Food Research 1 (3) : 67 - 76 (March 2017)
Journal homepage: *http://www.myfoodresearch.com*

FOOD RESEARCH

FULL PAPER

Risk of *Escherichia coli* O157:H7 infection linked to the consumption of beef

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Article history:
Received: 13 March 2017
Received in revised form: 25 March 2017
Accepted: 27 March 2017
Available Online: 12 May 2017

Keywords:
E. coli O157:H7
Beef
Quantitative microbial risk assessment

DOI:
http://doi.org/10.26656/fr.2017.3.011

Abstract

Escherichia coli O157:H7 is a major food-borne pathogen that has resulted in numerous outbreaks around the world. Widespread distribution of the organism in various ecological niches impedes the control measures. This study aimed to detect and quantify *E. coli* O157:H7 in beef sold in wet markets and hypermarkets in Malaysia and to determine the risk of *E. coli* O157:H7 infection linked to consumption of beef. The *rfb*O157 and *flic*H7 primers targeted on somatic antigen (O157) and flagellar antigen (H7) respectively of *E. coli* O157:H7 was used for the MPN-PCR method. A total of 99 beef samples were collected from local wet markets and hypermarkets. The highest *E. coli* O157:H7 contamination rate was observed in beef samples collected from wet markets (89.50%), whereas the contamination rate in hyper market A and B were compratively low (35.35 and 20% respectively). However, the microbial load was highest in the beef samples from hypermarket A (1100 MPN/g) while *E. coli* O157:H7 bacterial load in beef samples from hypermarket B and wet market ranged from 3 to 93 MPN/g and 3 to 240 MPN/g, respectively. Using the Quantitative Microbial Risk Assessment (QMRA) approach the risk was estimated incorporating the findings of the prevalence study and predictions based on home storage, cooking and consumption patterns. Three different exposure pathways were investigated to estimate the risk associated with contaminated beef and Monte Carlo simulation was used to determine the level of uncertainty. The developed model predicated that consumption of contaminated beef can be accountable for 1.83E+06 *E. coli* O157:H7 cases per year in Malaysia. The reliability of the model, data gaps and further research needs, is discussed. Through continuous improvement Quantitative Microbial Risk Assessment provides valuable insight into controlling and prevention strategies.

1. Introduction

According to the surveillance data of Malaysian Health Ministry, 50.33 cases per 100,000 people food and water-borne diseases were reported in Malaysia in year 2016 (MOH, 2016). Among the food and water-borne diseases, incidence of food poisoning cases were most prevalent with accounting for 47.34 cases per 100,000 populations in 2016 (MOH, 2016). However, the true burden of food-borne diseases in Malaysia need to be investigated (Lim, 2002). Under-diagnosis and under-reporting of food-borne disease in developing countries mainly resultant due to inadequate investigation and surveillance systems (Beuchat, 1998).

The majority of *E. coli* strains naturally inhabit the intestinal tract of the warm-blooded animals including humans, and most strains are non-pathogenic (Bell and Kyriakides, 1998). Every year more than *E.coli* O157:H7 estimated to cause around 73,000 illness and 250 deaths in United States (Newell *et al.*, 2010). *E. coli* O157:H7 has emerged as a key foodborne pathogen that leads to foodborne disease outbreaks and sporadic cases in people around the globe (Griff

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eISSN: 2550-2166

and Boyce, 1998; Woodward *et al.*, 2002). *E. coli* O157.H7 is a highly virulent strain that is categorised under the enterohemorrhagic *E. coli* (EHEC) which also referred to as verocytotoxin producing *E. coli* (VTEC) or Shiga-like toxin producing *E. coli* (STEC) (Meng *et al.*, 2007). The first public health significance of *E. coli* O157.H7 traced back to 1982, after reporting two unfamiliar enteric outbreaks connected to contaminate undercooked hamburger patties in U.S. (Riley *et al.*, 1983).

