Risk assessment
of *Campylobacter* spp.
in broiler chickens
and *Vibrio* spp. in seafood

Report of a Joint FAO/WHO Expert
Consultation

Bangkok, Thailand
5-9 August 2002

Contents

List of Abbreviations

CAC	Codex Alimentarius Commission
CCFFP	Codex Committee on Fish and Fishery Products
CCFH	Codex Committee on Food Hygiene
CFU	colony forming unit
CT	cholera toxin
ctx	cholera toxin gene
FAO	Food and Agriculture Organization of the United Nations
FDA-VPRA	The United States Food and Drug Administration draft risk assessment on the "Public Health Impact of *Vibrio parahaemolyticus* in Raw Molluscan Shellfish"
g	gram(s)
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
h	hour(s)
ISSC	Interstate Seafood Sanitation Conference
MPN	Most Probable Number
PCR	Polymerase Chain Reaction
ppt	parts per thousand
RAP	FAO Regional Office for Asia and the Pacific
TDH	Thermostable direct haemolysin
tdh	Thermostable direct haemolysin gene
TLH	Thermolabile haemolysin
TRH	TDH-related haemolysin
trh	tdh-related haemolysin gene
USFDA	United States Food and Drug Administration
WHO	World Health Organization

1 Introduction

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) convened an expert consultation on "Risk assessment of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood" in the FAO Regional Office for Asia and the Pacific (RAP), Bangkok, Thailand on 5 - 9 August 2002. The list of participants is presented in Annex 1.

Mr Dong Qingsong, FAO Deputy Regional Representative for Asia and the Pacific and Officer-in-charge, RAP, opened the meeting on behalf of the two sponsoring organizations. In welcoming the participants Mr Qingsong noted the increasing significance of microbiological hazards in relation to food safety. He noted that international trade had amplified the opportunity for these hazards to be disseminated from the original point of production to locations thousands of miles away, thereby permitting such food safety hazards to impact on public health and trade in more than one country. Mr Qingsong observed that this underlined the need to first consider microbiological hazards at the international level and provide the means by which they can then be addressed at regional and national levels. He highlighted the commitment of FAO and WHO to provide a neutral international forum to consider new approaches to achieving food safety, and in particular to address microbiological risk assessment.

As the meeting was convened in Asia, Mr Qingsong also highlighted the importance of the work on microbiological risk assessment for this region. He noted that seafood and poultry are important products in both domestic and international trade and provide a valuable contribution to the economy of the region. He also noted the concerns for public health raised by the microbiological hazards associated with these foods. Therefore, in conclusion he requested the expert consultation to consider, in particular, the practical application of their work so that the output would be of value to a range of end users and to countries in different stages of development.

The consultation appointed Dr Servé Notermans (the Netherlands) as chairperson of the consultation and Ms Dorothy-Jean McCoubrey (New Zealand) as rapporteur. Dr Notermans also served as chairperson of the campylobacter working group and Prof. Diane Newell (United Kingdom) served as rapporteur. Dr Mark Tamplin (United States of America) served as chairperson of the working group on vibrio and Dr Ron Lee (United Kingdom) as rapporteur of that group.

2 Background

Risk assessment of microbiological hazards in foods has been identified as a priority area of work for the Codex Alimentarius Commission (CAC). At its 32nd session the Codex Committee on Food Hygiene (CCFH) identified a list of pathogen-commodity combinations for which it requires expert risk assessment advice. In response, FAO and WHO jointly launched a programme of work with the objective of providing expert advice on risk assessment of microbiological hazards in foods to their member countries and to the CAC.

Dr. Hajime Toyofuku, WHO, and Dr. Sarah Cahill, FAO provided participants with an overview of the joint FAO/WHO activities on microbiological risk assessment. In their presentation, they also highlighted the objectives and expected outcomes of the current meeting.

The FAO/WHO programme of activities on microbiological risk assessment aims to serve two customers - the CAC and the FAO and WHO member countries. The CAC, and in particular the CCFH, needs sound scientific advice as a basis for the development of guidelines and recommendations as well as the answers to specific risk management questions on certain pathogen-commodity combinations. Member countries on the other hand need specific risk assessment tools to use in conducting their own assessments and, if possible, some modules that can be directly applied in a national risk assessment.

To implement this programme of work, FAO and WHO are convening a series of joint expert consultations. To date three expert consultations have been held. The first of these was held on 17 - 21

July 2000[1] and the second on 30 April - 4 May 2001[2]. Both of these expert consultations addressed risk assessment of *Salmonella* spp. in broiler chickens and eggs and *Listeria monocytogenes* in ready-to-eat foods. In March 2001, FAO and WHO initiated risk assessment work on the two pathogen-commodity combinations being considered in this expert consultation. Two *ad hoc* expert drafting groups were established to examine the available relevant information on *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood and prepare documentation on both the exposure assessment and hazard characterization steps of the risk assessment. These documents were reviewed and evaluated by a joint expert consultation convened on 23 - 27 July 2001[3].

