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The risk assessment of *Vibrio parahaemolyticus* in cooked black tiger shrimps (*Penaeus monodon*) in Malaysia

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ABSTRACT

A microbiological risk assessment was conducted on *Vibrio parahaemolyticus* in order to estimate the risk of getting infected by consuming cooked shrimps (*Penaeus monodon*) for Malaysians. This study was based on the risk assessment framework developed by the Codex Alimentarius Commission. The risk estimate was calculated by using the data generated from this study and assumptions based on data taken from reports produced by the Ministry of Health Malaysia and from other studies. The @RISK software package, version 4.5 (2005 Palisade USA) in combination with Microsoft® Excel were used to run the simulations. All of the calculations were performed by the Monte Carlo method of simulations from specified input distributions and appropriately combining the sampled values to generate the corresponding output distributions. All simulations, consisting of 10,000 iterations were undertaken. The estimated illness per year was 123 people (aged from 18 to 59 years) for Malaysians. The 90% of distribution of illness due to the consumption of cooked shrimps lies between 49 and 197 cases per year. The incidence rate of illness/100,000 population/year (aged between 18 and 59 years) is 1.3 while 90% distribution lies between 0.5 and 2. In the case of shrimp that were cooked during meal preparation, the hazard has been significantly reduced. It is important to pay attention to temperature control during harvesting and post-harvest handling. It is also required to prevent cross-contamination during handling and preparation of shrimps and adequate cooking before consumption.

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1. Introduction

Shrimp is one of the most important seafood commodities in international trade and most shrimp come from developing countries. While high value shrimp is mostly exported by the developing countries to earn valuable foreign exchange, low value shrimp are consumed domestically (FAO, 2004). Yet, one of the most serious difficulties facing exporters is the different quality and safety standards and policies imposed by importers. These disparities concern regulations, standards and procedures, including border controls where seafood products can be rejected, destroyed or detained. In order to promote harmonization and equivalence among seafood-trading nations, such differences need to be reduced and ultimately removed and replaced by international control systems and standards based on scientific techniques such as risk assessment (Ababouch, Gandini, & Ryder, 2005).

According to WHO (2012), risk assessment is the scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazards. The process consists of the four steps: hazard identification, hazard characterization, exposure assessment and risk characterization. Hazard identification is the identification of known or potential health effects associated with a biological, chemical, or physical agent which may be present in food. Hazard characterization is the qualitative and/or quantitative evaluation of the nature of the adverse effects associated with a particular agent which may be present in food. For chemical agents, a dose–response assessment should be performed. For biological or physical agents, a dose–response assessment should be performed if the data is obtainable. Exposure assessment is the qualitative and/or quantitative evaluation of the degree of intake likely to occur in a given population. Risk characterization is the integration of hazard identification, hazard characterization and exposure assessment into an estimation of the adverse effects likely to occur in a given population. The definition includes quantitative risk assessment, which emphasizes reliance on numerical expressions of risk, and also qualitative expressions of

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risk, as well as an indication of the attendant uncertainties (WHO, 2012).

Vibrio parahaemolyticus is one of the major seafood-borne gastroenteritis causing bacteria and is frequently isolated from shellfish samples (Zhao, Zhou, Cao, Ma, & Jiang, 2011). Gastroenteritis caused by this organism is almost exclusively associated with seafood consumed raw or inadequately cooked, or contaminated after cooking. Black tiger shrimp (*Penaeus monodon*) which is sold in domestic markets is generally non-exportable grade and shares a small quantity. The incidence of food poisoning reported in Malaysia has gradually decreased over the last five years. In the year 1999 the incidence rate was 38.04 per 100,000 population with a gradual reduction to 36.61 in 2000, 29.99 in 2001, 28.63 in 2002 and 26.44 in the year 2003. One of the causative agents suspected in the food poisoning episodes was *V. parahaemolyticus*. In 1999 and 2000 there were no episodes suspected to be linked to *V. parahaemolyticus*, but in 2002, 2003 and 2004, there was one, two and one episode respectively. However, the issue of *V. parahaemolyticus* in seafood is getting more attention in Malaysia for economic and trade reasons. *V. parahaemolyticus* can be quantified in natural shrimp's samples using rapid and sensitive quantitative real-time PCR (Q-PCR). This assay could be proposed, in response to the demands of the European Commission as a tool for testing for the presence of *Vibrios* in crustaceans, making it possible to legislate in this domain (Robert-Pillot, Copin, Gay, Malle, & Quilici, 2010).

