*As. J. Food Ag-Ind.* **2010**, 3(06), 587-596

OPEN ACCESS

***Asian Journal of Food and Agro-Industry***

**ISSN 1906-3040**

Available online at www.ajofai.info

*Research Article*

# Assessing the risk of acquiring listeriosis from consumption of minimally processed vegetables using a step-wise risk assessment

**P. Jeyaletchumi, [1,*], R. Tunung [1], S.P. Margaret[1], L.C. Chai, [1], R. Son, [1], M.G. Farinazleen, [1], Y.K. Cheah, [2] , M. Nishibuchi, [3], Y. Nakaguchi [3] and K.M. Pradeep [4]**

[1]Center of Excellence for Food Safety Research, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan , Malaysia.

[2]Department of Biomedical Sciences , Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.

[3]Centre for Southeast Asian Studies, Kyoto University, 46 Shimoadachi –cho, Yoshida Sakyo-ku , Kyoto, 606-8501 Japan.

[4]Department of Food Safety Science, Institute of Food Research, Norwich University Park, NR4 7UA, Norwich, United Kingdom.

*Email: jeyakanesan@yahoo.com

## Abstract

The aim of this study was to assess the risk of acquiring listeriosis through the consumption of vegetables that are eaten in the minimally processed state as 'ulam', which is a kind of fresh salad that is popular in Malaysia. A microbial survey on the prevalence and concentration of *L. monocytogenes* in vegetables consumed in this manner was carried out over a one-year period (February 2008 to January 2009). To determine if this posed a risk warranting further attention, a simple risk assessment was initiated. From the study, it was found that the risk estimate of acquiring listeriosis for the healthy population was $2.3 \times 10^{-3}$ per 100,000 population. For susceptible population, the risk estimate was considerably higher, i.e. 2.015 per 100,000 population for AIDS patients, $5.82 \times 10^{-2}$ per 100,000 population for diabetics and $1.74 \times 10^{-2}$ per 100,000 for the elderly population.

**Keywords:** *Listeria monocytogenes*, contamination, hazard identification, exposure assessment, Malaysia

## Introduction

Foodborne illness is amongst the most widespread public health problem, creating social and economic burdens as well as human suffering, making it a concern that needs to be addressed. Food safety risk analysis has emerged as a structured model for improving our food control systems with the objectives of producing safer food, reducing the number of foodborne illnesses and facilitating domestic and international trade in food [1].

Risk assessments have been conducted for a range of bacterial pathogens and food types. Lindqvist and Westoo [2] carried out a risk assessment on *L. monocytogenes* in smoked fish; Robertson *et al.* [3] carried out a risk assessment on the risk of acquiring cryptosporisiosis or giardiosis from consumption of mung bean sprouts, while Cassin *et al.* [4] conducted a risk assessment on *E. coli* 0157 in hamburgers.

The step-wise risk assessment approach was developed for quantitative risk assessment associated with microbial hazards for food products and focuses on the aspects that quantitatively determine the risk to a significant extent and thus prevent important aspects from being overlooked [5]. The approach enables informed decision-making regarding risk management and risk communication and allows for incorporation of new information and research as they become available.

The current risk assessment uses data from a study on the prevalence of *L. monocytogenes* in vegetables that are consumed in the raw or minimally processed state as 'ulam', a kind of fresh salad that is eaten with boiled rice in Malaysia. The vegetables examined in the survey which had been carried out over a one-year period (February 2008 to January 2009) included cabbages, yard-long beans, Japanese parsley, carrot, sweet potato, cucumbers, tomatoes, wild parsley, Indian pennywort and winged bean [6]. Data from a subsequent kitchen simulation study that determined the decontamination rates based on current food handling practices was also used to estimate the microbial load prior to consumption [7]. The exposure assessment was carried out using information obtained from the Food Consumption Statistics of Malaysia, 2003. The available data were used here to provide a quantitative risk assessment of the exposure and risk of acquiring listeriosis following consumption of the vegetables using a step-wise risk assessment. The effect on different populations, particularly those more at risk, was also determined.

## Materials and methods

### *Statement of purpose*

The purpose of the risk assessment was to estimate the probability of contracting listeriosis from

consumption of minimally processed vegetables that are contaminated with the *L. monocytogenes* pathogen in Malaysia. Data generated from the hazard identification, exposure assessment and hazard characterization were used in a spreadsheet model to estimate the risk. At each step, assumptions and the amount of uncertainty surrounding inputs are stated. In this risk assessment, the exponential model will be used wherein it is assumed that all the ingested organisms have the same probability of being individually capable of causing an infection to a specific consumer.