Pathogenic *E. coli* O157:H7 is frequently found in various environment including, food (Hancock *et al.*, 1994; Cagney *et al.*, 2004), livestock (Elder *et al.*, 2000; Johnsen *et al.*, 2001), soil (Ibekwe *et al.*, 2014) and manure (Franz *et al.*, 2007). Cattle act as the main reservoir of *E. coli* O157:H7 (Bach *et al.*, 2002) while intermittently and seasonally shed the organism in faeces (Chapman, 2000). The majority of the human *E. coli* O157:H7 cases were due to food and water contaminated with cattle faecal matter (Gyles, 2007). Recent *E. coli* O157:H7 outbreaks were linked to undercooked ground beef (King *et al.*, 2014; Torso *et al.*, 2015), beef products (CDC, 2016), ground bison (Cronquist, 2014), pork (Cheng, 2015; Honish, 2017), raw milk (Logsdon *et al.*, 2015), bagged salad (Marder *et al.*, 2014), romaine lettuce (Slayton *et al.*, 2013), spinach (Sharapov *et al.*, 2016) and strawberies (Laidler *et al.*, 2013).

People from any age group can be affected by *E. coli* O157:H7 infection; while old people and young children can be more susceptible (Codex Alimentarius Committee, 2002). The initial clinical symptoms of *E. coli* O157:H7 infection includes abdominal cramps, non-bloody diarrhoea and more than 70% of the cases subsequently lead to bloody diarrhoea (Bell *et al.*, 1994). While some cases result in hemorrhagic colitis (grossly bloody diarrhoea) and hemorrhagic uremic syndrome (HUS) a systemic condition associated with acute or chronic kidney failure, thrombotic thrombocytopenic purpura (TTP) and neurological sequelae (Nataro and Kaper, 1998). The ultimate consequence of above complications can be an end-stage renal disease (ESRD) which is a severe chronic form that leads to death (Nataro and Kaper, 1998).

Low infective dose as 10-100 cells (Griffin and Tauxe, 1991; Nataro and Kaper, 1998; Harris *et al.*, 2003), stress resistance mechanisms (Price *et al.*, 2004) and production of toxins (Meng *et al.*, 2007) contribute to the severity of the *E. coli* O157:H7 infection. Further, efficient acid resistant methods facilitate survival and colonisation of the organism under the low acidic conditions in the gastrointestinal tract (Price *et al.*, 2004) and food with low pH value (Weagant *et al.*, 1994; Price *et al.*, 2004). Therefore, *E. coli* O157:H7 is considered as a serious threat to consumers (Kaper *et al.*, 2004; Meng *et al.*, 2007).

Undercooking, cross contamination, improper storage and handling can lead to contamination of food commodities with pathogenic organisms (Panisello *et al.*, 2000; Elexson *et al.*, 2017; New *et al*., 2017). Therefore, farm to fork investigation facilitates implementation of effective and efficient control measures. The quantitative microbial risk assessment (QMRA) is a scientific and systematic approach that can be used to quantify the risk associated with hazards at the various steps in the production process (Covello and Merkhofer, 1993). Previously, QMRA has been developed for Salmonella enterica serovar Enteriditis in pasteurised liquid eggs (Whiting *et al.*, 1997), *E. coli* O157: H7 in ground beef hamburgers (Cassin *et al.*, 1998) and Campylobacter in chicken meals (Pouillot *et al.*, 2012).

To date, no such model has been developed to estimate the human health risks associated with *E. coli* O157: H7 within Malaysia. Hence, this study investigated the prevalence of *E. coli* O157:H7 in raw beef samples and the generated quantitative data was used to develop a QMRA for *E. coli* O157:H7. This paper reports the first risk assessment model of *E. coli* O157: H7 linked to the consumption of beef in Malaysia.

2. Materials and methods

2.1 Sample collection

A total of 99 samples of imported beef (n= 27) from the supermarket and local beef from the supermarket (n=34) and night market (n=38) were purchased randomly from a supermarkets and night market in Selangor, Malaysia. Samples were collected into sterile plastic bags and analysed immediately upon arrival at the laboratory.

