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Risk assessment of acquiring listeriosis from consumption of chicken offal in Selangor, Malaysia

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Abstract

Listeria monocytogenes (L. monocytogenes) is an important foodborne pathogen which can cause foodborne listeriosis with high mortality rates especially in susceptible population groups such as pregnant women, elderly and immunocompromised individuals. The biosafety level of *L. monocytogenes* in chicken offal has becomes a great concern as chicken offal is a cheap source of protein and it is often served as side dishes in South East Asian countries. In Malaysia, the consumption of chicken offal has almost doubled from 5 g per capita per day in the early 1980s to 9 g per capita per day in 2009. In this study, risk assessment was conducted to estimate the risk of acquiring listeriosis from consumption of chicken offal in Malaysia. A microbial survey on the prevalence and concentration of *L. monocytogenes* in chicken offal were carried out in Selangor, Malaysia over a one-year period (November 2010 to October 2011). It was assumed that there were no seasonal changes in the prevalence and consumption pattern all year round. Assuming that 5.6 million people in Selangor, Malaysia consume a single serving (125 g) of chicken offal per week, it is estimated that in a year there could be 0.61 cases and 1.98×10^{-4} cases of listeriosis per 100,000 population of pregnant woman and immunocompromised individual, respectively. However, the potential for getting listeriosis among the healthy population was very low, only 1.39×10^{-8} cases per 100,000 population. This study demonstrated risk assessment model not only used as a tool to estimate the risk of acquiring illness but it can influence public health surveillance and providing data in setting appropriate level of protection.

Introduction

Foodborne illnesses affect millions of people worldwide which cause social and economic problems such as sufferings of infected patients and economic burdens due to medical costs and productivity loss (Harris, 1997; Wilcock *et al*., 2004; Jeyaletchumi *et al*., 2010). The emergence of food safety issues has resulted in the decline in public trust towards the food safety regulations and management (Houghton *et al*., 2008). Knowledge about the potential health hazards and risks of certain foods is important when establish an appropriate level of protection (ALOP) or in food safety risk analysis. Food safety risk analysis consist of three interrelated components, which are risk assessment, risk management, and risk communication. In the past 10 years, risk analysis emerged as a decision making model to improve food control system (FAO/WHO, 1997; Marvin *et al*., 2009; Jeyaletchumi *et al,* 2010).

Risk assessment is a scientific approach to provide sufficient scientific information regarding risk issues. In the 1950s and 1960s, risk assessment was started applied to chemical contaminants that may found in the environment and food. Later, in the past decade, much effort have been paid for the development and application of risk assessment to identify and estimate the risk of microbiological foodborne diseases in a given population (McLauchlin *et al*., 2004; Yang *et al*., 2006; Pouillot *et al,* 2007; Lenhart, 2008). Microbiological risk assessment has been divided into four main steps which are hazard identification, hazard characterization, exposure assessment, and risk characterization (WHO/FAO, 1995).

With the increasing in incidence of foodborne listeriosis, *Listeria monocytogenes* has become an important foodborne pathogen. *L. monocytogenes*

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infection caused high hospitalization rates (91%) and large outbreaks of human illness worldwide with about 500 death cases reported annually in USA (Mead *et al*., 1999; Sergelidis and Abrahim, 2009). In addition, EFSA and ECDC (2014) reported that foodborne listeriosis was the most frequent cause of death in Europe in the period of 2008-2012. Obviously, this foodborne pathogen poses public health threats and hence, risk management actions should be implemented immediately to reduce the current level of listeriosis. In this study, a risk assessment was developed to identify and determine the risk of acquiring listeriosis from consumption of chicken offal in Malaysia. This approach can be further improved when the new information or better data are become available.

Materials and methods

Statement of purpose

This study was aimed to estimate the probability of getting foodborne listeriosis from eating chicken offal in Malaysia. Data obtained from the hazard identification, exposure assessment and hazard characterization were used to evaluate the potential risk. All the assumptions and uncertainties surrounding inputs are clearly stated. In this risk assessment, exponential dose-response model is used to describe the risk of acquiring listeriosis and it was assumed that all the ingested foodborne pathogens have the same capability and chances to cause a bacterial infection to each individual.