Exports of block frozen black tiger shrimp (*P. monodon*) had been rejected from Malaysia due to the presence of *V. parahaemolyticus* by some EU countries (Mohammad, Hashim, Gunasalam, & Radu, 2005). The scope of this work was to assess the risk of acquiring gastroenteritis due to *V. parahaemolyticus* for Malaysian population as a result of consumption of cooked black tiger shrimp (*P. monodon*).

2. Materials and methods

The @RISK software package, version 4.5 (2005 Palisade USA) in combination with Microsoft® Excel were used to run the simulations. The risk estimate was calculated by using the data generated in this study and assumptions based on data from other studies (FAO/WHO, 2002). Other information required for the risk assessment was obtained from different sources as mentioned in Table 1.

Data generated in this risk assessment on the prevalence of total and virulent (*tdh+* and *trh+*) *V. parahaemolyticus* strains obtained from culture and PCR methods in frozen shrimp were used as input data in the module. The risk estimate was calculated by using the data generated in previous study (Sujeewa, Norrakiah, & Laina, 2009) and assumptions based on data from other studies elsewhere (FAO/WHO, 2002; FDA, 2001). Codex Alimentarius Commission (CAC) framework for microbiological risk assessment

was applied (CAC, 1999). All of the calculations were performed by the Monte Carlo method of sampling from specified input distributions and appropriately combining the sampled values to generate the corresponding output distributions. All simulations, consisting of 10,000 iterations were undertaken. To develop the model, the four steps of the risk assessment were included: hazard identification, hazard characterization, exposure assessment and risk characterization. This risk assessment is a quantitative product pathway analysis in which the key steps from processing through post-harvest handling and to consumption were modelled. The likelihood of illness following exposure to pathogenic *V. parahaemolyticus* from consumption of frozen black tiger shrimps (*P. monodon*) was calculated.

2.1. Hazard identification of V. parahaemolyticus

The hazard identification component of a microbial risk assessment is the identification of the pathogenic microorganism that is capable of causing adverse health effects and is present in a particular food or group of foods. The hazard in this risk assessment was focused on pathogenic *V. parahaemolyticus* in raw frozen black tiger shrimp (*P. monodon*) after cooking. The adverse health effects include gastroenteritis.

2.2. Hazard characterization of V. parahaemolyticus

This section focuses on evaluating the nature of adverse health effects associated with *V. parahaemolyticus* in seafood and how to quantitatively assess the relationship between the magnitude of the food-borne exposure and the likelihood of adverse effects occurring. The objective of the hazard characterization is to provide sufficient information to allow for a quantitative measurement of the public health risk from *V. parahaemolyticus* associated with the consumption of shrimps.

2.3. Exposure assessment of V. parahaemolyticus

The exposure assessment component of a microbial risk assessment is an evaluation of the likelihood of ingesting a pathogenic microorganism via food and the likely level of exposure. In this assessment, the likelihood of exposure to pathogenic *V. parahaemolyticus* from consumption of raw frozen black tiger shrimps (*P. monodon*) and also frozen black tiger shrimps after cooking was evaluated. The purpose of this exposure assessment is to quantify the exposure of consumers to pathogenic *V. parahaemolyticus* from the consumption of frozen shrimps after cooking. In this risk assessment study, a mathematical model was developed to estimate the likelihood and magnitude of exposure assessment for *V. parahaemolyticus*.

2.4. Risk characterization of V. parahaemolyticus

In the risk characterization, the estimated exposure is normally integrated with the dose–response model to provide a risk estimate and to determine the influence of different mitigation strategies on this risk estimate. The risk estimate was calculated by using the data generated in this study and assumptions based on data from other studies. The model simulations were implemented in @Risk (Palisade). All of the calculations were performed by the Monte Carlo method of sampling from specified input distributions and appropriately combining the sampled values to generate the corresponding output distributions. All simulations, consisting of 10,000 iterations were undertaken.

