistance
ml	mililiter
MPN	Most Probable Number
PCR	Polymerase Chain Reaction
Taq	*Thermus aquaticus* DNA (polymerase)
TBE	Tris-Borate EDTA electrophoresis buffer
TE	Tetracycline
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Foodborne disease or sometimes called the foodborne illness or food poisoning is a very common and costly problem faced by people. However, this issue is preventable if food is prepared and handle with extra care and hygiene. Every year, there is one among six Americans suffocate from foodborne disease by consuming contaminated foods or drinks (CDC, 2015). There are various factors causing food contamination in foods, which are microbes, pathogens, poisonous chemicals or even any harmful substances. Any of these organisms or substances can contaminate food and cause food poisoning if the foods consumed contain bacteria having sufficient infection dose to cause an infection. Food contamination can happen at any stage throughout the process of food production to consumption, which is also known as "farm to fork" (WHO, 2015). In Malaysia, foodborne disease is a common problem due to the hot and humid climate which is suitable for the growth of foodborne bacteria (Abdul-Mutalib, Syafinaz, Sakai, & Shirai, 2015). Yet, standard of food hygiene in Malaysia is relatively low, where the official reported figure for foodborne illness was probably just a tip on an iceberg (Lim, 2002). The exact incidence of foodborne infections in Malaysia is unknown with little effort in determining or finding out the severity of this problem.

Escherichia coli (*E. coli*) is one of the common organisms among various disease-causing organisms that contribute to food poisoning. Certain strains of *E. coli* which produce toxin can cause foodborne disease, which is also commonly refer as travelers' diarrhea. The onset time after ingesting toxin producing *E. coli* is 1 to 3 days and can cause watery diarrhea, abdominal cramps and even vomiting (FDA, 2016). These symptoms can last up to 7 days. One can infected with this foodborne disease after consuming human feces contaminated water or food. Other than toxin producing *E. coli*, there is another strain in *E. coli* which can cause a more severe infection which is the *E. coli* O15:H7 strain. It causes hemorrhagic colitis or the *E. coli* O157:H7 infection. It caused severe diarrhea, sometimes bloody diarrhea, abdominal pain and vomiting. Younger children infected with this disease can lead to kidney failure (CDC, 2014). The common food sources for *E. coli* O157:H7 are from undercooked beef, unpasteurized milk and juices, raw fruits and vegetables and even from contaminated water (Marler, 2011).

1.2 Problem statements

Disease caused by *E. coli* cannot be underestimated where detection of toxin producing *E. coli* and *E. coli* O157:H7 in food is extra important in reducing the risk of consumer

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in exposing to such bacteria. There are various ways in determining the presence of bacteria in foods, including biochemical test, polymerase chain reaction (PCR), or even real time PCR. Few decades back, scientist used to carry out biochemical test in order to determine the presence of specific bacteria in food. However, PCR is now leading in clinical laboratory where it gives sensitive and specific identification of microorganisms (Buchan & Ledeboer, 2014). Local beef and India buffalo were chosen as the samples in this study to determine the presence of Shiga-toxin producing *E. coli* and *E. coli* O157:H7 using Most Probable Number (MPN)-PCR method. Minimum research on Shiga-toxin producing *E. coli* and *E. coli* O157:H7 from local beef and India buffalo had been carried out for the past few decades in Malaysia. There was a research for the detection of *E. coli* O157:H7 in beef marketed in Malaysia was carried out by Radu and his fellow friends (1998), where the presence of *E. coli* O157:H7 strains from beef were tested using biochemical test and agglutination test. In this study, PCR will be used to replace the technique of biochemical test in order to obtain a more sensitive result. After obtaining the isolates of both Shiga-toxin producing *E. coli* and *E. coli* O157:H7, antibiotic susceptibility test was carried out in order to determine the antibiotic profile of each strains. Finally, kitchen simulation study was carried out as well to identify how holding time and washing process can make a difference in bacteria growth in food.

According to Pin (2014), there was a dramatic increment in the total imports of meat and meat preparation in Malaysia from 2009 to 2013, which is from 421 US million to 805 US million. This indicates the expanding demand of meat and also meat preparation by the people as time passed. Beef and also buffalo have now become a better choice in their food basket is due to the high nutrient of beef and buffalo, where they contribute to less calories, at the same time providing good value of protein and other vitamins such as zinc, iron phosphorus and riboflavin. Other than that, the prices of beef and buffalo are consumer friendly as well, where average family could afford them. Hence, local beef and India buffalo were selected as the sample in this research.

1.3   Objectives of this study

a. To examine the prevalence of Shiga-toxin producing *E. coli* and *E. coli* O157:H7 from local beef and India buffalo using MPN-PCR method.
b. To evaluate the antibiotic profile of Shiga-toxin producing *E. coli* and *E. coli* O157:H7 isolated from local beef and India buffalo.
c. To evaluate the effect of holding time and washing technique on the growth pattern of *E. coli* O157:H7 on beef samples.

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