Hazard identification

L. monocytogenes is an opportunistic pathogen and widely spread in the environment. It can cause a severe illness of listeriosis and this foodborne infection was responsible for 3.8% of total foodborne disease hospitalizations and 27.6% deaths among the foodborne disease deaths (Mead *et al.,* 1999). Although the incidence of listeriosis is relatively rare, it contributed for high mortality rate (20-30%) as compared to other microbial pathogens such as Campylobacter spp. and Salmonella spp. (Berche, 2005). This foodborne illness largely affects those from susceptible populations such as pregnant women, unborn or neonates, older persons, adults with weakened immune system, and HIV/ AIDS patients (Slutsker and Schuchat, 1999; Jeyaletchumi *et al*., 2010). FAO /WHO (2004) found that the elderly (60 years old and above) and perinatals were 2.6 times and 14 times more susceptible than the healthy population, respectively. Besides, due to the psychrotrophic characteristic, *L. monocytogenes* can remain alive and multiply to significant number in the cold conditions when given sufficient time (Lin *et al.*, 2004). Listeriosis cases often reported in industrialized countries. However, the differences in incidence rates between developed and developing countries are not clear (FAO/WHO, 2004). Although this incidence in Malaysia is relatively low and rarely reported, it should not be considered as low risk to public health because *L. monocytogenes* had been isolated from various types of food such as poultry, seafood, vegetables, ready-to-eat foods, beef offal and chicken offal in Malaysia over the past 20 years (Arumugaswamy *et al*. 1994; Endang *et al.* 2003; Jeyaletchumi *et al*., 2010b; Marian *et al.*, 2012, Kuan *et al.*, 2013a, Kuan *et al.*, 2013b).

Hazard characterization

The hazard characterization describes the characteristic of microbial pathogen and infectious host that contribute to the *Listeria* infection (FAO/ WHO, 2004). There are two forms of illness linked with infection by *L. monocytogenes,* such as serious invasive listeriosis and non-invasive gastroenteritis. Invasive listeriosis usually happens amongst the immunosuppressed individual with high fatality rate (Mead *et al*., 1999). The non-invasive listerial gastroenteritis is normally observed during a number of outbreaks and it was associated with mild 'flu-like' symptoms, diarrheal, fever and headache (Dalton, 1997, Aureli *et al*., 2000). These incidences generally involve the ingestion of large numbers of *L. monocytogenes* cells by healthy individuals. The occurrence rate and the elements that contributed to the onset of this non-invasive form are not known. Hence, this risk assessment only looked on invasive listeriosis as the outcome of exposure.

Dose-response relations model have been developed to describe the interaction between dose, infectivity and the probability of getting adverse health effects associated with the hazard in an exposed population (Walls, 2006). A major assumption for this modelling is that every single microbial cell has an equal probability of causing infection. Dose-response relationship cannot developed from human volunteer studies with *L. monocytogenes* because it is not ethical as listeriosis is a severe illness and it may not practical and meaningful if conducted only in healthy individuals which are not from high risk groups (Walls, 2006). Due to this reason, dose-response relationship model that developed for *L. monocytogenes* invasive infection was based on data in animal studies, expert citations, human illness, outbreak and surveillance data (FAO/WHO, 2004; Walls, 2006).

Table 1. Summary of dose-response model for *L. monocytogenes* reported in several studies

Population	Model	Parameters	Description	Reference
General population	Exponential model	r-value = 8.5×10^{-16}	This model is interpreted that major portion of the population is not susceptible and incorporating the distribution for strain virulence.	FAO/WHO (2004)
Immunocompromised	Exponential model	r-value = 1.2×10^{-10}	Model developed based on an estimate of immunocompromised individuals. This model is conservative and assumed that all listeriosis cases were caused by a single type of food.	Buchanan *et al.* (1997)
Immunocompromised	Exponential model	r-value = 5.6×10^{-10}	Model developed based on severe listeriosis and the annual foodborne disease statistics and food survey.	Lindqvist and Westwoo (2000)
Pregnant women	Exponential model	r-value = 3.7×10^{-7}	Model developed based on an outbreak study in Mexican-style cheese in pregnant women	FDA/FSIS(2003)
Elderly, above 60 years of age	Exponential model; multiple mathematical models	r-value = 8.4×10^{-15}	Model developed based on mice lethality and human fatality statistics. This model includes population over 60 years of age and incorporating the distributions for strain virulence.	FDA/FSIS(2003)