2.2 Most probable number (MPN) method

In a sterile stomacher bag, 10 g of sample was added with 90 ml of Tryptic Soy Broth (TSB; Bacto, France) and homogenised for 60 s. The mixture was incubated at 37°C for 24 h. To perform the MPN, 100-fold and 1000-fold dilutions of the stomacher fluids were prepared. One ml aliquot from each dilution was removed into triplicate MPN tubes; next tubes were incubated at 37°C for 24 h. The positive MPN tubes were then subjected to PCR for the detection of *rfb*O157 and *flic*H7 genes specific for *E. coli* O157:H7.

eISSN: 2550-2166

2.3 DNA extraction and PCR amplification

MPN tubes showing visible turbidity were used for DNA extraction using a modified boil cell method. Briefly, 1 mL of aliquot from each MPN tube was centrifuged at 12,000 x *g* for 3 min. The pellet was resuspended in 500 µL of TE buffer and after mixing was boiled for 10 min. Boiled mixture was cooled at -20°C for 10 min and centrifuged at 12,000 x *g* for 5 min. Final supernatant was used as a template for PCR amplification. *rfb*O157 and *flic*H7 genes were detected by multiplex PCR. The sequence of the two primer pairs was shown in Table 1. The *rfb*O157 primer is specific for somatic antigen (O157) and *flic*H7 primer is specific for the flagellar antigen (H7).

PCR amplification was performed in a 25 µL reaction mixture consisting of 5 µl of 5 x PCR buffer, 0.5 µl of deoxynucleoside triphosphate (10 mM), 2 µl of $MgCl_2$ (25 mM), 0.2 µl of Taq polymerase (5U/µL), 2 µl of DNA template and 1.25 µl of each primer (0.5 µM). The thermal cycling started with pre-denaturation at 94°C for 2 min, followed by 35 cycles of 1 min denaturation at 94°C, 1 min primer annealing at 55°C, 1 min elongation at 72°C, and the final extension at 72°C for 10 min. Amplified products were electrophoresed on 1.0% agarose gel at 100 V for 30 min. PCR products were visualised under UV light after staining with ethidium bromide. The expected sizes of amplicons for *rfb*O157 and *flic*H7 genes were 259 bp and 625 bp respectively.

2.4 Risk assessment

2.4.1 Statement of purpose

The objective of this primary risk assessment is to estimate the probability of infection due to consumption of *E. coli* O157:H7 contaminated beef. Data generated from the hazard identification, exposure assessment and hazard characterization were used to estimate the risk.

A conceptual model was developed conduct the risk assessment and the @risk Version 7.5 software package (Palisade Corporation, USA) together with Microsoft Excel was applied to run the simulations. The Monte Carlo simulation was adopted to estimate probabilities by performing a total of 100,000 iterations for simulation. The output of this risk assessment was the probability of human *E. coli* infection associated with consumption of beef. All the variables and probability distributions used for this quantitative risk assessment were presented in Table 2.

2.4.2 Exposure assessment

The risk assessment of *E. coli* O157:H7 was conducted separately to assess the risk associated with consumption of beef purchased from hypermarket A, B and wet market.

Table 1. Primer sequences for the detection of *Escherichia coli* O157:H7 using a multiplex PCR

Primers	Primer sequence (5' to 3')	Target gene	Amplicon size	Reference
*flic*H7-F	GCG CTG TCG AGT TCT ATC GAG	*flic*H7	625 bp	Sarimehmetoglu *et al.*, 2009
*flic*H7-R	CAA CGG TGA CTT TAT CGC CAT TCC			
*rfb*O157-F	CGG ACA TCC ATG TGA TAT GG	*rfb*O157	259 bp	Jamshidi *et al.*, 2008
*rfb*O157R	TTG CCT ATG TAC AGC TAA TCC			

Table 2. Description of parameters and distributions in the model for exposure assessment of *E. coli* O157:H7

