**UNIVERSITI PUTRA MALAYSIA**

# MICROBIOLOGICAL RISK ASSESSMENT OF *LISTERIA MONOCYTOGENES* IN MINIMALLY PROCESSED VEGETABLES

**P. JEYALETCHUMI A/P S. PONNIAH**

**FSTM 2011 6**

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**By**

**P. JEYALETCHUMI A/P S. PONNIAH**

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

**May 2011**

**Dedicated to my late mother, Retnam Thambipillai**

**my husband, Kanesan Sathianathan**

**my sons, Thivagar and Gajendra**

**for their endless love and support**

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

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**May 2011**

**Chairman: Professor Son Radu, PhD**

**Faculty: Food Science and Technology**

The purpose of this study was to carry out a microbiological risk assessment on *Listeria. monocytogenes* in vegetables that are consumed in the minimally processed state in Malaysia. The prevalence and number of *Listeria* spp. and *L. monocytogenes* were determined in raw vegetables from pre-harvest and retail level. Environmental samples of soil, water and animal manure were also obtained from vegetable farms. Characterisation of strains isolated from vegetables was carried out by phenotypic (antibiotic resistance) and genotypic (Random Amplification of Polymorphic DNA) methods. A kitchen simulation study was conducted to provide decontamination and cross-contamination data and information for estimation of the risk of acquiring listeriosis from consumption of minimally processed vegetables using a step-wise risk assessment as well as a stochastic approach using simulation software.

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At the retail level, 306 vegetable samples were examined over a one-year period (February 2008 to January 2009). The prevalence of *Listeria* spp. was 33.3% while the prevalence for *L. monocytogenes* was 22.5 %. *L. monocytogenes* was frequently found in yardlong bean (n=32) at 31.3% and Japanese parsley (n=33) at 27.2%. At the farm level, 50 vegetable samples were taken. Both *Listeria spp* and *L. monocytogenes* were detected in 16% and 6 % of the samples respectively. Among the environmental samples (n=94), *Listeria* spp. and *L. monocytogenes* were detected in 47.6% and 38.1 (n=21) of soil samples; 77.8% and 61.1% of manure samples (n=18); 25% and 12.5% of environmental swabs (n=40). It was not however detected in samples of the irrigation water (n=15).

From the kitchen simulation study, it was found that the mean percent transfer rate from vegetables (n=45) to the wash water ranged from 32.4% to 60.2%, from wash water to cucumber was 24.9% to 66.3%; from vegetables to chopping board was 18.9 to 32.2%; from chopping board to cucumber was 5.4 to 75.3%. Washing of the vegetables in tap water caused a 0.3-log reduction of *L. monocytogenes* attached to the vegetables.

Characterization of 71 strains isolated from the 306 samples of vegetables was done by Random Amplification of Polymorphic DNA (RAPD). It was found that the strains could be grouped into 6 clusters and 1 solitary isolate. This shows that the strains that have been isolated demonstrate genetic variability and is of importance to the Microbial Risk Assessment as the different strains would have variations in virulence and pathogenicity. In terms of antimicrobial susceptibility, the Multiple



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Antibiotic Resistance Index (MARI) of the strains ranged from 0.06 to 0.63. Only 14 % of the strains had MARI values higher than 0.2. MARI values less than 0.2 indicate strains from origins where antibiotics are seldom or never used. The importance of the antimicrobial study to the Microbial Risk Assessment would be that some of the strains exhibit multi-resistance to drugs used in the treatment of listeriosis.

The estimation of risk of acquiring listeriosis from consuming minimally processed vegetables was done using a deterministic and stochastic approach. An exponential dose-response model was used to estimate the probability of illness in low risk and high risk group of consumers. The estimated mean risk per serving for salads was $1.42 \times 10^{-5}$ per 100 000 population for the healthy low-risk population. For the high risk group, the risk estimate was $1.23 \times 10^{-2}$ per 100 000 population for AIDS patients, $3.55 \times 10^{-4}$ per 100 000 population for diabetics and $1.09 \times 10^{-4}$ per 100 000 population for the elderly population respectively.

The exposure assessment model was most sensitive to the input distribution describing the serving size. This implies that the serving size was the input parameter that most strongly influenced the risk and would be the primary control option in trying to reduce risk. In conclusion, a risk assessment has been conducted that can lay the foundation for more comprehensive studies as well as alert health authorities to be more vigilant about listeriosis especially among the vulnerable population.



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

**PENILAIAN RISIKO MIKROBIOLOGI KE ATAS *LISTERIA MONOCYTOGENES* PADA SAYUR-SAYURAN YANG DIPROSES SECARA MINIMUM**

Oleh

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**Mei 2011**

**Pengerusi : Profesor Son Radu , PhD**

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Tujuan kajian ini adalah untuk menjalankan penilaian risiko mikrobiologi ke atas *Listeria monocytogenes* pada sayur-sayuran yang dimakan dan diproses secara minimum di Malaysia. Prevalens dan kuantiti *Listeria* spp. dan *L. monocytogenes* ditentukan pada sayur-sayuran mentah dari kebun dan di peringkat peruncitan. Sampel-sampel persekitaran yang terdiri daripada tanah, air dan baja dari haiwan juga diperolehi dari kebun sayur. Pencirian pencilan dari sayur-sayuran dilakukan secara kaedah fenotip (resistan antibiotik) and genotip ('Random Amplification of Polymorphic DNA'). Kajian simulasi di dapur turut dijalankan untuk memberikan data dan maklumat mengenai proses nyahkontaminasi dan kontaminasi silang sebagai anggaran risiko dijangkiti penyakti *listeriosis* akibat dari memakan sayur-sayuran yang diproses secara minima dengan menggunakan "step-wise risk assessment" dan juga pendekatan stokastik menggunakan perisian simulasi.