(Source: FAO/WHO, 2004)

The FAO/WHO (2004) proposed a dose response model for risk assessment study which described by (Haas, 1983; Vose, 1998):

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

where r is a variable that describe the dose/response relationship and N is the amount of microbes ingested. In this model, it was assumed that all of the ingested microbial pathogens have the equal chance (variable r) to cause an infection to a specific population group. Variable "r" is depend on exposed population groups which reflect to different susceptibilities among high risk group populations, and usually are around the 10^{12}-10^{14} level (Jeyaletchumi et al., 2010). This model is known as single hit model where the likelihood of getting foodborne illness is closely related with the amount of microbes ingested. Therefore, the probability of illness is almost the same whether there is small amount of cells are eating regularly or large amount of cells are eating at once. Table 1 shows the r-values for risk characterization in different population groups.

On the other hand, there is lack of firm evidences to prove that severity of foodborne listeriosis is caused by certain strains. For this reason, all *L. monocytogenes* strains were assumed to be pathogenic and have the same potential in causing listeriosis infection (McLauchlin *et al.*, 2004).

Exposure assessment

When characterizing exposure in microbiological risk assessment, data are needed on the frequency of contamination (prevalence), amount of microbes (concentration) found in a specific food and amount of food that is ingested (Walls, 2006). The prevalence and concentration data used in this study were referred from a previous study on *L. monocytogenes* in chicken offal that were sold at retail level in Selangor, Malaysia (Kuan *et al.*, 2013a). A total of 216 samples of raw chicken offal were randomly purchased from four wet markets and three hypermarkets over a one-year period (November 2010 to October 2011). It was assumed that there were no seasonal changes in the prevalence and consumption pattern all year round. The prevalence of *L. monocytogenes* in 216 samples of chicken offal was 26.39% and the mean concentration were 2.02 MPN/g for raw chicken offal. Besides, it was assumed that all types of chicken offal (e.g., chicken liver, chicken heart and chicken gizzard) were share equal mean concentration of *L. monocytogenes*. However, this mean concentration data was based on raw chicken offal. Due to Malaysians do not consume raw chicken offal, hence, worst-case scenario was carried out in this study and assumed all the *L. monocytogenes* cells (2.02 MPN/g) survived under cooking process.

Based on the food consumption statistics from FAO (FAOSTAT), the consumption of chicken offal among Malaysians has increased from 5 g per capita per day in the early 1980s to 9 g per capita per day in 2009 (FAO, 2013). Based on this statistical data, it was assumed that half of Selangor population (approximately 2.8 million people, without taking into consideration of age) are consuming a single serving (125 g) of chicken offal per week.

In order to evaluate the risk for different population groups, information on the Selangor population was referred from the Department of Stattistics Malaysia's database. Based on the latest available census and estimates, Selangor population

Table 2. Population of specific groups in Selangor, Malaysia based on references and assumptions

Demography	Year	Number of people	Reference
General population	2011	5,600,000	Department of Statistics Malaysia (2015a)
Age 60 and above	2010	327,703	Department of Statistics Malaysia (2015b)
Pregnant women	2011	[1]103,600	Department of Statistics Malaysia (2015a)
Immunocompromised	NA	[2]280,000	Laura (2008)

[1]Total numbers of pregnant women was assumed equal with the number of birth in a year at 2011 in Selangor, Malaysia.
[2]It was assumed that there was 5% of immunocompromised population in Selangor, Malaysia. This assumption was made based on US statistical data of 3.6% reported by Laura, (2008).

consists of 5.6 million people in 2011 (Department of Statistics Malaysia, 2015a). Meanwhile, the total population of pregnant women was estimated from the number of birth in a year in 2011. There were 103,600 live births in Malaysia in 2011 (Department of Statistics Malaysia, 2015a) and it was assumed that each woman gave birth to a baby at least once. Hence, it was assumed that there were a total of 103,600 pregnant women. However, there is no data available for immunocompromised population in Malaysia. Therefore, estimation on the total population for the immunocompromised group was based on assumption again. Laura (2008) reported that total immunocompromised population in United States is 3.6% of the total population but it most probably an underestimation due to the large portion of the population that consumes immunosuppressive drugs for other disorders such as rheumatoid arthritis and inflammatory bowel disease. The immunocompromised population in Selangor was estimated at 5% of the total population (approximately 0.28 million people) as Malaysia is a developing country and may not have good health care system and advance medical facilities compared to United States. Table 2 summarizes the population for specific groups in Malaysia.