In October 2001, the report of that expert consultation was delivered to CCFH. Additional risk management guidance was sought from the Committee in relation to the finalization of the risk assessments. In response to this, the Committee established risk management drafting groups to consider the approach it could take towards providing guidance on managing problems associated with *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood. A limited amount of feedback was received; therefore the risk assessment drafting groups, FAO and WHO met early in 2002 to consider how to complete the risk assessment work. Both risk assessments have been progressed towards completion, although to varying extents. The risk assessment documents that have been developed to date were reviewed and evaluated by the expert consultation.

The purpose of this report is to present the summary of the draft documents on risk assessment of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood as well as the discussions and recommendations of the expert consultation.

3   Objectives of the consultation

The expert consultation examined the information provided by the expert drafting groups on risk assessment of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood with the following objectives;

- To critically review the documents prepared by the *ad hoc* expert drafting groups giving particular attention to;

    - the risk characterization component and the manner in which the risk assessment outputs were generated;

    - the assumptions made in the risk assessment;

    - the associated uncertainty and variability;

    - the potential application of the work.

- To provide scientific advice to FAO and WHO member countries on the risk assessment of *Vibrio* spp. in seafood and *Campylobacter* spp. in broiler chickens based on the available documentation and the discussions during the expert consultation.

- To respond to the risk management needs of Codex.

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[1] FAO. 2000. Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods, FAO Headquarters, Rome, Italy, 17 - 20 July 2000. FAO Food and Nutrition Paper 71.
http://www.fao.org/es/ESN/food/risk_mra_campylobacter_en.stm / http://www.who.int/fsf/Micro/index.htm

[2] FAO. 2001. Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods: Risk characterization of *Salmonella* spp. in eggs and broiler chickens and *Listeria monocytogenes* in ready-to-eat foods, FAO Headquarters, Rome, Italy, 30 April - 4 May 2001. FAO Food and Nutrition Paper 72.
 http://www.fao.org/es/ESN/food/risk_mra_salmonella_en.stm / http://www.who.int/fsf/Micro/index.htm

[3] WHO. 2001. Report of the Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods; Hazard identification, exposure assessment and hazard characterization of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood. WHO Headquarters, Geneva, Switzerland 23 - 27 July 2001. WHO 2001.
http://www.fao.org/es/ESN/food/risk_mra_campylobacter_en.stm / http://www.who.int/fsf/Micro/index.htm

4 Summary of the general discussions

The drafting groups presented overviews of the risk assessment documents on *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood to the expert consultation. A summary of the draft risk assessments and the discussions of the expert consultation are given in sections five and six of this report. However, a number of issues relevant to risk assessment in general that were addressed are summarized below.

It was anticipated that the campylobacter and vibrio risk assessments will prove useful for decision makers such as Codex committees, food industries, food control authorities, regulators and other stake holders. It was not the task of the risk assessment groups to undertake risk profiling or determine acceptable risk levels – this is the role of risk managers. However, the outputs of this work should provide tools and information useful to both risk managers and risk assessors.

It was not possible at this stage to complete full quantitative risk assessments for all pathogens, inclusive of full validation exercises. This was due to the lack of extensive quantitative data being available, the uncertainties that exist and the need to make assumptions e.g. that all strains of *Campylobacter* spp. are pathogenic. However, it was noted that even preliminary quantitative and good qualitative risk assessments would be very useful in decision making. The risk assessment of *Vibrio parahaemolyticus* in bloody clams provides an example of how readily accessible data can be collated into the risk assessment framework to give valuable information that could be used towards improving public health.

The models on *Campylobacter* spp. and *Vibrio* spp provided examples of how risk assessment can be applied to pathogen-commodity combinations. However, it is important that countries do not just transfer any conclusions from this risk assessment work to make risk management decisions without giving due consideration to the effect of different species, environments and populations in their own country.

The challenge will be to show the world, including developing countries, the advantages of undertaking risk assessment. Training, complete with easy to understand guidelines on how to apply the risk assessments was considered extremely important. The training and guidelines material should be written in simple language that is applicable to different audiences and countries with different levels of development.

There is concern by many countries that not having a full risk assessments will create trade barriers. Assistance may need to be given to many developing countries to generate and collect data and undertake modelling exercises so that such concerns can be addressed.

The consultation formed two working groups to address the risk assessment documentation on *Campylobacter* spp. and *Vibrio* spp. respectively. The composition of the two working groups is outlined in the table below.

Campylobacter spp. in broiler chickens

Independent experts	Expert members of the drafting group	Members of the secretariat
John Cowden	Bjarke Christensen	Sarah Cahill
Heriberto Fernández	Aamir Fazil	Karen Huleback
Geoffrey C. Mead	Emma Hartnett	Jeronimas Maskeliunas
Diane G. Newell	Anna Lammerding	Hajime Toyofuku
Servé Notermans	Maarten Nauta	
Sasitorn Kanarat	Greg Paoli	
Paul Brett Vanderlinde		

Vibrio spp. in seafood

Independent experts	Expert members of the drafting group	Members of the secretariat
Nourredine Bouchriti	Anders Dalsgaard	Lahsen Ababouch
Jean-Michel Fournier	Angelo DePaola	Peter Karim BenEmbarek
Ron Lee	I. Karunasager	Maria de Lourdes Costarrica
Carlos A. Lima dos Santos	Thomas McMeekin	
Dorothy Jean McCoubrey	Ken Osaka	
Marianne Miliotis	John Sumner	
Mitsuaki Nishibuchi	Mark Walderhaug	
Pensri Rodma		
Mark Tamplin		

5 Risk Assessment of thermophilic *Campylobacter* spp. in broiler chickens

5.1.1 Summary of the Risk Assessment

5.1.2 Introduction

An understanding of thermophilic *Campylobacter* spp., and specifically *Campylobacter jejuni* in broiler chickens is important from both public health and international trade perspectives. In order to achieve such an understanding, evaluation of this pathogen-commodity combination by quantitative risk assessment methodology was undertaken.