***Hazard identification***

*L. monocytogenes* is a gram-positive, facultatively anaerobic, non-sporulating, rod-shaped bacteria with tumbling motility at 20-25$^0$C. The organism is psychotrophic and grows over a temperature range of 0$^0$C to 45$^0$C, with an optimum around 37$^0$C. It can cause two forms of disease in humans, i.e. a serious invasive disease (listeriosis) and non-invasive gastroenteritis. Foodborne listeriosis is a relatively rare but serious disease with high fatality rates (20-30%) [8]. The disease often affects specific segments of the population with increased susceptibilities, such as pregnant women, the elderly, immunocompromised individuals, unborn or newborn infants, HIV/AIDS patients [9]. Among the risk groups, the worldwide fatality rates for listeriosis are estimated to be as high as 36% [10, 11].

*L. monocytogenes* is widespread in the environment including soil, vegetation, water and sewage. It is also carried asymptomatically in the faeces of 2-6% of the population [12]. Food is the primary route of transmission for human exposure to this pathogen. *L. monocytogenes* is a psychrotrophic microorganism that can grow at refrigeration temperatures. It is frequently present in raw food of both plant and animal origin and can become endemic in food processing environments. It has been isolated from food such as raw and pasteurized milk, cheese, ice-cream, raw vegetables, raw and fermented meat and raw and fermented seafood [12, 13].

In Malaysia, there have been no reported cases of listeriosis although a handful of cases have been reported in neighbouring Singapore [14]. Due to the non-specific clinical manifestations of listeriosis, the long incubation period prior to onset of symptoms as well as diagnostic limitations, many countries face constraints in identifying the disease and this may cause the disease to go undetected in the population.

***Hazard characterization***

There are two types of disease associated with infection by *L. monocytogenes*; invasive and non-invasive. The invasive disease is called listeriosis and normally occurs in people with weakened immune systems. The non-invasive disease is usually called febrile gastroenteritis i.e. gastroenteritis associated with mild 'flu-like' symptoms and can occur in healthy people if large numbers of *L. monocytogenes* cells are consumed.

To cause listeriosis, ingested *L. monocytogenes* cells penetrate the intestinal tissue and become exposed to phagocytic cells of the immune system. A portion of the *L. monocytogenes* cells

survive and multiply within the host phagocytes. They then move throughout the host via blood or the lymphatic system. The populations most at risk from this disease are the elderly, the immunocompromised and the perinatal. Perinatal infections occur primarily as a result of transplacental transmission to the foetus following infection of the mother. The perinatal group includes foetuses or neonates and infection can occur before or after birth. The symptoms experienced by the mother are usually only a mild fever.

The incubation time for this disease varies between 1 and 90 days while the mean is 30 days. Symptoms include 'flu'-like symptoms (e.g. fever, headache), diarrhoea and vomiting. In perinatal cases, clinical outcomes for the foetus or newborn include general septicaemia, intrauterine death, premature birth, still-birth. In non-perinatal cases, symptoms commonly include bacteraemia and meningitis. The disease can have long term effects. Pre-term infants may suffer from excess fluid in the brain and partial paralysis.

*L. monocytogenes* is susceptible to a number of antibiotics, but penicillin and ampicillin optionally with an aminoglycoside (e.g. gentamicin) is considered to be the combination of choice for treatment of listeriosis.

The non-invasive form of listeriosis was recognised during the 1990s. It has an incubation time of 11 hours to 7 days while the median is 18 hours. Symptoms of this disease include diarrhoea, fever, muscle pain, headache and less frequently with abdominal cramps and vomiting. Attack rate has been reported to be upwards of 74%.

With regards to dose response, it is becoming increasingly realised that the only completely safe dose of *L. monocytogenes* is zero, even in healthy people. However the probability of invasive disease following exposure to even moderate levels of cells is very low.

The FAO/WHO risk assessment used a dose response model described by:

$$P_{illness} = 1 - \exp^{-r*N}$$

where r is a variable that defines the dose/response relationship and N is the number of cells consumed. The values of r vary depending on population group (to reflect different susceptibilities) but are around the $10^{-12}$ - $10^{-14}$ level. The model is a single hit model which means that there is a probability of illness associated with each cell consumed. It is therefore total consumption of cells that dictates risk; there is no "infectious dose", and there is no difference to risk if a small number of cells are eaten frequently or many cells eaten at the same time as long as the total eaten is the same. Table 1 shows the estimated values for r for different categories of the population.