Results

Available data from the exposure assessment were fed into the exponential dose-response model to calculate the risk of acquiring listeriosis. Results presented in Table 2 were derived by using the following steps in a simple spreadsheet model.

As reported in the Exposure Assessment section, the probability of a positive sample (prevalence) is 0.264 and the mean concentration was 2.02 MPN/g of raw chicken offal. There is no data available on the consumption of chicken offal in Malaysia. It was estimated from the FAO statistical database (FAOSTAT) that there are half of Selangor population (approximately 2.8 million) are consuming a single serving (125 g) of chicken offal per week (FAO, 2013).

In order to calculate the dose per serving, following formula is used:

Dose per serving = (Number/gram) x (gram/serving)

With the aid of dose-response relationship model, dose ingested to a risk of illness can be translated by using the formula:

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

Variable r from this exponential dose-response model describes the dose/ response relationship whereas variable N is the mean dose or amount of microbes consumed (Teunis *et al*., 1996; FAO/WHO 2004; Robertson *et al*., 2005). The value of r for different population groups was referred to Table 1.

On the other hand, in order to determine the overall probability of listeriosis infection, the probability of infection from a contaminated serving needs to be combined with the probability of actual consuming a contaminated serving (prevalence data). In this risk assessment, it was assumed that all *L. monocytogenes* infections will be converted into symptomatic foodborne illness. Besides, it was also assumed that there were no seasonality and consumption pattern changes throughout the year. Hence, the exposure risk was equivalent over 52 weeks. The probability of illness per year was calculated as:

$$\text{Probability of illness per year} = 1\text{- } (1\text{- } P_{inf}\text{ per individual})^{52}$$

The expected number of listeriosis cases per year was calculated by multiplying the exposed population with the probability of illness per year (Robertson *et al*., 2005).

Discussion

In this study, data were obtained from multiple sources, either by previous studies or assumptions were made in order to predict the risk of getting listeriosis from consumption of chicken offal in Selangor, Malaysia. It was found that the estimated risk of getting listeriosis illness for the general population was very low, only 1.39×10^{-8} cases per 100,000 population. However, susceptible groups such as

Table 3. Risk assessment of *L. monocytogenes* in chicken offal for different population groups in Selangor, Malaysia

Health conditions	Baseline (General population)	Immuno-compromised	Pregnant women	Elderly (Above 60 years of age)
Prevalence				
Number of samples	216	216	216	216
Prevalence (%)	26.4	26.4	26.4	26.4
Probability of a positive sample	0.264	0.264	0.264	0.264
Concentration				
Mean concentration (MPN/g)	2.02	2.02	2.02	2.02
Dose				
Serving size (g)	125	125	125	125
Dose (org/serving)	252.03	252.03	252.03	252.03
Probability of infection per serving				
Exponential dose parameter (r-value)	8.5×10^{-16}	1.2×10^{-10}	3.7×10^{-7}	8.4×10^{-15}
Probability of infection per positive serving	2.00×10^{-15}	2.88×10^{-10}	8.88×10^{-7}	2.02×10^{-14}
Probability of infection per serving	5.28×10^{-16}	7.60×10^{-11}	2.34×10^{-7}	5.33×10^{-15}
Probability of infection per year				
Number of servings per year	52	52	52	52
Probability of infection per year	2.77×10^{-13}	3.95×10^{-9}	1.22×10^{-5}	2.77×10^{-12}
Estimated number of listeriosis cases per 100,000 population				
Population	5,600,000	280,000	103,600	327,703
Number consuming (50% of target group)	2,800,000	140,000	51,800	163,852
Expected number of cases	7.76×10^{-7}	5.53×10^{-4}	0.63	4.54×10^{-7}
Rate per 100 000 population	1.39×10^{-8}	1.98×10^{-4}	0.61	1.39×10^{-7}

elderly, pregnant women and immunocompromised individuals are more likely to be infected, e.g., 0.61 cases per 100,000 pregnant women, 1.98×10^{-4} cases per 100,000 immunocompromised individuals and 1.39×10^{-7} cases per 100,000 older people who over 60 years old.