Variable	Description	Distribution
P_{prev}	Prevalence of *E. coli* O157:H7 at retail	Beta (s+1, n-s+1)
C_{conc}	Concentration of *E. coli* O157:H7 at retail	Pert (min, med, max)
L_{cont}	Level of *E. coli* O157:H7 contamination at retail	Discrete (P_{prev}, C_{conc})
H_{temp}	Holding temperature	Triangle (min, med, max)
H_{time}	Holding time duration	Triangle (min, med, max)
N_{max}	Maximum density population	MAX(Normal((L_{cont} + k X H_{time}),SQRT((L_{cont} + k X H_{time}))))
T_{cook}	Cooking time	Triangle (min, med, max)
A_{serv}	Serving amount	Normal (µ , σ)

s = number of positive samples, n = total number of sample
µ = mean value in $\log_{10}$, σ = standard deviation value in $\log_{10}$
min = minimum, med = median, max = maximum

2.4.2.1 Retail beef

The initial prevalence of *E. coli* O157:H7 contamination (P_{prev}) in beef was characterised as a Beta (s+1, n-s+1) distribution in @risk. The concentrations of *E. coli* O157:H7 (C_{conc}) in beef was estimated using a PERT distribution where included the minimal, the median and the maximal value of *E. coli* O157:H7 concentration in log MPN/g data generated from this study. The level of *E. coli* O157:H7 contamination (L_{cont}) at retail was estimated as a discrete distribution (Table 2).

2.4.2.2 Holding time of beef at home

Bacterial growth can occur during the time period between retail to consumption. Estimated data on home storage duration (H_{time}) and temperature (H_{temp}) of beef at Malaysian household level were fitted by using triangular distribution. The growth rate of *E. coli* O157:H7 was calculated using the below equation.

$$k = \alpha (T-T_{min})^{1.5}$$

In which k is the growth rate of bacteria (log MPN/g) as a function of T is the holding temperature, T_{min} is the minimum temperature and α is a constant. The maximum population density with storage (N_{max}) was modelled as a normal distribution using the square root value of the level of *E. coli* O157:H7 contamination (L_{cont}) at retail, bacterial growth rate (k) and on home storage duration (H_{time}).

2.4.2.3 Cooking

The cooking duration of beef by Malaysian was based on expert opinion and used as input data to estimate cooking time (T_{cook}) using triangular distribution. The reduction of *E. coli* O157:H7 in beef during cooking was based upon the following equation.

$$C_{cook} = (-T_{cook} / D) + (N_{max})$$

Where C_{cook} corresponds to bacteria present after cooking and D represents the D-value 0.2 minutes at 70°C.

2.4.2.4 Serving

The concentration of *E. coli* O157:H7 per serving was estimated by multiplying the serving amount (A_{serv}) and concentration of the bacteria present after cooking C_{cook}. The serving amount (A_{serv}) was estimated by incorporating into a normal distribution.

2.4.2.5 Direct cross contamination to hand

To estimate the cross contamination (C_{hand}) of *E. coli* O157:H7 , multiplied the transfer rate (Rtran) either by level of *E. coli* O157:H7 contamination (L_{cont}) at retail in exposure pathway 01 or maximum population density with storage (N_{max}) in exposure pathway 02. Transfer rate (R_{tran}) was adopted from previous studies.

2.4.3 Hazard characterization

An exponential model was used to calculate the probability of infection (P_{inf}) following exposure to *E. coli* O157:H7.

$$P_{inf} = 1- \exp (- rN)$$

In which, P_{inf} corresponds probability of infection, N is the number of pathogens per exposure, and r is an exponential dose−response relationship. Acute gastroenteritis, fever, abdominal cramps are some of the common symptoms associated with *E. coli* O157:H7 infection (Bell *et al.*, 1994).

2.4.4 Risk characterization

The final result of a risk assessment model is the risk characterization, in which combines exposure assessment and hazard characterization to estimate the probability of an adverse effect in a known population resultant due to the hazard (Schlundt *et al.*, 2004). The probability of acquiring *E. coli* O157:H7 infection (N_{inf}) by Malaysians was estimated by the equation given below.

$$N_{inf} = P_{inf} X P_{cons}$$

Considered that Malaysian population was 30,000,000 (Department of Statistics Malaysia, 2013) and consumer data (P_{cons}) from Malaysian health ministry was used to calculate the exposed population (Ministry of Health, 2003).

