PREVALENCE OF *SALMONELLA*, *ESCHERICHIA COLI* O157:H7, *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS* IN RAW BEEF IN KELANTAN

NIK NUR FAZLINA BINTI NIK MOHD FAUZI

MASTER OF SCIENCE

2014

Prevalence of *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* in Raw Beef in Kelantan

by

Nik Nur Fazlina binti Nik Mohd Fauzi

A thesis submitted in fulfillment of the requirements for the degree of Master of Science

Faculty of Veterinary Medicine
UNIVERSITI MALAYSIA KELANTAN

2014

THESIS DECLARATION

I hereby certify that the work embodied in this thesis is the result of the original research and has not been submitted for a higher degree to any other University or Institution.

☐ **OPEN ACCESS** I agree that my thesis is to be made immediately available as hardcopy or on-line open access (full text).

☐ **EMBARGOES** I agree that my thesis is to be made available as hardcopy or on-line open access (full text) for a period approved by the Post Graduate Committee.
Dated from _____ until _____

☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*

☐ **RESTRICTED** (Contains restricted information as specified by the organization where research was done)*

I acknowledge that Universiti Malaysia Kelantan reserves the right as follows.

1. The thesis is the property of University Malaysia Kelantan.
2. The library of Universiti Malaysia Kelantan has the right to make copies for the purpose of research only.
3. The library has the right to make copies of the thesis for academic exchange.

_____ _____

SIGNATURE SIGNATURE OF SUPERVISOR

_____ _____

I/C/PASSPORT NO. NAME OF SUPERVISOR

Date: Date:

ACKNOWLEDGEMENT

BISMILLAHIRRAHMANIRRAHIM…

In the name of Allah Subhanahu Wa Ta'ala, the Most Gracious and Most Merciful. An utmost gratitude goes to Allah, with His blessed and strength given to me, I'm able to complete this dissertation after going through two years of challenging and stressful period. The completion of this thesis would not have been possible without the guidance and support of many people. I would like to take this opportunity to express my appreciation to their efforts and kindness.

I would first like to express my deep and sincere appreciation to my supervisor, Assoc. Prof. Dr. Mohd Mokhtar Arshad for his wide knowledge and superb ideas have been great value in this study. His understanding, encouraging, advices and supports have inspired me to finish my research and thesis successfully. Deepest thanks to my co-supervisor, Prof. Dr. Imad Ibrahim Ali Al-Sultan who kindly provided me with his knowledge, guidance and advice in completion of this thesis. I also would like a special thanks to my former supervisor, Dr. Jasbir Singh for his idea carrying out this study.

A million thanks to Department of Veterinary Services Kota Bharu for approval our co-operation with Division of Veterinary Enforcement and Division of Bacteriology Laboratory in collecting samples, training and guidance throughout the study. Sincere thanks to all lab assistant of Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, who is very helpful to provide equipments and apparatus required during the study.

Deeply appreciated and thanks goes to my truly friends especially Qayyum, Ziela, Izzah, Ain, Sis Azwani, Sis Zalina and Sis Zetty for their willingness to help, brilliant ideas, advice and great supports from beginning until the end of the study. Our friendships will remain in my memory forever and ever. Last but not least, my precious appreciation goes to my beloved family for their prayer, constant patience and moral supports especially to my beloved mother, Che Rahmah Che Salleh, father, Nik Mohd Fauzi Wan Ismail and siblings. I could not ask for a better one as without them, my study would have never been possible.

ALHAMDULILLAH…

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMC	Amoxycillin-clavulanate
AMP	Ampicillin
a_w	Water activity
BAM	Bacteriological Analytical Manual
BCIG	5-bromo-4-chloro-3-indolyl- b-D-glucuronide
C	Chloramphenicol
cfu	Colony forming unit
CIP	Ciprofloxacin
CLSI (formally NCCLS)	Clinical and Laboratory Standards Institute
CN	Gentamycin
CRO	Cefotaxime
CT-SMAC	Cefixime and tellurite sorbitol MacConkey agar
DA	Clindamycin
DNA	Deoxyribonucleic acid
E	Erythromycin
FDA	Food and Drug Administration
FOX	Cefoxitin
H_2O	Water
HUS	Hemolytic uremic syndrome
K	Kanamycin
MAR	Multiple Antibiotic Resistance
MDR	Multi Drug Resistance
mw	Molecular weight
mpn	Most probable number
NA	Nalidixic acid
NCBI	National Centre for Biotechnology Information
NCCLS	National Committee for Clinical Laboratory Standards
No.	Number

