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MICROBIAL RISK ASSESSMENT OF THERMOPHILIC *CAMPYLOBACTER* SPP. IN RAW VEGETABLES FROM FARM TO TABLE

CHAI LAY CHING

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By

CHAI LAY CHING

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

August 2008

Dedicated to my late father and my beloved family for their endless love and support

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

MICROBIAL RISK ASSESSMENT OF THERMOPHILIC *CAMPYLOBACTER* SPP. IN RAW VEGETABLES FROM FARM TO TABLE

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August 2008

Chairman: **Associate Professor Fatimah Abu Bakar, PhD**

Faculty: **Food Science and Technology**

The first aim of this study was to determine the prevalence and number of thermophilic *Campylobacter* spp. (*Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter fetus*) in raw vegetables (ulam) at pre-harvest and retail level, soil and animal manure in an organic and a traditional vegetable farm. The biosafety of *Campylobacter jejuni* was assessed by phenotypic (antibiotic resistance) and genotypic (presence of virulent and toxin genes) as well as RAPD-PCR characteristics of the strains isolated from vegetables. A kitchen simulation study was conducted to provide decontamination and cross-contamination data and information for estimation of the risk of acquiring campylobacteriosis from consumption of ulam using a step-wise risk assessment.

The prevalence of thermophilic *Campylobacter* spp. in 309 (number of samples) raw vegetables purchased from two supermarkets and a wet market was relatively high, 29% to 68%. *Campylobacter jejuni* (25.5% to 67.7%) and *C. coli* (21.6% to 65.7%) were predominant species isolated; while *C. fetus* was only detected in two samples (1.9%) from one of the supermarkets. Only 18.3% of *Campylobacter*-MPN-PCR positive samples were recovered by enumeration-plating method indicating that routine enumeration-plating methods has very low recovery rate for *Campylobacter* spp. from vegetables.

The study was extended to investigate the level of contamination with *Campylobacter* spp. in vegetables farms. A total of 172 samples of animal manure (n=18), soil (n=60), irrigation water (n=45) and vegetables (n=49) samples were collected from both an organic and a conventional vegetable farm. The organic vegetable farm (20.5%) was found to have a higher prevalence of *Campylobacter* spp. compared to the vegetable farm practicing conventional farming (2%). The low contamination level in the conventional farm was most probably due to the bed-burning practice and the use of composted manure in the farm. *Campylobacter coli* was not detected in all the samples from both farms. Soil (30.4%) and animal manure (57.1%) sampled

from the organic vegetable farm were found to harbor *Campylobacter* spp. and *C. jejuni*. However, none of the irrigation water samples examined from both farms were positive for *Campylobacter* spp.

RAPD-PCR fingerprinting and antibiotic resistance profiling indicated that multi-resistant *Campylobacter* spp. might be wide-spread in the study area. Clustering of *C. jejuni* isolates based on RAPD-PCR profiles suggested that some isolates from different sources and locations were genotypically closely related. Clusters A2, A3, A5 and A6 comprised *C. jejuni* strains isolated from raw vegetables in the supermarkets and a wet market. All clusters including B1 and B3, which comprised strains only from supermarkets, were actually consisted of isolates from different sources. The isolates showed multi-resistance to as many as 10 antibiotics tested. All the isolates were detected to carry the virulent genes, *cad*F, *ceu*E and *fla*A. However, toxin genes detection indicated only 16.1% and 10.7% of the isolates carry *cdt*B and *cdt*C toxin genes, respectively; while none of the isolates carry *cdt*A gene.

The potential of raw salad vegetables as a vehicle in *C. jejuni* transmission was demonstrated by a step-wise risk assessment. Based on the

assumptions used in the step-wise risk assessment, the annual number of cases of campylobacteriosis acquired from the consumption of ulam is estimated to be 4992/100,000 of Malaysian population, assuming that 10% of *Campylobacter* spp. infection translates into illness. However, the risk estimate was predicted to reduce to 175/100,000 if an extra blanching step was incorporated into the model. In conclusion, there is an immediate need for further investigation to look into the wide-spread problem of *Campylobacter* spp. in ready-to-eat foods, such as salad and ulam, in Malaysia.

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

BIO-KESELAMATAN TERMOFILIK *CAMPYLOBACTER* SPP. DALAM SAYURAN MENTAH DARI KEBUN KE PEMAKANAN

Oleh

CHAI LAY CHING

Ogos 2008

Chairman: Profesor Madya Fatimah Abu Bakar, PhD

Faculty: Sains dan Teknologi Makanan

Matlamat utama kajian ini adalah untuk menentukan prevalens dan kuantiti termofilik *Campylobacter* spp. (*Campylobacter jejuni, Campylobacter coli* dan *Campylobacter fetus*) dalam sayuran mentah (ulam) pada tahap pra-penuaian dan jualan, tanah dan baja haiwan dari sebuah kebun sayur organik dan sebuah kebun sayur tradisi. Bio-keselamatan *Campylobacter jejuni* juga dikaji dengan pencirian fenotipik (kerintangan antibiotik) dan genotipik (kehadiran gen virulen dan toksin) dan juga pencirian RAPD-PCR bagi pencilan-pencilan yang diperolehi dari sayuran. Suatu kajian simulasi dalam dapur telah pun dijalankan untuk mendapat data dan informasi mengenai nyah-kontaminasi dan kontaminasi semula bagi anggaran risiko

dijangkiti kampylobakteriosis dengan makan ulam dengan menggunakan kajian risiko berperingkat (step-wise risk assessment).