3. Results

3.1 Prevalence

The prevalence level of *E. coli* O157:H7 in beef samples collected from various retail dwellings including hypermarket and wet market were given in Table 3. Overall, *E. coli* O157:H7 was isolated 54 (54.54%, CI: 49.54, 59.54) out of 99 beef samples with a concentration ranging from 3- 1100 MPN/g. Among the markets, highest *E. coli* O157:H7

eISSN: 2550-2166

prevalence of 89.5% (34 out of 38) was detected in beef samples from the wet market. While *E. coli* O157:H7 was positive in 35.3% and 20% beef samples from hypermarket A and B respectively.

Table 3. Prevalence of *E. coli* O157:H7 in beef

Place	Number of samples	Prevalence rate (%)	95% CI[a]	MPN range (MPN/g)
Hypermarket A	51	35.30	28.61, 41.99	3-1100
Hypermarket B	10	20.0	7.35, 32.65	3-93
Wet market	38	89.50	84.52, 94.48	3-240
Total	99	54. 54	49.54, 59.54	

All 27 samples of imported beef were obtained from hypermarkets while 72 samples of local beef were collected from both wet market (n=38) and hypermarkets (n=34). In the present study, 18.5% imported beef harboured *E. coli* O157:H7 and prevalence in local beef samples from wet market and hypermarkets were positive at 89.5% and 44.1%, respectively (Figure 1).

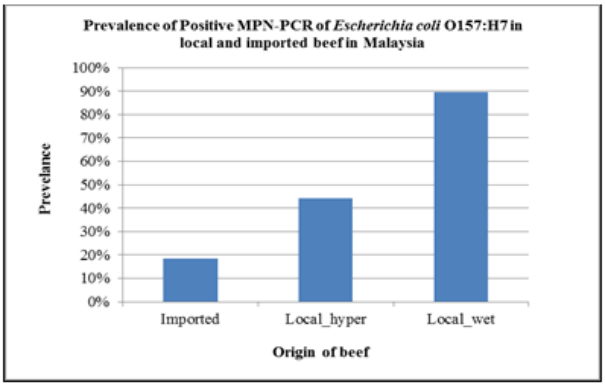

Figure 1. Origin of the *E. coli* O157:H7 contaminated beef samples

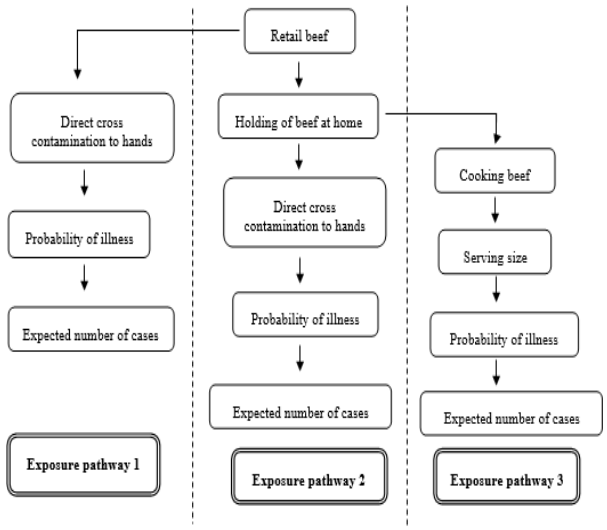

Figure 2. A conceptual model for risk assessment of *E. coli* O157:H7 in beef

3.2 Risk estimation

The present study can be identified as the first quantitative risk assessment intended to estimate the likelihood of acquiring *E. coli* O157:H7 infection by Malaysians with consumption of beef. A conceptual model as outlined in Figure 2 was developed considering exposure pathways to *E. coli* O157:H7, on which the risk assessment was conducted.

The model assumed that *E. coli* O157:H7 can be transmitted to humans via three different exposure routes. In exposure pathway, one considers direct contamination of pathogen via raw beef from retail, while the exposure 2 and 3 consider the growth of bacteria during home storage. The risk associated with beef was estimated based on the above exposure routes and according to the retail market type (Table 4).

