
**UNIVERSITI PUTRA MALAYSIA**

# *EVALUATION OF QUALITY AND SAFETY OF COW AND GOAT MILK FROM ESCHERICHIA COLI O157 AND CAMPYLOBACTER SPP*

**YUSRALIMUNA BINTI NORDIN**

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By

**YUSRALIMUNA BINTI NORDIN**

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**November 2018**

**Chair : Professor Son Radu, PhD**
**Faculty : Food Science and Technology**

High demand for milk has been observed amongst the Malaysian public. Hence, research in milk is essential to assure food safety in milk consumption. This study evaluates the quality of locally-produced milk and present of bacterial hazards in cow and goat milk. A total of 120 milk samples (30 raw cow milk, 30 pasteurised cow milk, 30 raw goat milk and 30 pasteurised goat milk) were collected from dairy farms, delivery milkman, marts and markets in Selangor. The sampling has been carried out from May 2016 until August 2017. The bacteriological quality of milk was evaluated for the presence of mesophilic, coliform and *Escherichia coli* bacteria. An acceptable standard limit of $< 1 \times 10^5$ CFU/ml for the total bacterial count was used to indicate good quality of milk. The standard is according to Malaysian Food Act 1983 and Food regulations 1985. Additionally, the bacteriological safety of milk was assessed. The selected pathogenic *E. coli* O157 and *Campylobacter* species were identified using most-probable number and polymerase chain reaction (MPN-PCR) for quantification and species identification. The behaviour of *E. coli* O157 also has been investigated in this study. The *E. coli* O157 counts were evaluated over 24 hours of incubation at different temperatures. Overall, all type of milks exceeded 100,000 CFU/ml. Approximately half of milk samples were contaminated with coliform bacteria. A proportion has exceeded the acceptable limit of 50 CFU/ml. The presence of *E. coli* was detected in over 44% of samples. Further screening detected pathogenic *E. coli* O157 in 10 samples (raw cow milk 13.33%; other type of milks 6.67%). *Campylobacter* spp. were not detected in samples. In addition, *E. coli* O157 was able to survive and proliferate at refrigerated and ambient temperature. The presence of pathogenic *E. coli* O157 in milk signify a threat to public health. The finding is expected to aid the risk profile for *E. coli* O157 as there is no complete data available for assessment of the microbiological quality.

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

**EVALUASI KUALITI DAN KESELAMATAN MAKANAN DALAM SUSU LEMBU DAN KAMBING DARIPADA *ESCHERICHIA COLI* O157 DAN *CAMPYLOBACTER* SPP**