**Table 1. Estimation of r-value for different categories of the population.**

| Condition of population | Relative susceptibility | Calculated r-value |
|---|---|---|
| Transplant | 2584 | $1.41 \times 10^{-10}$ |
| Cancer-Blood | 1364 | $7.37 \times 10^{-11}$ |
| AIDS (A) | 865 | $4.65 \times 10^{-11}$ |
| Cancer Gastrointestinal and liver | 211 | $1.13 \times 10^{-11}$ |
| Cancer Bladder and prostrate | 112 | $5.99 \times 10^{-12}$ |
| Cancer Gynaecological | 66 | $3.53 \times 10^{-12}$ |
| Diabetes (Type 2) ( B) | 25 | $1.34 \times 10^{-12}$ |
| Over 65 years old (C) | 7.5 | $4.01 \times 10^{-13}$ |
| Healthy adult (Baseline) | 1 | $5.34 \times 10^{-14}$ |

The FDA/FSIS modelled value of R accounts for variation of virulence in the types of *L. monocytogenes* existent in the population. It is known that certain serotypes of *L. monocytogenes* appear to be associated with human disease, but there is no certainty that any one isolate will be pathogenic to humans just because it belongs to a particular serotype. A recent study has grouped *L. monocytogenes* into three distinct lineages [15], and there did appear to be some differences between the contributions that the lineages made to human disease. However, these lineages are not based on serotyping. The conservative approach is to treat all isolates as potentially capable of causing disease, but modelling of variability will be a more accurate reflection of real life.

***Exposure assessment***

The hazard in the food supply was assessed using prevalence data obtained from the previous study on *L. monocytogenes* in minimally processed vegetables that were sold at the retail level [7]. A total of 306 raw vegetables that were consumed in the minimally processed state were purchased from hypermarkets and wet markets in Selangor, Malaysia. Of the 306 samples, 22.5% were found to harbour *L. monocytogenes.* The mean concentration of *L. monocytogenes* according to the previous chapter was 15.8275 MPN/g of raw salad vegetables. Based on the kitchen simulation study, washing, which is the only treatment that the vegetables undergo, reduces the microbial load by 0.3 log reduction. Therefore, it is estimated that the mean concentration after washing would be 9.4543 MPN/g. Although *L. monocytogenes* displays strain variation in virulence and pathogenicity, an assumption was made in the assessment that all cells were pathogenic.

The changes in the frequency and extent of contamination in the food between retail marketing and the point of consumption were estimated based on the kitchen simulation study carried out

[8]. This enabled an estimation of the contaminant in the food at the point of consumption. Information on the serving size was obtained from the Food Consumption Statistics of Malaysia, 2003.

The Food Consumption Statistics provides comprehensive information on the food consumption pattern for Malaysian adults aged 18-59 years according to geographical zones in Malaysia, stratum, ethnic groups and sex. This information is an important requirement in assessing the exposure of contaminants through the dietary intake by the population. Based on the statistics, it was estimated that the standard single serving size of 'ulam' was 18.5g.

**Results**

Using the available data, the results presented in Table 2 were derived using the following steps in a simple spreadsheet model:

- The prevalence data are as reported in the Exposure Assessment Section. The probability of a positive sample, as a result, is 0.225
- The mean concentration as reported in the Exposure Assessment Section was 15.8275 MPN/g of raw salad vegetables. Based on the results of the kitchen simulation study, the mean concentration of washed vegetables would be 9.4543 MPN/g.
- The Food Consumption Statistics 2003 suggest that the typical serving size for 'ulam' would be 18.5g. From estimates of the concentration per gram and the serving size in grams, the dose per serving would be calculated as:
  (Number/gram) x (gram/serving) = Number/serving
- To translate the dose consumed to risk of illness, a dose-response relationship is required.
  - The exponential dose-response model has been used to characterize the dose-response relationship for *L. monocytogenes* by the FAO/WHO, 2003.
    This model can be expressed as:

$$P_{illness} = 1 - \exp^{-r*N}$$

    - where r is a variable that defines the dose/response relationship and N is the number of cells consumed or mean dose.
    - The values of r vary depending on population group (to reflect different susceptibilities) but are around the $10^{-12}$ -$10^{-14}$ level.
- Based on this model, the probability of illness per serving for the healthy population would be $7.97 \times 10^{-12}$
- The probability of illness per year for the healthy population was obtained by calculating the estimated annual consumption and multiplying this with the probability of illness per serving.
- The population of Malaysia is 27,728,700. Assuming half the population consume

'ulam', then the expected number of cases would be 0.0023 per 100,000 population for the healthy population.

- Similarly, the expected number of cases for the susceptible population has been calculated.