Table 4. Risk estimation of *E. coli* O157:H7 in beef

Parameter	Hypermarket A	Hypermarket B	Wet market
Level (Log MPN/g)	1.97	1.53	1.15
Annual probability of illness-exposure 1	2.28E-04	2.26E-04	2.24E-04
Annual expected cases- exposure 1	419	415	412
Annual probability of illness- exposure 2	2.30E-04	2.28E-04	2.26E-04
Annual expected cases- exposure 2	424	420	416
Annual probability of illness- exposure 3	9.97E-01	8.84E-01	5.93E-01
Annual expected cases- exposure 3	1.83E+06	1.63E+06	1.09E+06

The Monte Carlo simulation conducted using the @Risk software, defined various levels of contamination with probability distributions. According to the calculations, the mean contamination level was 1.97 and 1.53 MPN/g beef from hypermarket A and B respectively. While the lowest level of contamination (1.15 MPN/g) was detected in beef from the wet market. Though the highest prevalence of *E. coli* O157:H7 was detected in wet markets, the concentration was the lowest comparative to other markets. The level of contamination was simulated as a discrete distribution of concentration and prevalence.

The dose-response model estimated the probability of illness per serving and multiplied by estimated 365 servings per year to compute the annual probability of illness. The annual likelihood of illness ranged from 2.24E-04 to 2.28E-04 for the exposure route 1 for beef from three markets. The highest probability of illness (2.30E-04) for exposure

route 2 was reported for beef from hypermarket A. Overall probability of illness was highest in the exposure route 3, of which beef products from hypermarket A resulted in the highest (9.97E-01) number of diseases.

According to the estimates of the consumer data of MOH, Malaysia (2003), that 61.29% of the population consumed beef and that calculated to be 18,387,000 people. With this assumption, the simulation model estimated that annual *E. coli* O157:H7 cases ranged from 412-419 and 416-424 following exposure to route 1 and 2 respectively. However, the expected number of *E. coli* O157:H7 cases with consumption of contaminated beef estimated to be 1.09E+06, 1.63E+06 and 1.83E+06 for beef purchased from the wet market, hypermarket B and hypermarket A, respectively. The growth of the bacteria in the home storage and serving size may be associated with the high number of cases in exposure route 3 which in comparative to the other exposure methods which only estimated the risk associated with the cross contamination.

4. Discussion

E. coli O157:H7 is a zoonotic foodborne disease with a high public health significance and one of the most frequently reported foodborne infections in the world (Newell *et al.*, 2010). The *E. coli* O157:H7 infection is routinely investigated by the developed countries but not in most of the developing countries. In Africa, South America, and Asia, annually million infant deaths were estimated subsequently to enterohemorrhagic *E. coli* infection (University of Bradford, 1999). Although beef is a commonly consumed staple food in Malaysia, there are only limited studies conducted to identify the prevalence of *E. coli* O157:H7.

Previously, Radu *et al.* (1998) detected *E. coli* O157:H7 in 36% of beef samples collected from Malaysia. However, prevalence level of *E. coli* O157:H7 in ground beef from studies in Argentina (Chinen *et al.*, 2001) and North America (Doyle and Schoeni, 1987) was able to detect only in 3.8% and 3.7% respectively. Recently, a study conducted in by Sukhumungoon and colleagues (2011) found a prevalence of STEC in 27% of buffalo, 23% of cattle, and 38.5% of goat meat exported from Malaysia to Thailand. Both locally produced beef and imported beef are available at the retail level in Malaysia. Therefore, beef samples used in this study had imported and local origin.

Certifying the safety of food commodities is the main challenge faced by the local authorities as the safety of food is related to public health and economy of the country (Signorini *et al.*, 2009). Traditional food safety measures based on penalties and withdrawing hazardous food commodities are not feasible for current and emerging food related hazards safety as those methods do not adopt or simulate any precautionary measures. With the introduction of risk analysis, insight into foodborne infections could be comprehended in a more scientific basis and preventive practice methodology (Hoornstra and Notermans, 2001). Risk analysis is a scientific method that evaluates, manage and communicate risk with the assistance of related stakeholders. Based on the outputs of the risk analysis the interested parties and the regulatory authorities can implement control measures to ensure safety (Signorini *et al.*, 2009).