Table 1
Information used for risk assessment.

Information	Sources	Values
Estimated intake of shrimp	MOH, 2006a	3 g/day ([a]SD = 0.08)
Estimated population who consumed shrimp (aged 18–59 years)	MOH, 2006a	9,572,128
Average meal size for shrimp	MOH, 2006b	18 g
Percentage virulent *V. parahaemolyticus* in frozen shrimp	Mohammad, Hashim, Gunasalam, & Radu, 2005	8

[a] SD – Standard Deviation.

3. Results

3.1. Hazard identification of V. parahaemolyticus

V. parahaemolyticus has been recognised as a major cause of food-borne gastroenteritis. Gastroenteritis caused by this organism is almost exclusively associated with seafood consumed raw or inadequately cooked, or contaminated after cooking. A new *V. parahaemolyticus* clone of O3:K6 serotype emerged in Calcutta in 1996 and it has spread throughout Asia and to the United States elevating the status of *V. parahaemolyticus* to pandemic (Matsumoto et al., 2000). In Australia, in 1990 and 1992, there were two outbreaks of gastroenteritis caused by *V. parahaemolyticus* in chilled, cooked shrimps imported from Indonesia (Kraa, 1995). In France, one outbreak of *V. parahaemolyticus* has been reported. In April 1997, 44 persons developed diarrhoea after consuming shrimp imported from Asia (Lemoine, Germanetto, & Giraud, 1999). In Thailand, there were 15 incidents of outbreaks involving 1650 patients during the 1995–2001 periods (Anon, 2001). Between 1986 and 1995, 197 outbreaks of food-borne disease were caused by *V. parahaemolyticus* in Taiwan (Pan, Wang, Lee, Chien, & Horng, 1997) while in 1997 over 200 outbreaks were reported, including an outbreak of 146 cases acquired from boxed lunches (Anon, 1999).

The incidence of food poisoning reported in Malaysia has gradually decreased over the last five years. In the year 1999 the incidence rate was 38.04 per 100,000 population with a gradual reduction to 36.61 in 2000, 29.99 in 2001, 28.63 in 2002 and 26.44 in the year 2003. One of the causative agents suspected in the food poisoning episodes was *V. parahaemolyticus*. In 1999 and 2000 there were no episodes suspected to be linked to *V. parahaemolyticus*, but in 2002 there was one, 2003 two and in 2004 one episode. These episodes had occurred in different residential school hostels involving various types of food as shown in Table 2 (Mohammad et al., 2005).

3.2. Hazard characterization of V. parahaemolyticus

The hazard characterization presents dose–response curves for *V. parahaemolyticus*. Infection by *V. parahaemolyticus* is characterised by an acute gastroenteritis. Therefore, the end-point of the dose–response curve is defined as gastroenteritis. The quantitative measurement of public health risk is accomplished by the determination of dose–response relationships for *V. parahaemolyticus* based upon the best available data. Fig. 1 shows the most probable dose–response curve for *V. parahaemolyticus*. These data are from healthy human volunteer studies where gastrointestinal illness was used as the endpoint response (FAO/WHO, 2002). Symptoms of

Table 2
The incidence of reported food poisoning cases believed to be caused by *V. parahaemolyticus* in Malaysia from 1999 to June 2004.