OX	Oxacillin
P	Penicillin G
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
pH	Measure of the acidity of a solution in terms of the activity of hydrogen (H+)
RD	Rifampin
RNA	Ribonucleic acid
S	Streptomycin
spp.	Species
STEC	Shiga toxin-producing *Escherichia coli*
S3	Sulphonamides
SXT	Trimethoprim-sulfamethoxazole
TE	Tetracycline
TPC	Total plate count
UK	United Kingdom
US	United States
vs	Versus
W5	Trimethoprim
XLT-4	Xylose Lactose Tergitol™ 4

LIST OF SYMBOLS

%	Percent
°C	Degree Celsius
°	Degree
β	Beta
®	Copyright
™	Trademark
g	Gram
h	Hour
L	Liter
ml	Milliliter
mg	Milligram
mm	Millimeter
min	Minute
n	Sample size
ng	Nanogram
r.p.m.	Revolutions per minute
sec	Second
U	Unit
V	Volt

Kelaziman *Salmonella, Escherichia coli* O157:H7, *Listeria monocytogenes* dan *Staphylococcus aureus* dalam Daging Lembu Mentah di Kelantan

ABSTRAK

Penyakit bawaan makanan disebabkan oleh *Salmonella, Escherichia coli* O157: H7, *Listeria monocytogenes* dan *Staphylococcus aureus* telah dikaitkan dengan pengambilan daging lembu kurang masak dalam makanan telah dilaporkan di seluruh dunia. Di Kelantan, penyembelihan lembu di rumah sembelih persendirian biasa diamalkan kerana rumah sembelih kerajaan berdaftar adalah terhad. Objektif kajian ini adalah untuk; i) Menentukan kualiti bakteriologi pada daging lembu mentah, ii) Menentukan kelaziman bakteria bawaan makanan (*Salmonella*, *E. coli* O157: H7, *L. monocytogenes* dan *S. aureus*) dalam daging lembu mentah dari kedai, rumah sembelih persendirian dan rumah sembelih kerajaan, iii) Mengenalpasti corak daya tahan bakteria terhadap antibiotik. Sampel daging lembu mentah dikumpulkan dari pelbagai premis berdasarkan kepada kiraan bakteria plat (TPC), kiraan koliform, analisis *Salmonella*, *E. coli* O157: H7, *L. monocytogenes* dan *S. aureus*. Pencilan bakteria seterusnya melalui ujian antibiotik dengan menggunakan kaedah *Disc diffusion* (Kirby-Bauer). Sebelas daripada 23 (47.8%) daging lembu mentah dari kedai tepi jalan mempunyai TPC melebihi had yang dibenarkan (<1.0 x 10^6 cfu/g). Manakala tiada daripada 19 (0%) daging lembu dari rumah sembelih kerajaan. Lapan belas daripada 23 (78.3%) sampel daging lembu dari kedai tepi jalan mempunyai kiraan koliform melebihi had yang dibenarkan (1000 mpn/g). Manakala satu daripada 19 (5.3%) daging lembu dari rumah sembelih kerajaan mempunyai kiraan koliform melebihi had yang dibenarkan. *Salmonella* spp. dikesan 44.0% (11/25), 16.7% (2/12), 5.3% (1/19) dalam daging lembu mentah dari kedai tepi jalan, rumah sembelih persendirian dan rumah sembelih kerajaan masing-masing. Daripada 14 *Salmonella* dipencil, yang paling tinggi ialah *S.* Mbandaka (5/14), *S.* Weltevreden (5/14) diikuti *S.* Albany (4/14).Tiada *E.coli* O157: H7 dan *L. monocytogenes* dikesan dalam 25 sampel daging lembu dari kedai tepi jalan, 12 rumah sembelih persendirian dan 19 rumah sembelih kerajaan. *S. aureus* dikesan 32% (8/25), 33.3% (4/12) dalam daging lembu dari kedai tepi jalan dan rumah sembelih persendirian masing-masing. Daripada 14 pencilan *Salmonella*, 21% (3/14) adalah sensitif kepada semua antibiotik yang diuji, 36% (5/14) tahan kepada satu kelas antibiotik, 14% (2/14) tahan kepada 2 kelas antibiotik dan 29% (4/14) adalah MDR. Analisis MAR indeks menunjukkan 2 pencilan *S.* Albany mempunyai nilai MAR indeks tertinggi (1.00) diikuti 2 pencilan *S.* Albany yang lain (0.69). Kesimpulannya, kajian ini menunjukkan bahawa pengguna di Kelantan mungkin terdedah kepada kualiti bakteriologi dan tahap keselamatan daging lembu mentah yang rendah dari kedai tepi jalan dan mengandungi *Salmonella* yang tahan rintangan antibiotik. Keputusan yang dibentangkan dalam kajian ini dapat membantu agensi yang berkaitan untuk dijadikan garis panduan bagi meningkatkan kualiti bakteriologi dan keselamatan daging lembu mentah runcit di Kelantan. Ia juga boleh dijadikan sebagai asas perbandingan untuk kajian pada masa hadapan.