**Table 2. Step-wise risk estimation for *L. monocytogenes* in salad vegetables for healthy and susceptible individuals in the Malaysian population.**

| | Baseline (Healthy) | AIDS | Diabetic (Type2) | Elderly |
|---|---|---|---|---|
| ***Prevalence*** | | | | |
| Number of samples | 306 | 306 | 306 | 306 |
| Number positive | 74 | 74 | 74 | 74 |
| Prevalence | 22.5 | 22.5 | 22.5 | 22.5 |
| Probability of a positive sample | 0.225 | 0.225 | 0.225 | 0.225 |
| ***Concentration*** | | | | |
| Mean concentration/g | 15.8275 | 15.8275 | 15.8275 | 15.8275 |
| ***Consumption pattern*** | | | | |
| No.of serving (per week) | 3 | 3 | 3 | 3 |
| Serving size (g) | 18.6 | 18.6 | 18.6 | 18.6 |
| ***Decontamination*** | | | | |
| Rate of microbial reduction after washing | 0.31 | 0.31 | 0.31 | 0.31 |
| Mean concentration after washing | 9.4543 | 9.4543 | 9.4543 | 9.4543 |
| ***Dose*** | | | | |
| Dose (org/serving) | 149.33 | 149.33 | 149.33 | 149.33 |
| ***Probability of illness per serving*** | | | | |
| Exponential dose parameter | $5.34 \times 10^{-14}$ | $4.65 \times 10^{-11}$ | $1.34 \times 10^{-12}$ | $4.01 \times 10^{-13}$ |
| Probability of illness per serving | $7.97 \times 10^{-12}$ | $6.94 \times 10^{-9}$ | $2.00 \times 10^{-10}$ | $5.98 \times 10^{-11}$ |
| ***Probability of illness per year*** | | | | |
| Number of servings per year | 2901.6 | 2901.6 | 2901.6 | 2901.6 |
| Probability of illness per year | $2.31 \times 10^{-8}$ | $2.015 \times 10^{-5}$ | $5.82 \times 10^{-7}$ | $1.74 \times 10^{-7}$ |
| ***Estimated number of cases per 100,000 population*** | | | | |
| Population | 27 728 700 | 940 | 1 176 000 | 1220062.8 |
| Number consuming (50% of target group) | 13 864 350 | 470 | 588 000 | 610031 |
| Expected number of cases | 0.32026 | 0.00948 | 0.34222 | 0.106 |
| Rate per 100 000 population | 0.0023 | 2.015 | 0.0582 | 0.0174 |

## Discussion

The step-by-step risk assessment for acquiring listeriosis from consumption of vegetables that are eaten in the minimally processed state in Malaysia has utilized data from diverse sources. It was found that the risk estimate of acquiring listeriosis for the healthy population was 2.3 x $10^{-3}$ per 100,000 population based on the prevalence and mean concentration obtained from our study. For susceptible population, the risk estimate was considerably higher, i.e. 2.015 per 100,000 population for AIDS patients, 5.82 x $10^{-2}$ per 100 000 population for diabetics and 1.74 x $10^{-2}$ per 100,000 for the elderly population.

There is little information on the incidence of listeriosis in Malaysia. Generally, listeriosis has been mainly observed in industrialized countries where it afflicts 0.3-0.6 per 100,000 population. However, the findings of the risk assessment indicate that listeriosis may be afflicting the Malaysian population, especially the vulnerable groups, albeit at low numbers. Therefore, improved surveillance of cases with clinical symptoms similar to that of listeriosis would be recommended.

There are a number of limitations in this risk assessment that should be acknowledged to facilitate better understanding of the risk assessment as well as enable its correct interpretation and use. The risk assessment focused on only ten types of vegetables from five categories that are consumed in the minimally processed state, although there are about 120 vegetables that are consumed in a similar manner by the local population.

The risk characterization results are subject to uncertainty associated with a modelled representation of reality involving simplification of the relationship among prevalence, cell number, growth, consumption characteristics and the adverse response to consumption of some number of *L. monocytogenes* cells.

## Conclusion

The risk estimate of acquiring listeriosis for the healthy population was quite low at 2.3 x $10^{-3}$ per 100,000 population based on the prevalence and mean concentration obtained from our study. For susceptible population, the risk estimate was considerably higher, i.e. 2.015 per 100,000 population for AIDS patients, 5.82 x $10^{-2}$ per 100,000 population for diabetics and 1.74 x $10^{-2}$ per 100,000 for the elderly population. Therefore, improved surveillance of cases with clinical symptoms similar to that of listeriosis would be recommended.