Year	Location (State)	Type of locality	No. of cases	Age (years)	Food type	Status
1999	NA	–	–	–	–	–
2000	NA	–	–	–	–	–
2001	NA	–	–	–	–	–
2002	Kelantan	Residential	2	NA	Clam	Suspected
2003	Penang	School hostel	124	5–19	Prawn & sambal	Suspected
	Kedah	Residential	14	0–4 = 3 5–19 = 9 20–54 = 2	Chicken sambal/Kerabu tauge (bean sprout)	Suspected
2004	Kedah	School hostel	128	5–19	Prawn & sambal/Fried mee (noodle)	Suspected

NA-Not available. Source: Mohammad, Hashim, Gunasalam, and Radu (2005).
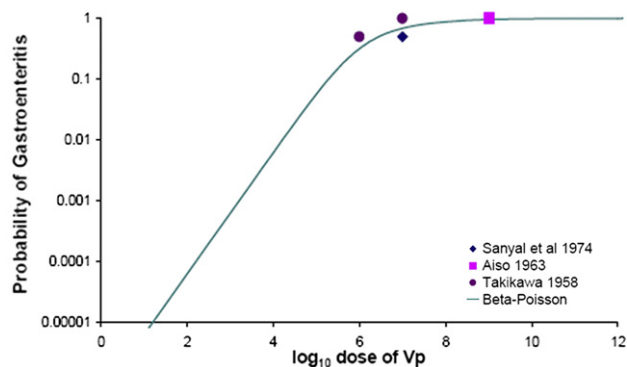

Fig. 1. Beta-Poisson dose–response curve for *V. parahaemolyticus* (Vp) (endpoint modelled is gastrointestinal illness). Source: FAO/WHO, 2002.

gastroenteritis are abdominal cramps, explosive watery diarrhoea and sometimes nausea, vomiting and fever. Symptoms last from 1 to 7 days, occasionally longer. The mean duration is 2.5 days, usually self-limiting (Lake, Hudson, & Cressey, 2003).

3.2.1. Assumptions regarding the dose–response curve

While providing a framework for understanding the relationship of risk to various parameters, the development of the risk assessment model necessarily requires certain assumptions to fill the data gaps as described: healthy volunteer response to oral challenge of *V. parahaemolyticus* is representative of the general population; the virulence of the pathogen or susceptibility of the host does not vary; the Beta-Poisson dose–response model is reasonable for use in characterizing the risk of illness following exposure to *V. parahaemolyticus*; since there was no available data to lead dose–response curve for *V. parahaemolyticus* consumption by Malaysian, the same Beta-Poisson parameters that were estimated by FDA (2001) to model the probability of illness per cooked meal were assumed.

3.3. Exposure assessment of V. parahaemolyticus

The exposure assessment was divided into two modules: post-harvest, and consumption. The post-harvest module predicts the probability of total and virulent ($tdh+$ and $trh+$) *V. parahaemolyticus* in frozen shrimp. The output of the exposure assessment was fed into hazard characterization to produce the risk characterization. For this risk assessment, the likelihood and severity of illness (gastroenteritis) from the consumption of shrimps containing pathogenic *V. parahaemolyticus* was predicted on both per serving and per annum basis.

3.3.1. Post-harvest module

The post-harvest module determines the role of post-harvest processing and handling on the numbers of pathogenic *V. parahaemolyticus* at consumption. Fig. 2 shows the conceptual model for post-harvesting practices. Retail component was not included for this. The number of *V. parahaemolyticus* in frozen shrimps was multiplied by the meal size (18 g), in order to get the number of *V. parahaemolyticus* per meal. This was multiplied by the percentage of virulent *V. parahaemolyticus* (8%) in frozen shrimp (Mohammad et al., 2005), in order to get the number of virulent *V. parahaemolyticus* in frozen shrimps.