The estimated number of listeriosis cases per year calculated from this study was relatively low as compared to incidences reported in few previous studies, e.g., 1.7 to 2.4 cases per million between 1995 and 1999 in England and Wales; 5.4 and 9.4 cases per million in France and the United States (Mead *et al*., 1999; Goulet *et al*., 2001; Smerdon *et al*., 2001; McLauchlin *et al*., 2004). The low number of estimated cases might due to purposefully conservative dose-response model was used in this study to determine the potential risk of acquiring listeriosis. For example, the exponential dose parameter (r-value) reported by Marchetti (1996) in healthy individuals was 5.34×10^{-14} whereas r-value used in this study was 8.5×10^{-16}. In addition, the low mean concentration (2.02 MPN/g) of *L. monocytogenes* in chicken offal also contributed to the low number of estimated cases.

Due to people rarely consume raw chicken offal, the estimated risk of acquiring listeriosis from consumption of chicken offal was based on worst-case scenario. It was assumed that all the *L. monocytogenes* cells (2.02 MPN/g) present in chicken offal will survive under cooking process and the infections will convert into foodborne listeriosis. Although the estimated incidences are low, the potential risks of acquiring listeriosis from consumption of chicken offal can increase sharply if the butchers do not follow appropriate slaughtering procedures and/ or the consumers do not follow good handling practices. Temperature abuse during storage or inadequate cooking of chicken offal may allow *L. monocytogenes* to survive for a long periods of time and proliferate to significant number (Lin *et al*., 2004). Thus, high risk groups should pay special attention when handling or cooking chicken offal because raw meats are well known as reservoirs for foodborne pathogens (Mead, 2007).

To date, there is lack of information on the occurrence of listeriosis outbreak in Malaysia. This could be due to the exclusion of the *L. monocytogenes* in the list of the reported pathogens because it was rarely detected in food as compared to other foodborne pathogens such as *Escherichia coli, Vibrio* spp.and *Salmonella* spp. (Kuan *et al*., 2013a). Besides, due to the mild symptoms of *Listeria* infections such as mild flu, fever and diarrheal which are not the feature of all listeriosis illness, therefore, subclinical listeriosis is more likely to be greatly underdiagnosed (McLauchlin *et al*., 2004). On the other hand, some cases of illness are diagnosed only at necropsy and did not performed on all the patients while early pregnancy losses are also rarely microbiologically investigated. In addition, blood cultures and culture media for the identification of *Listeria* isolated from the faeces are not routinely used for investigation of *Listeria* infection (McLauchilin *et al*., 2004).

There were a number of assumptions made in this study due to the lack of information. To refine the risk assessment and provide a better understanding of risk estimates, there is a necessity to collect more data and incorporate into probability distribution which can capture the uncertainties and reduce the number of assumptions (Robertson *et al.*, 2005). The more refined the data and the less of assumptions are made, the more accurate of risk estimates. Assumptions such as the infection to illness ratio, the total number of people from specific population in consuming chicken offal and the consumption pattern (serving size and the number of serving per year) can affect the final risk estimates.

A complete quantification and characterization of the uncertainties was not essential in this study due to the apparent lack of some basic information. Although this risk assessment only provides a crude estimate of risk of acquiring listeriosis, it serves as a template and a useful tool to demonstrate the potential risks from consumption of chicken offal and subsequently influence public health surveillance. As further data are available and data gaps are filled, this risk assessment model can be expanded and provide a more realistic risk estimate.

Conclusion

The estimated risk for a healthy individual to acquire listeriosis is very low, only 1.39×10^{-8} cases per 100,000 population. However, pregnant women are more likely to be infected, with estimated 0.61 cases per 100,000 population. From this result, it was suggested high risk groups especially pregnant women should avoid consume any potential high risk foods or follow safe food handling practices prior to consumption because listeriosis infections can lead to abortion or stillbirth. To minimize the threat posed by *L. monocytogenes*, appropriate preventive measures should apply in daily life such as cook the high risk foods especially meat products adequately before consumption. Although there were a number of uncertainties and variabilities in this study, the crude estimate of risk would be useful and provide important information to attract some degree of attentions from public health authorities for a better understanding of the potential risks from consumption of chicken offal and subsequently refine the analysis, implement control measures and improve surveillance system to reduce the potential hazards. Though the samples were taken from Selangor in this study, these results may further improve as a whole if the sampling is continue at other states in Malaysia.

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