The first steps of this process, hazard identification, hazard characterization and the conceptual model for exposure assessment were presented at an expert consultation in July 2001, and summarized in the report of that consultation[4]. The exposure assessment model described the production-to-consumption pathway for fresh and frozen broiler chicken carcasses prepared and consumed in the home.

The final step, risk characterization, integrates the exposure and dose-response information in order to attempt to quantify the human-health risk attributable to pathogenic thermophilic *Campylobacter* spp. in broiler chickens. The risk assessment model and results were presented to the present expert consultation in the working document MRA 02/01. Recommendations for modifications and/or areas for further development of the exposure assessment model, arising from the 2001 consultation were taken into consideration and, where possible, were incorporated during preparation of the MRA 02/01 report. Recommendations, that were not incorporated, are noted in the exposure assessment summary section below.

5.1.3 Scope

The purpose of the work was to develop a risk assessment that attempts to understand how the incidence of human campylobacteriosis is influenced by various factors during chicken rearing, processing, distribution, retail storage, consumer handling, meal preparation and finally consumption. A benefit of this approach is that it enables consideration of the broadest range of intervention

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[4] WHO. 2001. Report of the Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods; Hazard identification, exposure assessment and hazard characterization of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood. WHO Headquarters, Geneva, Switzerland 23 - 27 July 2001. WHO 2001. http://www.fao.org/es/ESN/food/risk_mra_campylobacter_en.stm / http://www.who.int/fsf/Micro/index.htm

strategies. The risk characterization estimates the probability of illness per serving of chicken associated with the presence of thermophilic *Campylobacter* spp. on fresh and frozen whole broiler chicken carcasses with the skin intact and which are cooked in the domestic kitchen for immediate consumption. A schematic representation of the risk assessment is shown in Figure 5.1.
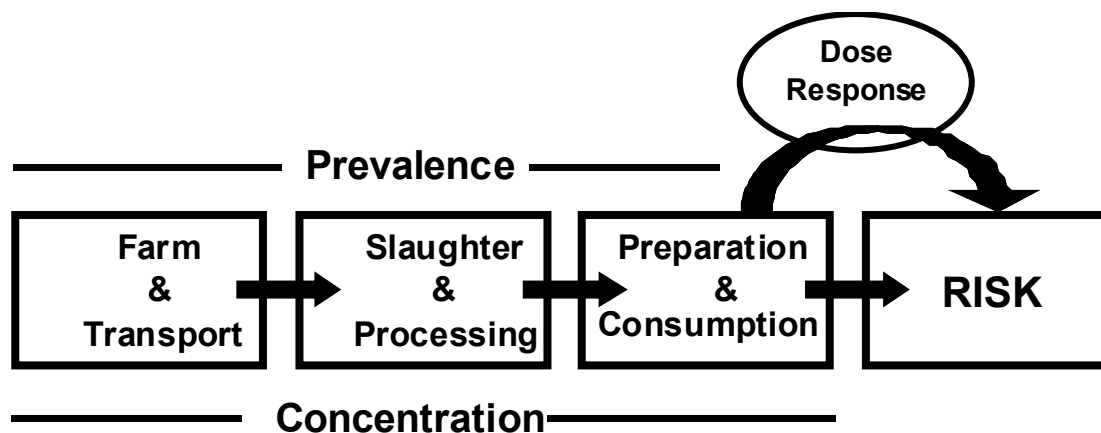
FIGURE 5.1: Schematic representation of the risk assessment of *Campylobacter* spp. in broiler chickens.

5.1.4 Approach

5.1.4.1 Hazard identification

Thermophilic *Campylobacter* spp. are a leading cause of zoonotic enteric illness in most developed countries (Friedman *et al*., 2000[5]). Human cases are usually caused by *C. jejuni,* and to a lesser extent, by *Campylobacter coli.* Information on the burden of human campylobacteriosis in developing countries is more limited (Oberhelman & Taylor, 2000[6]). Nevertheless, it is reported that asymptomatic infection with *C. jejuni* and *C. coli* is frequent in adults. In children, under the age of two, *C. jejuni, C. coli* and other *Campylobacter* spp. are all associated with enteric disease.