The mean level of virulent *V. parahaemolyticus* in frozen shrimp at purchase was 15.96 cfu per meal size (Fig. 3). It was assumed that frozen black tiger shrimps were thawed for 3 h before cooking. There was approximately a 1 log cycle increment of
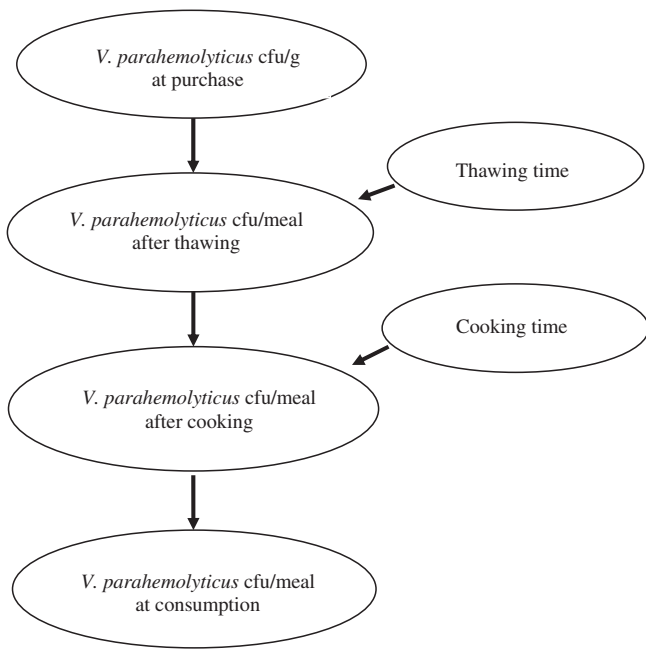
Fig. 2. Post-harvest module for exposure assessment of *V. parahaemolyticus* in cooked shrimps.

V. parahaemolyticus during 3 h thawing at 30 °C (Ariyawansa, Abdullah Sani, & Babji, 2006 and Fig. 4) which is mostly the ambient temperature in Malaysia. Typically, under local condition the shrimps are cooked in the home by heating. It was also assumed that shrimps are cooked at 60 °C for 5 min. It has been observed that 3-4 log cycle reduction of *V. parahaemolyticus* occurs during heating at 60 °C for 5 min (Twedt, 1989) (Fig. 5). After thawing at 30 °C, the number of *V. parahaemolyticus* was changed to 142.3 cfu per meal (Fig. 6). After cooking for 5 min, the level of *V. parahaemolyticus* was reduced to 0.036 cfu/meal (Fig. 7). This post-harvest module estimates the levels of pathogenic *V. parahaemolyticus* in a single serving of shrimp.

3.3.2. Consumption module

Fig. 8 represents the consumption module for cooked shrimps. Fig. 9 illustrates the simulated distribution for shrimp consumption/person/day. The level of *V. parahaemolyticus* cfu/meal (cooked) at consumption was the output of the post-harvest module. This ingested dose was used in the dose response to calculate the
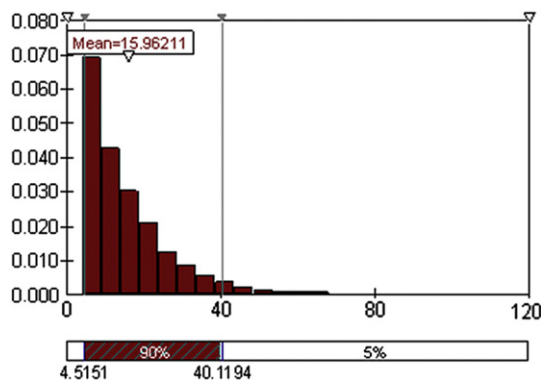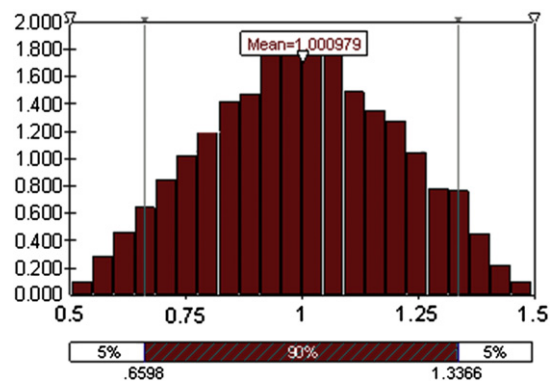

Fig. 4. Simulated distribution for log increase of *V. parahaemolyticus* during thawing.

probability of risk of illness associated with the consumption of a single cooked meal.

3.4. Risk characterization of V. parahaemolyticus

For Malaysians the estimated exposure for probability of illness was 4.8×10^{-6}. The number of persons become ill per annum was calculated by multiplying the probability of illness and estimated number of contaminated meals per year (Fig. 10). For the Malaysian consumers the mean estimated number of illnesses (age between 18 and 59 years) per annum was 123 (Fig. 11). The 90% of distribution of illness due to the consumption of cooked shrimps lies between 49 and 197 cases per year. The incidence rate of illness/100,000 population/year (age between 18 and 59 years) is 1.3, while 90% distribution lies between 0.5 and 2 (Fig. 12).