According to the findings, the initial concentration and prevalence of bacteria on beef were key variables in the model output. However, input data generated from this study does not represent the entire Malaysia. According to Gardner (2004), one of the drawbacks of risk assessment process particularly at the exposure assessment is the unavailability of representative data from various researches. Though probability distributions were incorporated to reduce the uncertainty, the estimations cannot be validated. Further, data from farm to fork is necessary to conduct a more robust exposure assessment (Lammerding and Fazil, 2000). In addition, before usage of third party data, the specificity, sensitivity, and reliability of sampling and testing methods should be assessed and should be properly acknowledge (Lammerding and Fazil, 2000).

The relationship between the dose and the response is scalable; the exponential model was used to estimate dose-response (Teunis and Havelaar, 2000). According to the exponential dose– response, probability of illness depends on the exponential dose-response relationship and number of pathogens per exposure. Hence, the precise dose is not necessary to estimate the risk (Teunis *et al.*, 2005). Available developed and validated, dose–response models were established only in developed countries. Therefore, application into local conditions may lead to inaccuracies (Haas and Eisenberg, 2001). Moreover, the current status of the art of QMRA is that this risk assessment method does not account for other factors that could influence the infection such as the degree of immunity of targeted people, or the distribution of infection over time due to the initial exposure (Huang and Haas 2009). The infectivity of *E. coli* O157:H7 is very low, and the dose-response model can assess the infection even at minute levels of infection or risk which may complement

eISSN: 2550-2166

epidemiological investigations (Ferrer *et al.*, 2012).

In the current model, influential factors such as level of immunity in the population and probability of infection prior to assessment were considered (Huang and Haas 2009). Availability of local consumer data and incorporation into the model was able to provide a more realistic estimation for expected number of cases in the country, rather than predicting based on data from developed countries. However, further studies are necessary to generate data on local consumer and cooking patterns. In addition, this study evaluated the risk of *E. coli* O157:H7 infection, which can be further categorised based on the type of infection including gastroenteritis, haemolytic uremic syndrome and mortality.

The current risk assessment was conducted with incorporating probability distributions rather than using point estimates. According to previous studies, if reliable data is at available usage for probability distributions, it would be more suitable (Nauta, 2000). Further, the probability distributions can consider the uncertainty associated with the assessment. In spite the limitations of risk assessment modelling; the technique was used to evaluate the risk associated with many aspects including health, water and food safety (WHO 2006; Mataragas *et al.*, 2010). Comparative to epidemiological studies, risk assessment process is less resource demanding and requires a limited amount of financial, personnel and time involvement. Therefore, risk estimation can be generated earlier than the epidemiological studies which may be beneficial in implementing control or preventive strategies.

5. Conclusion

In conclusion, the present research work is the first effort in modelling the risk of *E. coli* O157:H7 infection connected to beef in Malaysia. The developed quantitative risk assessment model quantified and provided understanding into the risk of *E. coli* O157:H7 infection and indicated that the risk could be controllable at the retail and home levels, especially during food preparation. The model can be used to evaluate the various mitigations options to control the risk of *E. coli* O157:H7 in beef including food sanitary measures and cooking procedures. With the experiential data collected, this model can be further improved to create microbial risk predictions applicable to Malaysia in the future.

Conflict of interest

There is no conflict of interest.

Acknowledgements

The research work was funded by Kakenhi Grant-in-Aid for Scientific Research (KAKENHI 24249038), Japan Society for the Promotion of Sciences and grant-in-aid of Ministry of Health, Labour and Welfare, Japan and in part Fundamental Research Grant Project (FRGS/1/2014/SG05/UPM/01/2/5524559) from the Ministry of Education, Malaysia.

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eISSN: 2550-2166