Campylobacter spp. may be transferred to humans by direct contact with contaminated animals or animal carcasses or indirectly through the ingestion of contaminated food or water. The principal reservoirs of thermophilic *Campylobacter* spp. are the alimentary tracts of wild and domesticated mammals and birds. *Campylobacter* spp. are commonly found in poultry, cattle, pigs, sheep, wild animals and birds, and in dogs and cats. Foodstuffs, including, poultry, beef, pork, other meat products, raw milk and milk products, and, less frequently, fish and fish products, mussels and fresh vegetables can also be contaminated (Jacobs-Reitsma, 2000[7]). Findings of analytical epidemiological studies are conflicting. Some have identified handling raw poultry and eating poultry products as important risk factors for sporadic campylobacteriosis (Friedman*, et al.,* 2000[5]), however others have found that contact in the home with such products is protective (Adak *et al*., 1995[8]).

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[5] Friedman, C., Neimann, J., Wegener, H. & Tauxe, R. 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In *Campylobacter 2nd Edition*, pp. 121-138. Edited by I. Nachamkin & M. J. Blaser. Washington DC: ASM Press.

[6] Oberhelman, R. & Taylor, D. 2000. Campylobacter infections in developing countries. In *Campylobacter 2nd Edition*, pp. 139-154. Edited by I. Nachamkin & M. Blaser. Washington DC: ASM Press.

[7] Jacobs-Reitsma, W. 2000. Campylobacter in the food supply. In *Campylobacter 2nd Ed.*, pp. 467-481. Edited by I. Nachamkin & M. J. Blaser. Washington DC: ASM Press.

[8] Adak, G. K., Cowden, J. M., Nicholas, S. & Evans, H. S. 1995. The Public Health Laboratory Service national case-control study of primary indigenous sporadic cases of campylobacter infection. *Epidemiology and Infection* 115, 15-22.

Information for the hazard identification section was compiled from published literature and from unpublished data submitted to FAO and WHO by public health agencies and other interested parties.

5.1.4.2 Hazard characterization

Hazard characterization provides a description of the public health outcomes following infection, including sequelae, pathogen characteristics influencing the organism's ability to elicit infection and illness, host characteristics that influence the acquisition of infection, and food-related factors that may affect the survival of *C. jejuni* in the human gastrointestinal tract. A dose-response model was derived that mathematically describes the relationship between the numbers of organisms that might be present in a food and consumed (the dose), and the human health outcome (the response). In order to achieve this, human feeding trial data (Black *et al.*, 1988[9]) for two strains of *C jejuni* were pooled and used to derive estimates for the probability of infection (colonization of the gastrointestinal track without overt symptoms) as well as the probability of illness once infected. Campylobacteriosis predominantly occurs as sporadic cases (Friedman, *et al.*, 2000[10]), and hence dose-response data from outbreak investigations are essentially non-existent.

The probability of infection upon ingestion of a dose of *C. jejuni* was estimated with the caveat that the data are from a feeding study involving healthy volunteers, and using a milk matrix and a limited number of campylobacter strains. Whether the probability of infection and/or illness are different for immune individuals or those individuals with an increased susceptibility to illness (e.g. immunosuppressed, very young, etc.) compared to the volunteers could not be determined from the feeding trial data. The probability of illness following infection was also estimated based upon these investigations, and assumed to be dose-independent. Again, the impact of other factors, such as susceptibility, on the probability of illness cannot be quantified due to a lack of adequate epidemiological data and resolution to this level. The progression of the illness to more serious outcomes and the development of some sequelae can be crudely estimated from the approximate proportions reported in the literature, but these were not included in the present work. For the purposes of this model it was assumed that *C. coli* has the same properties as *C. jejuni*.

5.1.4.3 Exposure assessment

The exposure model describes the stages Rearing and Transport, Slaughter and Processing, and Preparation and Consumption (Figure 5.1). This modular approach estimates the prevalence and concentration of thermophilic *Campylobacter* spp., and the changes in these associated with each stage of processing, and refrigerated and frozen storage and consumer handling of the product.

Routes contributing to initial introduction of *Campylobacter* spp. into a poultry flock[11] on the farm have been identified in epidemiological studies. However, these remain poorly understood and the phenomenon may be multi-factorial, and hence, colonization was modelled based on an unspecified route of introduction. However, an epidemiology module has been created as a supplement to the model, which allows evaluation of interventions aimed at changing the frequency with which risk factors occur. As such risk factors are usually interdependent and may be country specific, incorporation of this module into the current risk model was not appropriate: however, it does illustrate how such information may be used in a specific situation.

The effects of cooling and freezing, and adding chlorine during water chilling, are evaluated in the model. Effects of other processing treatments, e.g. lactic acid and irradiation, on reduction of *Campylobacter* spp. contamination on poultry carcasses were not explicitly evaluated as the quantitative data are lacking: however, if the level of reduction as a consequence of any type of

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[9] Black, R. E., Levine, M. M., Clements, M. L., Hughes, T. P. & Blaser, M. J. 1988. Experimental *Campylobacter jejuni* infection in humans. *Journal of Infectious Diseases* 157, 472-9.

[10] Friedman, C., Neimann, J., Wegener, H. & Tauxe, R. 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In *Campylobacter 2nd Edition*, pp. 121-138. Edited by I. Nachamkin & M. J. Blaser. Washington DC: ASM Press.

[11] A flock is a group of hens of similar age that are managed and housed together.

processing modification, can be quantified for any treatment, then the impact on risk can be readily evaluated within the risk model.