4. Discussions

There were no any reported incidences of gastroenteritis due to *V. parahaemolyticus* for the age group between 18 and 59 years in 2004 for Malaysians. Possible explanation is true incidence of gastroenteritis may be about 20%, though the mild nature of illness means it passes unnoticed and is hence unreported (Forsythe, 2002). However in a preliminary case study estimates the cases of illness (same age group) due to consumption of cooked shrimp ranged from 0.43 to 3.0 cases per 100,000 people per annum which is in agreement with findings of this study (Hashim, 2007).

From the hazard characterization, the health effect cause by *V. parahaemolyticus* is not severe, usually not life threatening, short duration and symptoms are self-limiting. Though complete risk elimination is an impossible goal, risk reduction is possible if
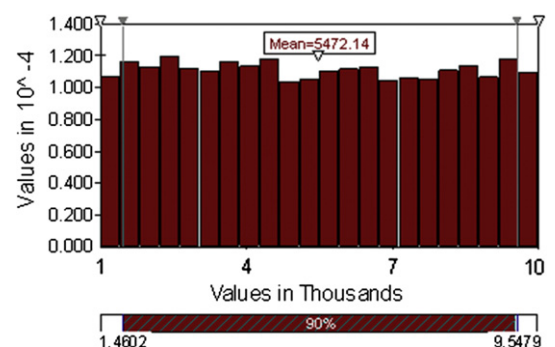

Fig. 3. Simulated distribution for estimated number of virulent *V. parahaemolyticus*/meal.

Fig. 5. Simulated distribution for reduction of (numbers) *V. parahaemolyticus* during cooking.
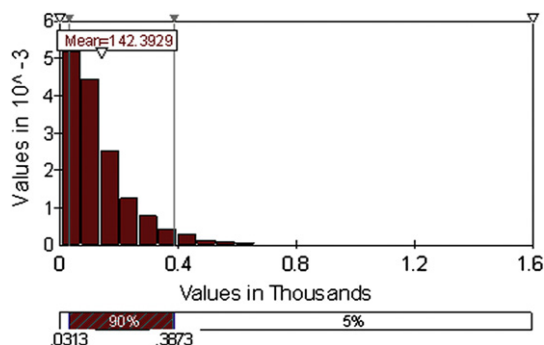
Fig. 6. Simulated distribution for level of *V. parahaemolyticus* after thawing.

guided by the best information (risk assessment) and the most transparent procedure available (risk management and risk communication).

The ubiquitous nature of *Vibrio* species in marine and estuarine environments makes it impossible to obtain seafood completely free of these bacteria. No epidemiological study has yet generated sufficient data to determine the relationship between infectious dose and the number of cases of illness resulting from the ingestion of pathogenic *V. parahaemolyticus* (whether for pathogenic forms only or for the total population of this species) (Robert-Pillot et al., 2010).

In the case of shrimp that were cooked during meal preparation, the hazard has been significantly reduced. It is important to pay attention to control temperature during harvesting and post-harvest handling. It is also required to prevent cross-contamination during handling and preparation of shrimps, and adequate cooking before consumption.