Oleh

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Terdapat permintaan yang tinggi untuk susu dalam kalangan rakyat Malaysia. Oleh itu, penyelidikan berkenaan susu adalah penting untuk memastikan keselamatan makanan susu. Kajian ini menilai kualti susu tempatan dan menilai potensi kehadiran bakteria berbahaya dalam susu lembu dan susu kambing. Sebanyak 120 sampel susu (30 susu lembu mentah, 30 susu lembu pasteur, 30 susu kambing mentah dan 30 susu kambing pasteur) telah dikumpulkan dari ladang tenusu, penghantar susu, pasar raya dan pasar basah di Selangor. Persampelan susu telah dijalankan pada Mei 2016 sehingga Ogos 2017. Bakteriologikal kualiti susu dinilai berdasarkan kehadiran bakteria mesophilik, koliform dan *Escherichia coli*. Had piawaian berdasarkan Malaysia Akta Makanan 1983 dan Peraturan Makanan 1985 sebanyak <1 X $10^5$ CFU/ml telah digunakan untuk mengira jumlah bakteria digunakan. Tambahan pula, kajian ini menilai keselamatan bakteria susu. Spesies patogen *E. coli* O157 dan *Campylobacter* telah dipilih untuk dikenal pasti menggunakan kaedah Bilangan Paling Mungkin – tindakbalas raintaian polymerase (MPN-PCR) untuk kuantifikasi dan mengenal identiti spesies. Karakter *E. coli* O157 juga telah disiasat dalam kajian ini. Hal ini dikaji berdasarkan pengiraan bilangan *E. coli* O157 selama 24 jam inkubasi pada suhu yang berbeza. Secara keseluruhan, semua jenis susu melebihi 100,000 CFU/ml. Kira-kira separuh sampel susu tercemar dengan bakteria koliform. Sebahagiannya telah melebihi had yang boleh diterima iaitu sebanyak 50 CFU/ml. *Escherichia* coli juga dikesan dalam lebih daripada 44% sampel. Tambahan juga, kajian ini mengesan patogenik *E. coli* O157 dalam 10 sampel (susu lembu mentah 13.33%; jenis susu lain 6.67%). *Campylobacter* spp. tidak dikesan dalam mana-mana sampel. Di samping itu, *E. coli* O157 dilihat mampu hidup dan membiak pada suhu sejuk dan ambien. Kehadiran patogen *E. coli* O157 dalam susu menandakan ancaman kepada kesihatan awam. Penemuan ini diharapkan dapat membantu profil risiko untuk *E. coli* O157.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| BAM | Bacteriological Analysis Manual |
| bp | base pair |
| CDC | Centers for Disease Control and Prevention |
| CFU | colony forming unit |
| DNA | deoxyribonucleic acid |
| dNTPs | deoxynucleotide triphosphates |
| DVS | Department of Veterinary Services, Malaysia |
| EMB | Eosin Methylene Blue |
| EDTA | ethylenediaminetetra-acetic acid |
| FAO | Food and Agricultural Organisation |
| FDA | Food Drug Administration |
| FSA | Food Standards Agency |
| FSANZ | Food Standards Australia New Zealand |
| GMP | good manufacturing practice |
| HUS | hemolytic uremic syndrome |
| ISI | innovative steam injection |
| MCC | Milk Collection Centre |
| $MgCl_2$ | Magnesium Chloride |
| mL | milliliter |
| µL | microliter |
| mM | millimolar |
| MPN | Most Probable Number |
| PCA | Plate Count agar |
| PCR | Polymerase Chain Reaction |
| spp. | species |
| *Taq* | Thermus aquaticus DNA (polymerase) |
| TBE | Tris-borate-EDTA |
| TTP | thrombotic thrombocytopaenic purpura |

| | |
|---|---|
| TPC | total plate count |
| TSB | Tryptic Soy broth |
| UHT | ultra high temperature |
| VRB | Violet Red Bile |
| WHO | World Health Organisation |
| $^{o}C$ | degree celsius |
| % | percentage(s) |

# CHAPTER 1

# GENERAL INTRODUCTION

## 1.1 Introduction

The dairy industry is one of the largest global industries and has been the backbone for agro-food sector in particular countries. In Malaysia, this sector is an important emerging industry. The niche of cow milk is growing steadily while the goat milk industry is relatively small yet have potential to be developed (Stoney & Francis, 2001). The number of dairy cows and goats have grown significantly, suggesting that commercial dairy industry will be getting larger (MGCC, 2015).

In many cultures, the cow and goat milk is one of the fundamental food. It has been part of compulsory diet for pregnant women and growing children (Reta & Addis, 2015). The milk is an important diet item because of its high nutritional values. Some has defined milk as "the most nearly perfect food" as it consists of calcium, phosphorus and vitamins that are good for bones and teeth (O'Mahony, 1988).

Raw milk consumption has become more well-known with the trend of consuming naturally and purchasing locally (Tremonte et al., 2014). More people preferred to consume raw milk as it is said to be minimally processed food. Some claimed that heat treatment through pasteurisation can damage the nutritional and health benefits in milk (Paul, Van Hekken, & Brewster, 2013; Tremonte et al., 2014). This has underscore the fact that raw milk harbours many pathogenic bacteria like *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes* (Hill, Smythe, Lindsay, & Shepherd, 2012; Claeys et al., 2013).