The home preparation module considered two routes of ingestion of viable organisms: via consumption of contaminated, undercooked chicken meat, and via cross-contamination, from contaminated raw poultry onto another food that is eaten without further cooking, or directly from the hands (Figure 5.2). As a result of recommendations from the previous expert consultation[12] the "protected areas" cooking approach and the drip-fluid model approach for cross-contamination were selected. The "protected areas" approach to modelling the cooking step considers that campylobacter cells may be located in parts of the carcass that reach cooking temperature more slowly than other locations. The drip-fluid model for cross-contamination relates to the spread of campylobacter cells via drip-fluid from raw carcasses.

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                    ┌─────────────────┐
                    │  Raw Chicken    │
                    └─────────────────┘
                      │             │
          ┌───────────────────┐  ┌───────────────────┐
          │ Cross contamination│  │  Under cooking    │
          └───────────────────┘  └───────────────────┘
                      │             │
                    ┌─────────────────┐
                    │    Exposure     │
                    └─────────────────┘
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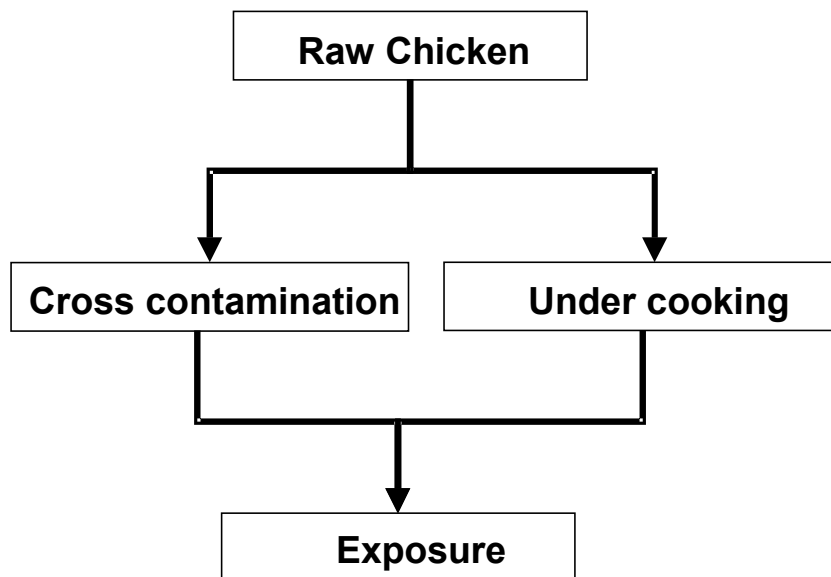
FIGURE 5.2: Schematic representation of the home preparation module.

5.1.4.4 Risk characterization

The risk characterization step integrates the information collected during the hazard identification, hazard characterization, and exposure assessment steps to arrive at estimates of adverse events that may arise due to consumption of chicken (Figure 5.1). This step links the probability and magnitude of exposure to campylobacter associated with consumption of chicken to adverse outcomes that might occur. The resulting risk is expressed as individual risk or the risk per serving of chicken. Although this model does not address risk to a specific population, data on amounts of chicken consumed can be incorporated into the model to arrive at estimates of the population-based risk.

The current model is unable to provide a central estimate of risk due to virtually unbounded uncertainty[13] on two key components of the model, namely the impact of undercooking and the impact of cross-contamination. Since these processes are the ultimate determinant of the final exposure of the consumer, the unbounded and unresolvable nature of the uncertainty undermines the establishment of a central estimate of consumer risk. In addition, the model has not yet been applied

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[12] WHO. 2001. Report of the Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods; Hazard identification, exposure assessment and hazard characterization of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood. WHO Headquarters, Geneva, Switzerland 23 - 27 July 2001. WHO 2001. http://www.fao.org/es/ESN/food/risk_mra_campylobacter_en.stm / http://www.who.int/fsf/Micro/index.htm

[13] As the upper and lower boundaries of the values describing the impact of undercooking and cross-contamination are unknown the distribution of possible values is potentially infinite and therefore unbounded.

to explore the upper and lower bound estimates of risk that are suggested by the uncertainty. However, the model still provides a useful means of studying potential exposure pathways and how these contribute to the risk of illness posed by campylobacter associated with broiler chickens.

The risk characterization involves the following elements:

1) A baseline model

A baseline model was explored in detail, to clarify the input-output relations between the different modules of the model and also to explore the credibility of the model. The baseline model was defined as the processing of fresh carcasses from both positive and negative flocks (with an overall flock prevalence of 80%), which are air chilled at the end of slaughter.

The model indicated that the external campylobacter load per chicken increased during transport and evisceration, and decreased at the other processing steps studied, with an overall reduction of the mean load from farm to fork of about 4 to 5 logs (Figure 5.3). The prevalence of campylobacter-contaminated chickens from positive flocks appears to drop from 100% of live birds to 20% of chicken meat servings (Figure 5.4). For negative flocks, prevalence increases during transport, defeathering and evisceration, indicating the effect of cross-contamination during processing. Prevalence later drops to a value of about 3% of servings at the moment of consumption.