Prevalens termofilik *Campylobacter* spp. dalam 309 (jumlah sampel) sayuran mentah yang dibeli dari dua pasar raya dan satu pasar borong tempatan adalah amat tinggi, 29% ke 68%. *Campylobacter jejuni* (25.5 ke 67.7%) dan *C. coli* (21.6% ke 65.7%) adalah spesies dominant yang dipencil, manakala *C. fetus* hanya dijumpai dalam dua sampel (1.9%) dari salah satu pasar raya sahaja. Hanya sebanyak 18.3% *Campylobacter* MPN-PCR positif sampel dapat dikesan positif bagi *Campylobacter* spp. dengan cara "enumeration-plating" dan ini menunjukkan bahawa kadar pengesanan *Campylobacter* spp. dalam sayuran dengan cara "enumeration-plating" adalah sangat rendah.

Kajian ini dilanjutkan untuk menyiasat tahap kontaminasi di kebun-kebun sayur dengan *Campylobacter* spp. Sebanyak 172 sampel yang tediri daripada baja haiwan (n=18), tanah (n=60), pengairan (n=45) dan sayuran (n=49) telah dikutip dari sebuah kebun sayur organik dan sebuah kebun sayur tradisi. Kebun sayur organik (20.5%) telah didapati mempunyai prevalens *Campylobacter* spp. yang lebih tinggi berbanding dengan kebun sayur yang mempraktik perkebunan tradisi (2%). Tahap kontaminasi yang rendah di

kebun sayur tradisi kemungkinan besar disebabkan oleh pengamalan kaedah "bed-burning" dan penggunaan baja kompos di kebun. *C. coli* tidak dijumpai dalam sampel-sampel dari kedua-dua kebun sayur. Tanah (30.4%) dan baja haiwan (57.1%) dari kebun sayur organik telah didapati membawa *Campylobacter* spp. dan *C. jejuni*. Walaubagaimana pun, tiada satu pun sampel pengairan dari kedua-dua kebun yang dikaji didapati positif bagi *Campylobacter* spp.

RAPD-PCR dan profil kerintangan antibiotik menunjukkan bahawa multi-rintangan *Campylobacter* spp. kemungkinan mempunyai sebaran luas di kawasan kajian. Pencilan *C. jejuni* telah menunjukkan multi-rintangan kepada sebanyak 10 antibiotik yang diuji. Kelompokan *C. jejuni* berdasarkan RAPD-PCR profil mencadangkan bahawa sebahagian daripada pencilan dari pelbagai sumber and lokasi adalah genotipik berkait rapat. Kelompok A2, A3, A5 dan A6 terdiri daripada pencilan *C. jejuni* yang didapati dari pasar raya dan pasar borong. Semua kelompok termasuk B1 dan B3, yang mana terdiri daripada hanya pencilan dari pasar raya tetapi dari sumber yang lain. Semua pencilan-pencilan didapati membawa gen virulen, *cad*F, *ceu*E dan *fla*A. Walaupun demikian, pengesanan gen tosin hanya menunjukkan sebanyak 16.1% dan 10.7% daripada pencilan tersebut

masing-masing mengandungi gen toksin *cdt*B dan *cdt*C. Tiada pencilan yang membawa gen *cdt*A.

Potensi sayuran mentah sebagai pembawa *C. jejuni* dalam penyebaran penyakit telah ditunjukkan dengan menggunakan kajian risiko berperingkat (step-wise risk assessment). Dengan merujuk kepada andaian yang digunakan dalam kajian risiko berperingkat (step-wise risk assessment) ini, jumlah kes kampylobakteriosis akibat memakan ulam dianggar sebanyak 4992/100,000 daripada populasi Malaysia, jika hanya 10% daripada jangkitan *Campylobacter* spp. terjemah kepada penyakit. Walaubagaimana pun, anggaran risiko ini dapat diturunkan kepada 175/100,000 jika langkah merebus ditambah kepada model. Sebagai kesimpulan, penyiasatan lanjutan adalah amat diperlukan untuk meneliti masalah penyebaran luas *Campylobacter* spp. dalam makanan-sedia-dimakan, seperti salad dan ulam, di Malaysia.

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I certify that an Examination Committee has met on 22th August 2008 to conduct the final examination of Chai Lay Ching on her Doctor of Philisophy thesis entitled "Microbial Risk Assessment of Thermophilic *Campylobacter* spp. in Raw Vegetables from Farm to Table" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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Date: 13 November 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is nor concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

_____

CHAI LAY CHING

Date: 22 August 2008

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