Healthy dairy animals can produce good quality milk, with only a few bacteria (FAO, 2013). However, the milk can easily be spoiled because of its high water content, nearly neutral pH and consists of variety important nutrients (Reta & Addis, 2015). These provide a good medium for microorganism growth and multiplication, including pathogens. Additionally, sick dairy animals, unsanitary milk handling and improper milk storage are among the possible sources of milk contamination (Lim, Hassan, Abdul Aziz, & Bejo, 2011).

Although milk quality and safety has been a great concern in various researches, issues on raw milk has been circulating especially in non-scientifically based information like on the internet (Claeys et al., 2013). Some highlights on possible benefits of drinking raw milk while there is a significant

concern from regulatory and health organisation on the risk of raw milk being contaminated with pathogens. It is essential to address this food safety of milk in context to protect consumer health and at the same time to support the economics of the producers (FAO, 2013).

Recently, in December 2016, the Malaysia Ministry of Health has urged the public to heat the milk before consume. The sale of unpasteurised milk was considered violation against Regulation 51(1A) under the Food Hygiene Regulations 2009 and Food Act 1983 (Bernama, 2016). This is a part of Malaysia government's effort to control the spread of milk-borne outbreak associated from drinking raw milk contaminated with pathogenic bacteria. Additionally, it was reported that 33.5% of local raw cow milk has been contaminated with *E. coli* O157 (Chye, Abdullah, & Ayob, 2004). Meanwhile, in local raw goat milk, 7.32% of milk samples contained *E. coli* O157 (Lye et al., 2013). No published researches on bacteriological quality of local pasteurised milk. Hence, true risk posed by pasteurised milk remained unknown.

Microbial contamination has influence the quality of milk. High quality of milk enables the farmers to get concession price from Milk Collection Centre (MCC) (De Silva, Kanugala, & Weerakkody, 2016). This ranking milk prices may result economic losses to the local farmers if their milk contained high bacterial counts (Suguna, Rajeev, & Wan Nadiah, 2012). Hence, it is important for farmers to produce high quality of milk.

A significant demand for food safety and quality of milk arise from rapid urbanization, changes in food habits by means of health awareness and improved in the purchasing power as a consequence from better economic status (Kumar, Thapa, Roy, & Joshi, 2017). For that reason, microbiological analysis has been routinely carried out to assess the quality of milk by monitor the level of microorganism. This includes the prevalence of spoilage and pathogenic bacteria for safety reasons (Suguna et al., 2012).

The total plate count (TPC) is used as the routine criterion to measure the quality of milk. It has been used world widely for example, in countries like US, Europe and Malaysia due to easy method and cheap (Claeys et al., 2013; FDA, 2015). One of the primary routes for pathogen transmission in milk is through fecal contamination during milking process. Coliform bacteria and *E. coli* are the common indicators use for fecal or soil contamination. Hence, another method that is used in milk analysis is the coliform counts.

Knowledge regarding the food safety and quality of pasteurised milk in Malaysia is still scarce. Thus, this study will provide the insight of milk safety in Malaysia and denote the related health authorities on quality and safety of locally produced raw and pasteurised milk from farms to marketplace.

## 1.2 Objectives

General objective

To evaluate the bacteriological quality and safety of locally produced raw and pasteurised milk collected from cow and goat

Specific objectives

1. To assess the total bacterial count, coliform count and *E. coli* count of raw and pasteurised milk collected from cow and goat
2. To identify and quantify selected milk-borne pathogens : *E. coli* O157 and *Campylobacter* in raw and pasteurised milk of cow and goat
3. To investigate the bacteriological quality and growth of *E. coli* O157 on storage life of raw and pasteurised milk under different temperatures

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