The correlation between the input and output of the models of the different processing steps has been graphed to illustrate the variability in load per chicken along the process. These plots indicate that the load on a chicken at the stages before evisceration appears to be a bad predictor for the final load, and thus for the risk per serving of that particular chicken carcass.

2) Scenario analysis

In a scenario analysis, the effects of alterations to the baseline are studied by changing one or two of the model parameters. By doing so the impact of uncertainty in parameter estimates can be explored, and the potential of risk mitigation strategies can be evaluated.

Eight alternative scenarios were explored, to illustrate the ability of the model to investigate the effect of changing specific model inputs. Each individual scenario involved a specific change in one parameter, as follows:

Mitigation related scenarios

1. Between flock[14] prevalence (80% prevalence reduced to 50%)
2. Within flock prevalence (100% prevalence reduced to 10%)
3. Scalding (hard scald (56-58°C for 2-2.5 minutes)and soft scald (50-52 °C for up to 3.5 minutes))

Alternate model assumption scenarios

4. Shorter freezing storage time range (1 day to 6 weeks storage time compared to 1 day to 1 week storage time)
5. Undercooking (5°C lower temperature in coolest spot in the chicken during cooking)
6. Defeathering (an increase the magnitude of cross-contamination)
7. Contamination during water chilling without chlorine (a decrease of the contamination added)
8. Contamination during water chilling with chlorine (decrease of the contamination added)

The largest effects were observed with lower undercooking temperature, which led to an increase in risk and lower within flock prevalence, which led to a decrease in risk.

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[14] Between flock refers to different groups of hens (i.e. different flocks).
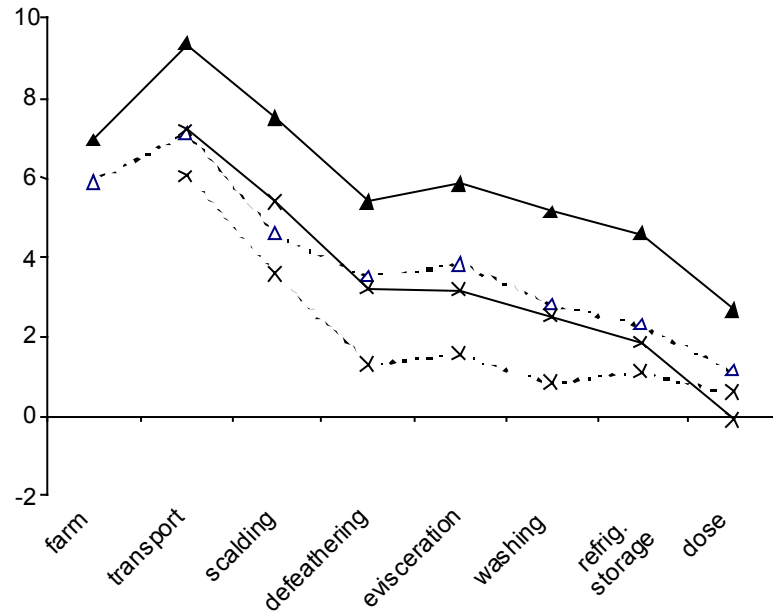
FIGURE 5.3: Numbers of campylobacter cells per carcass during processing of fresh air-chilled carcasses, from both positive and negative flocks. (Negative flocks get contaminated during transport). Both the mean of the logs and the log of the means are given. (The differences between these is as a result of the skewness[15] of the distribution of values and the fact that 'zero'- values cannot be incorporated in calculations of the mean of logs (which is therefore only about the positive carcasses)).

──▲──	log mean of positive flocks
-----ρ------	mean log of positive flocks
──x──	log mean of negative flocks
------x------	mean log of negative flocks
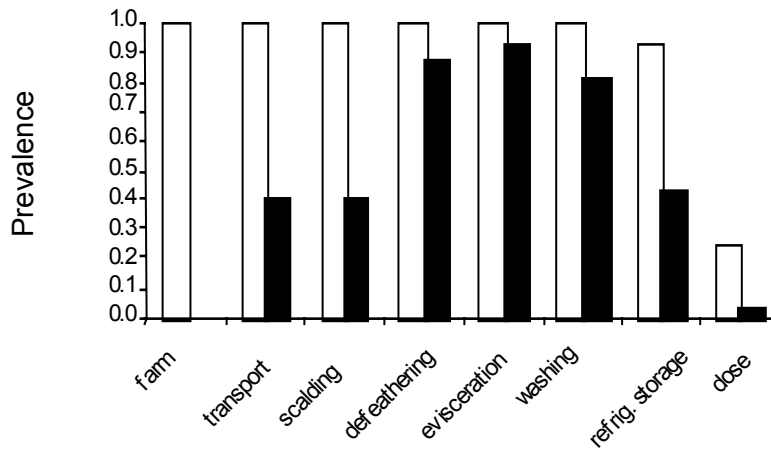
FIGURE 5.4: The prevalence of campylobacter on fresh air-chilled broiler chicken carcasses coming from flocks that were positive and negative for campylobacter at the farm level.

☐ Flocks positive for campylobacter at the farm

■ Flocks negative for campylobacter at the farm

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[15] Skewness is a statistical term that refers to the lobsideness of a distribution.