Prevalence of *Salmonella, Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* in Raw Beef in Kelantan

ABSTRACT

Foodborne illness caused by *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* associated with the consumption of undercooked beef or foods containing beef have been reported worldwide. In Kelantan, slaughtering of cattle in backyard slaughterhouse is common practice because there are limited numbers of government abattoir. The objectives of this study were to; i) Determine the bacteriological quality of raw beef, ii) Determine the prevalence of foodborne bacteria (*Salmonella*, *E. coli* O157:H7, *L. monocytogenes* and *S. aureus*) in raw beef from retail stores, backyard slaughterhouses and government abattoirs, iii) Determine the antibiotic resistant pattern of the bacteria. Raw beef samples were collected from different types of premises and subjected to TPC, coliform count and analysis for *Salmonella*, *E. coli* O157:H7, *L. monocytogenes* and *S. aureus*. The bacteria were then subjected to antibiotic susceptibility test by using the standard disc diffusion method (Kirby-Bauer). Eleven of 23 (47.8%) raw beef from retail stores had TPC above the acceptable limit (<1.0 x 10^6 cfu/g). Whereas none of 19 (0%) raw beef from government abattoir had TPC above the acceptable limit. Eighteen of 23 (78.3%) beef samples from retail store had coliform count above the acceptable limit (1000 mpn/g). Whereas one of 19 (5.3%) raw beef from government abattoir had coliform count above the acceptable limit. *Salmonella* spp. was detected in 44.0% (11/25), 16.7% (2/12), 5.3% (1/19) of raw beef from retail stores, backyard slaughterhouses and government abattoir respectively. Of 14 *Salmonella* isolated, the most common were *S.* Mbandaka (5/14), *S.* Weltevreden (5/14) followed by *S.* Albany (4/14). No *E.coli* O157:H7 and *L. monocytogenes* were detected in raw beef from all different types of premises. *S. aureus* was detected in 32% (8/25), 33.3% (4/12) of raw beef from retail stores and backyard slaughterhouses respectively. Of 14 isolates of *Salmonella*, 21% (3/14) were susceptible to all antibiotics tested, 36% (5/14) presented a single type of resistance, 14% (2/14) were resistance to 2 classes of antibiotics and 29% (4/14) were MDR. The MAR index analysis indicated that 2 isolates of *S.* Albany had highest MAR index value (1.00) followed by another 2 isolates of *S.* Albany (0.69). In conclusion the results of this study indicate that consumers in Kelantan may access to raw beef from retail stores that are of low bacteriological quality and safety level and contain MDR *Salmonella*. The results presented in this study can help relevant agencies to establish guidelines to improve the bacteriological quality and safety of retail raw beef in Kelantan. It can also serve as a baseline information for future studies.