As part of a semi-quantitative risk assessment of 10 seafood hazard/product combinations, a risk assessment tool was used to generate a Risk Ranking. The tool is in a spreadsheet software format and provides a risk estimate, which is scaled between 0 and 100, where 0 represents no risk and 100 represents all meals containing a lethal dose of the hazard. A full description of the tool is contained in Summer and Ross (2002). Based on their ranking, seafoods in Australia fell into three risk categories. Hazard/product pairs with ranking <32 included mercury poisoning (Relative Risk = 24), *Clostridium botulinum* in canned fish (RR = 25), or in vacuum-packed cold-smoked fish (RR = 28), parasites in sushi/sashimi (RR = 31), viruses in shellfish from uncontaminated waters (RR = 31), enteric bacteria in imported cooked shrimp (RR = 31) and algal biotoxins from controlled waters (RR = 31). It is noted that
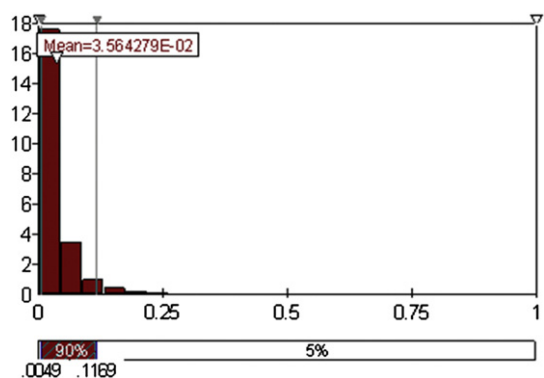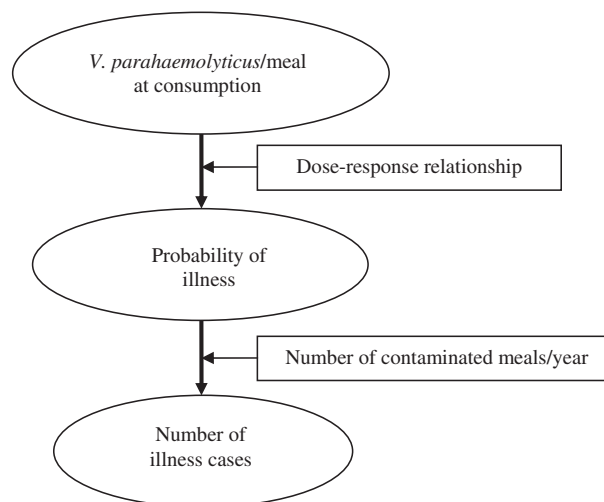

Fig. 8. Consumption module for exposure assessment of *V. parahaemolyticus* in cooked shrimps.

there have been no documented cases of food-borne illness from any of the above hazard/product pairings in Australia. Those with rankings 32–48 included *V. parahaemolyticus* in cooked prawns (RR = 37) and *Vibrio cholera* in cooked prawns (RR = 37). Almost all the hazard/product pairs in this category have caused the outbreaks of food poisoning in Australasia (Summer & Ross, 2002).

Although pathogenic *Vibrio* spp. are often found in bivalve molluscs and on crustaceans, the incidence of illness is low. For healthy individuals, doses of organisms higher than those normally found on food are required. The risk of contamination is seasonal, corresponding to the increased levels of *Vibrio* spp. in growing areas as water temperatures rise. The risk of thermal abuse also increases during summer. The FSANZ relative Risk Ranking for *V. parahaemolyticus* is low but *Vibrio vulnificus* and *Vibrio cholera* are rated medium based on severity of illness. The ranking for *V. parahaemolyticus* might change if the pandemic O3:K6 strain naturalises in Australian waters (NSW Food Authority, 2009).

The limitations of this study was that the data gathered from farm to frozen shrimps were very sparse since they were collected randomly from few farms in a selected area and also the number of samples analysed may not reflect the actual situation in Malaysia. More data are needed for other areas. In this study the shrimps cultured in aquaculture environments were considered. Contamination level for offshore wild caught shrimp can be different than this study. The dose–response relationship was based only on previous studies on *V. parahaemolyticus* administered with a pH-
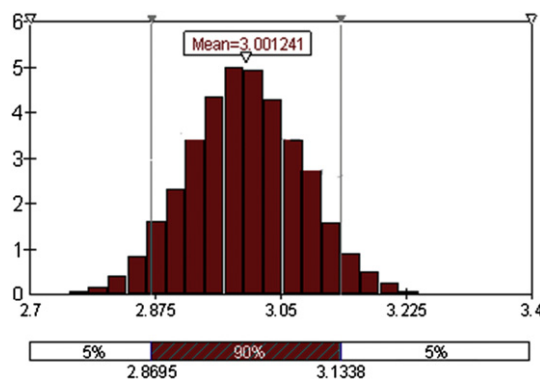

Fig. 7. Simulated distribution for level of *V. parahaemolyticus* in cooked shrimps per meal.