To further detail the model analysis, and to determine the effect of introducing different mitigations, five series of simulations were run. Using the baseline model settings one of following parameters was changed in each series of simulations:

- flock prevalence;
- campylobacter load on carcasses after transport;
- campylobacter load in caeca;
- campylobacter load in both caeca and on carcasses after transport;
- campylobacter load on carcasses after evisceration.

A linear relationship between flock prevalence and probability of illness was found. Thus a two-fold reduction in flock prevalence would result in a corresponding two-fold reduction in the probability of illness. This effect is mainly caused by a reduction in the number of campylobacter-positive meals. Both external contamination and colonization need to be reduced concurrently to achieve a substantial impact on risk. This is due to the potential for large numbers of campylobacter cells contaminating the carcass as a result of damage to the viscera at evisceration, thus undermining any benefits achieved in reducing the external numbers at an earlier processing step. Use of the model has identified two key ways of achieving this reduction: 1) reducing colonization early enough to impact external contamination and 2) intervening post-evisceration to reduce carcass contamination.

5.1.5 Key findings

1. There is a lack of systematic and fundamental investigation into the key processes throughout the production-to-consumption continuum that may lead to human infection as a result of chicken consumption. This was evidenced by the extensive knowledge acquired and data gaps identified during this risk assessment.

2. Quantifying and characterizing uncertainty, although often recommended, needs to be recognized as a task that can be unfeasible, and relatively unrewarding, in situations where the model contains:

    a. many uncertain parameters (lack of data and information to inform the parameters of the model),

    b. a large amount of uncertainty (lack of data and information to inform the mathematical description of how the system works, including causal relationships and dependencies), or

    c. a risk assessment simulation model that is complex (constraints in computing ability in order to perform the quantification).

    In the case of this risk assessment, all three of these properties apply. Although there were numerous quantifiable uncertainties, it was believed that model uncertainties (point b, above) would dominate the total uncertainty. The extent of the model uncertainties was such that the magnitude of the quantifiable uncertainties would be rendered irrelevant.

3. It was difficult to model cross-contamination in the home as a result of the lack of a clear understanding of the pathways and lack of data quantifying the magnitude and frequency of cross-contamination events. Further improvement of this module and validation may be extremely difficult given the complexity of cross-contamination, the many possible pathways by which it can occur, and the variability in the behaviour of individuals in the kitchen.

4. Based on thermal inactivation calculations, it was difficult to reconcile the assumed importance of undercooking as a cause of human exposure to campylobacter, if the contamination of broiler carcasses with campylobacter is on the external surface of the carcass (or very close to the surface). Resolution of this inconsistency requires the allocation of some amount of contamination to sub-surface sites within the carcass where the temperature increases much more slowly. While it is possible to demonstrate that campylobacter will, on occasion, be found in such places, it is very difficult to quantify the frequency and extent of this particular mode of contamination.

5. Overall campylobacter concentration on chicken carcasses decreased through processing, with temporary increases occurring during transport and evisceration.

6. The prevalence of campylobacter-positive carcasses from negative flocks increased up to and including evisceration and decreased at later stages. This decrease after evisceration was also found for positive flocks, depending upon the method of chilling.

7. The campylobacter load on a chicken at the stages before evisceration was identified as a bad predictor for the final load, and thus for the risk per serving of that particular chicken.

8. Assuming that cooking performance is independent of the chicken being fresh or frozen, frozen chicken posed a lower risk via consumption than fresh chicken.

9. The washing-off effect associated with water chilling translated to water-chilled chickens posing a lower risk than air-chilled chickens. However, there was uncertainty associated with the degree of cross-contamination that occurs in the chill tank during water chilling that would have an impact on this comparison and may be affected by the addition of chlorine to the chill water.

10. Undercooking was estimated to have a higher risk than cross-contamination using one set of assumptions. The cooking and cross-contamination modules are based on plausible theoretical constructs, but knowledge and data related to these two pathways are essentially unavailable. Since the set of assumptions on which this comparison relied was only one, of many, plausible sets, analysis of this component of the model remains inconclusive.

11. Unlike many other mitigation scenarios, there was very little uncertainty that reduction of the between-flock prevalence of campylobacter would reduce any associated public health risk. A linear relationship was found to exist between flock prevalence and probability of illness, i.e. a two-fold reduction in flock prevalence would result in a corresponding two-fold reduction in the probability of illness.

12. In order to meaningfully reduce the bacterial load on processed carcasses, interventions would be required to address the bacterial load both internally and externally, since efforts directed at only one of these contamination reservoirs can be readily undermined by high levels of contamination from the other.