**Acknowledgements**

This study was funded by the Food Safety and Quality Division of the Ministry of Health of Malaysia under the Food Contamination Monitoring Programme. The work was also supported by Grant-in-Aid for Scientific Research (KAKENHI 191010) from the Japan Society for the Promotion of Sciences.

**References**

1. Joint FAO/WHO (1997). Consultation Risk Management and Food Safety-FAO Food and Nutrition Paper 65,Report of a Joint FAO/WHO Consultation Rome, Italy, January 27-31, 1997. http://www.fao.org/docrep/W4982E/w4982e00.htm.Accessed October 2,2009

2. Lindqvist, R. and Westoo, A. (2001). Quantitative risk assessment for *Listeria monocytogenes* in smoked or gravad salmon and rainbow trout in Sweden. ***International Journal of Food Microbiology,*** 58:181-196.

3. Robertson, L.J., Greig, J.D., Gjerde, B.and Fazil, A. (2005). The potential for acquiring cryptosporidiosis or giardiosis from consumption of mung bean sprouts in Norway: a preliminary step-wise risk assessment. ***International Journal of Food Microbiology,*** 98:291-300.

4. Cassin, M.H., Lamnerding, A.M., Todd, E.C., Ross, W. and McColl, R.S. (1998). Quantitative risk assessment for *Escheria coli* 0157:H7 in ground beef hamburgers. ***International Journal of Food Microbiology,*** 41(1):21-44.

5. van Gerwen, S.J.C., te Giffe, M.C., van t Riet, K., Beumer, R.R. and Zwietering, M.H. (2000). Stepwise quantitative risk assessment as a tool for characterization of microbiological food safety. ***Journal of Applied Microbiology,*** 88;938-951.

6. Jeyaletchumi Ponniah, Tunung Robin, Margaret Selina Paie, Son Radu, Farinazleen Mohammed Ghazali, Cheah Yoke Kqueen, Mitsuaki Nishibuchi, Yoshitsugu Nakaguchi and Pradeep Kumar Malakar. (2010). Listeria monocytogenes in raw salad vegetables sold at retail level in Malaysia. ***Food Control,*** 21,774-778

.

7. Jeyaletchumi Ponniah, Tunung Robin, Margaret Selina Paie, Son Radu, Farinazleen Mohammed Ghazali, Cheah Yoke Kqueen, Mitsuaki Nishibuchi, Yoshitsugu Nakaguchi and Pradeep Kumar Malakar (2010). Assessment of *Listeria monocytogenes* in Salad Vegetables through a Kitchen Simulation Study**.** ***Journal of Tropical Agriculture and Food Science,*** 64:43-51.

8. Rocourt, J. and Bille, J. (1997). Foodborne listeriosis. ***World Health Statistics Quarterly,*** 50: 67-73.

9. Slutsker, L. and Schuchat, A. (1999). Listeriosis in humans. In E.T. Ryser and E.H. Marth ed. Listeria, listeriosis and food safety New York, NY(2nd Edition):Marcel Dekker, Publishers; 85-109.

10. Seeliger, H.P.R. and Jones, D. (1986). Genus *Listeria pirie* 1940. In Bergey's Manual of systematic bacteriology Vol.2, Ed.PHA Sneath, Mair,NS, Sharpe, ME and Holt,JG Baltimore. The Williams and Wilkins Co. Publishers, 1235-1245.

11. Siegman-Igra, Y., Levin, R., Weinberger, M., Golan, Y., Schwartz, D., Samra, Z., et al. (2002). *Listeria monocytogenes* infection in Israel and review of cases worldwide. ***Emerging Infectious Diseases,*** 2002; 8: 305–310.

12. Farber, J. and Peterkin, P.I. (1991). *Listeria monocytogenes*, a foodborne pathogen, ***Microbiology Reviews,*** 61: 3872-3874.

13. Buchanan, R.E., Stahl, H.G. and Archer, D.L. (1987). Improved plating media for simplified, quantitative detection of *Listeria monocytogenes* in foods. ***Food Microbiology,*** 4:269.

14. Epidemiological News Bulletin (2007) Epidemiological News Bulletin, Singapore. Retrieved on 20 August 2009 from: http://www.env.gov.sg/cop/qed2/enb_news.htm

15. Jeffers, G.T., Bruce, J.L., McDonough, P., Scarlett, J., Boor, K.J. and Wiedmann, M. (2001). Comparative genetic characteristics of L. monocytogenes isolates from human and animal listeriosis cases. ***Microbiology,*** 147:1095-1104.