Fig. 9. Simulated distribution for shrimp consumption/person/day for Malaysians.
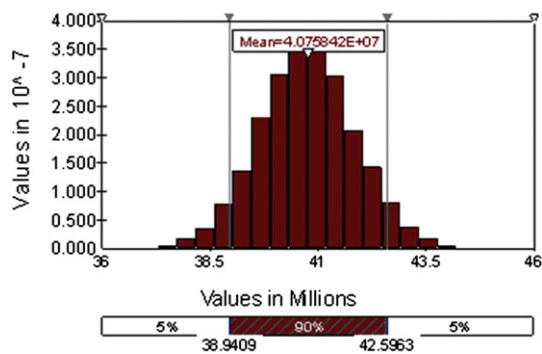
Fig. 10. Simulated distribution for estimated number of contaminated meals/year for Malaysians.
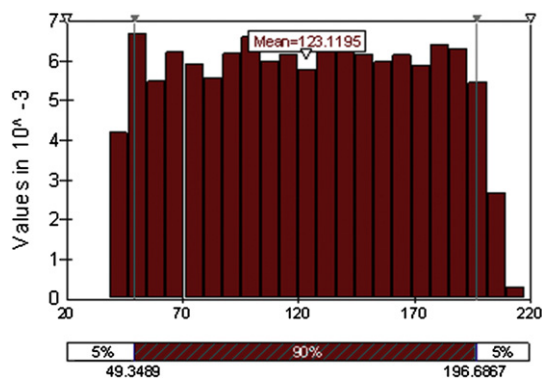

Fig. 11. Simulated distribution for illness per year for Malaysians.

neutralizing buffer rather than with a food matrix as described in the FAO/WHO (2002) publication.

In undertaking this assessment, some gaps were identified. This included a lack of quantitative data on the levels of the microorganism at various points along the production to consumption chain. The effect of the food matrix on the effective dose of pathogen is an important data gap. Only a certain group of consumers were considered (18–59 years age group for Malaysians). It needs to consider the other group of consumers (for example population below 18 years old). There was no specific data on the type of shrimps consumed, the retail component, consumer practices in preparation and handling of shrimps to determine to which extend their different pathways may contribute to exposure. There is no data available for prevalence and concentration of virulent *V. parahaemolyticus* for various types of marine wild prawns.
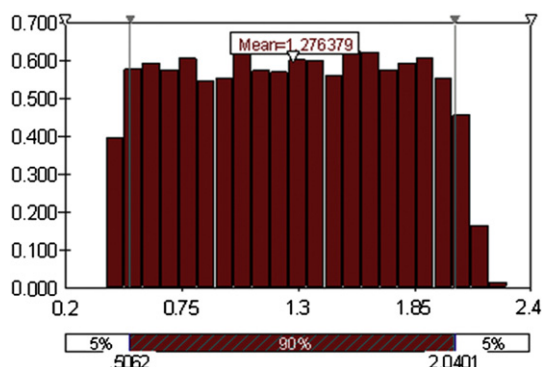

Fig. 12. Simulated distribution for illness per 100,000 persons/year for Malaysians.

5. Conclusions

For Malaysians the mean estimated number of illnesses (aged between 18 and 59 years) due to consumption of cooked shrimps per annum was 123. The 90% of distribution of illness due to the consumption of cooked shrimps lies between 49 and 197 cases per year. The incidence rate of illness/100,000 population/year (aged between 18 and 59 years) was 1.3 while 90% distribution lies between 0.5 and 2.

From the hazard characterization, the health effect caused by *V. parahaemolyticus* is not severe, usually not life threatening, short duration and symptoms are self-limiting. In the case of shrimp that were cooked during meal preparation, the hazard has been significantly reduced. It is important to pay attention to temperature control during harvesting and post-harvest handling. It is also required to prevent cross-contamination during handling and preparation of shrimps and adequate cooking before consumption.

Acknowledgements

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