Di peringkat peruncitan, sebanyak 306 sampel sayur telah dikaji sepanjang tempoh satu tahun (Februari 2008 hingga Januari 2009). Prevalens *Listeria* spp. adalah



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33.3% sementara bagi *L. monocytogenes,* prevalens adalah 22.5%. *L. monocytogenes* kerap dijumpai pada kacang panjang (n=32) dengan prevalens 31.3% dan sayur selom (n=33) pada prevalens 27.2%. Di peringkat kebun, sebanyak 50 sampel telah diambil. Kedua-dua *Listeria* spp. dan *L. monocytogenes* telah dikesan sebanyak 16% dan 6% dari sampel sayur-sayuran mentah. Di antara sampel-sampel persekitaran (n=94), *Listeria* spp. dan *L. monocytogenes* yang telah dikesan sebanyak 47.6% dan 38.1% dari sampel tanah (n=21), 77.8% dan 61.1% dari sampel baja (n=18), 25% dan 12.5% swab persekitaran (n=40). Walau bagaimanapun, tiada yang dapat dikesan di dalam air saliran (n=15).

Dari kajian simulasi dapur, purata peratus kadar perpindahan dari sayur-sayuran (n=45) ke air basuhan adalah diantara julat 32.4% hingga 60.2%,; dari air basuhan ke timun berada diantara julat 24.9% hingga 66.3%; dari sayur-sayuran ke papan pemotong berada diantara julat 18.9% hingga 32.2%; dari papan pemotong ke timun berada dalam julat 5.4% hingga 75.3%. Proses membasuh sayur-sayuran dengan air paip menyebabkan pengurangan sebanyak 0.3-log kandungan *L. monocytogenes* pada sayur-sayuran tersebut.

Pencirian 71 pencilan yang dipencilkan daripada 306 sampel sayur-sayuran telah dijalankan dengan kaedah 'Random Amplification of Polymorphic DNA' (RAPD). Didapati bahawa pencilan dapat dikelaskan kepada 6 kluster dan 1 pencilan tunggal. Ini menunjukkan bahawa pencilan menunjukkan variabiliti genetik dan ciri ini adalah penting kepada penilaian risiko mikrobiologi kerana stren yang berbeza akan menunjukkan perbezaan dari segi virulens dan pathogenisiti. Dari segi 'antimicrobial



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susceptibility', 'Multiple Antimicrobial Susceptibility Index' (MARI) berada diantara julat 0.06 hingga 0.63. Hanya 14% dari stren mempunyai nilai MARI melebihi 0.2. Nilai MARI kurang dari 0.2 menandakan stren berasal dari tempat-tempat di mana antibiotik tidak atau jarang digunakan. Kepentingan kajian antimikrobiologi kepada penilaian risiko mikrobiologi adalah beberapa stren mungkin resistan terhadap ubat antibiotik yang digunakan dalam rawatan penyakit listeriosis.

Anggaran risiko mendapat penyakit 'listeriosis' dari memakan sayur-sayuran yang diproses secara minima dikira dengan menggunakan pendekatan deterministik dan stokastik. Model "exponential dose-response" digunakan untuk menganggarkan kebarangkalian dijangkiti pada kumpulan pengguna berisiko rendah dan kepada pengguna berisiko tinggi. Anggaran purata risiko bagi setiap hidangan ulam adalah $1.42 \times 10^{-5}$ bagi 100 000 populasi penduduk bagi golongan sihat dan berisiko rendah. Bagi golongan yang berisiko tinggi, anggaran risiko adalah $1.23 \times 10^{-2}$ bagi 100 000 penduduk bagi pesakit AIDS, $3.55 \times 10^{-4}$ bagi 100 000 penduduk bagi pesakit diabetes dan $1.09 \times 10^{-4}$ bagi 100 000 penduduk bagi golongan wargatua. Sebagai kesimpulan, sayur-sayuran yang dimakan dan diproses secara minimum didapati mempunyai keupayaan untuk menyebabkan penyakit listeriosis. Oleh itu, usaha pemantauan perlu dilakukan untuk memastikan sayur-sayuran ini tidak membawa mudarat kesihatan. Model 'exposure assessment' paling sensitif terhadap saiz hidangan. Ini menandakan saiz hidangan adalah parameter yang paling mempengaruhi risiko dan harus dikawal untuk mengurangkan risiko. Sebagai kesimpulan, penilaian risiko telah dijalankan yang merupakan asas kepada kajian



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lebih lanjut dan ia juga dapat memberi kesedaran kepada pihak kesihatan mengenai penyakit listeriosis di kalangan penduduk berisiko tinggi.



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I certify that a Thesis Examination Committee has met on 27 May 2011 to conduct the final examination of P.Jeyaletchumi A/P S.Ponniah on her thesis entitled 'Microbiological Risk Assessment of *Listeria monocytogenes* in Minimally Processed Vegetables' in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia (P.U.(A) 106) 15 March 1998. The Comittee recommends that the student be awarded the Doctor of Philosophy.

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## 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 not concurrently, submitted for any other degrees at Universiti Putra Malaysia or other institutions.

_____

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Date: 27 May 2011

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