5.1.6 Risk assessment and developing countries

The current risk assessment document also addressed the issue of whether it is possible for developing countries to apply the concepts and use the components from this model to conduct their own quantitative risk assessment. The relative complexity of the current model was recognized. It is relevant to poultry produced and processed under conditions similar to those described in the risk assessment and so, in particular, may be applicable to the large-scale production and processing facilities in developing countries. However, many of the exposure elements in developing countries - the consumption patterns, slaughter processes and farming practices - may be quite different from those described here, thus limiting the applicability of the risk assessment. Furthermore, there are few, if any, data on exposure routes, risk factors and human illness associated with campylobacter in developing countries. Thus, the possibility of performing a national quantitative microbial risk assessment may require a capacity that does not currently exist in many developing countries. There are steps that developing countries can take to aid future risk assessment efforts. A knowledge of risk management activities, which initiate and facilitate the risk assessment process, are important. The "Guidelines for incorporating quantitative risk assessment in the development of microbiological food hygiene standards, guidelines and related texts"[16] currently being elaborated by FAO and WHO include guidance on preliminary risk management activities which are critical in structuring the risk

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[16] The process of elaborating FAO/WHO "Guidelines for incorporating quantitative risk assessment in the development of microbiological food hygiene standards, guidelines and related texts" began at a consultation convened in Kiel, Germany on 18 - 22 March 2002. The draft guidelines are currently being finalized and will be available on the FAO and WHO webpages at the end of 2002. http://www.fao.org/es/ESN/food/risk_mra_riskmanagement_en.stm / http://www.who.int/fsf/Micro/index.htm

assessment process. Data collection, one of the most important related activities, received particular attention and focus was given to the main tasks that need to be undertaken.

5.1.7 Limitations and caveats

The expert consultation identified features of the assessment that have an impact on the acceptability of the model and the appropriateness of using this model. The model was developed so as not to be representative of any specific country or region. Yet many of the model inputs were based on data and processing practices in one country. Prior to using the model to predict risk for a specific country, data, that is representative of that country, should be used to determine model inputs.

A hypothetical baseline scenario was considered in order to evaluate the relative merit of control strategies for campylobacter in broiler chickens. This scenario consists of a compromise of input assumptions derived from a range of countries. The findings from the baseline scenario should not be used to draw inferences about a particular nation or region. To make specific inferences it would be necessary to collect inputs that were directly relevant to the particular population of interest.

Although the uncertainty associated with several parameters in the consumption portion of the risk assessment was accounted for, a full analysis of statistical and model uncertainty was not performed. This is explained in section 5.1.4. (under 2).

The data available for generation of the dose-response curve was limited to one feeding trial study (see section 5.1.3.2). An alteration to our current understanding of the dose-response relationship, which may occur if for example additional dose-response information became available, would result in changes in the risk estimates generated by the model.

5.1.8 Gaps in the data

In the course of undertaking this risk assessment, it was found that appropriate data was not always available and this limited the extent to which the risk assessment could be completed. The main data gaps that were identified are outlined below.

Exposure assessment: On-farm

- Survey data on the prevalence of campylobacter-positive flocks for slaughter, that includes information on sample size, test methods etc.
- Data on the probability of contamination of birds during transport.
- Studies on the dynamics of within-flock transmission of campylobacter.
- Data on the routes of campylobacter infection in broilers.

Exposure assessment: Processing

- Prevalence and enumeration data for campylobacter on carcasses before and after various processing steps such as scalding, defeathering, evisceration, washing and chilling.
- Prevalence and enumeration data for campylobacter on carcasses comparing various methods of chilling (e.g. air chilling, water chilling, water chilling with chlorine).
- Prevalence and enumeration data for campylobacter on carcasses comparing different scalding temperatures or alternate scalding configurations (e.g. multi-tank scalding systems).
- Data describing the actual cross-contamination between positive and negative flocks and within flocks during the different slaughter processes.

Exposure assessment: Post-processing and consumer handling

- Additional data on the cooking of chicken that addresses areas of the chicken where campylobacter may be protected from heat.
- Survey data and direct observational data on consumer practices in preparation and handling of chicken that especially detail the frequency and degree that transfer of campylobacter and subsequent ingestion of campylobacter could occur.

Hazard Characterization

- Data on strain variability regarding virulence/pathogenicity and survival during processing.
- Studies on the mechanisms of infectivity, virulence/pathogenicity of campylobacter in the human host.
- Quantitative information about infection and illness rates at low doses of *C. jejuni*, and also at a range of doses of different strains of *C. jejuni* and strains of *C. coli*, from 100 to 10^9 organisms.
- Complete epidemiological data from outbreak studies including enumeration of thermophilic campylobacter in suspected food items or in drinking water, numbers of people exposed, attack rates, and demographics of those exposed, particularly immunocompromised population groups and children under the age of five.
- Data describing the impact of and longevity of acquired immunity resulting from recent exposure to thermophilic campylobacter.

5.2 Review of the Risk Assessment

The expert consultation reviewed the document entitled "Preliminary Report - A Draft Risk Assessment of *Campylobacter* spp. in Broiler Chickens" (MRA02/01) and the presentation of additional data by the drafting group. It acknowledged the extensive work undertaken by the risk assessment drafting group and reviewed the components of the risk assessment document, the outcome of which is summarized below.

5.2.1 Introduction:

The expert consultation highlighted the importance of including in the introduction of the final risk assessment document a succinct, clear description of the history of this exercise: by whom it was initiated; what reports have been produced, and when. The objectives of the risk assessment should be more clearly stated, with reference to who suggested them. Objectives which have been specifically excluded should also be listed. It would be useful to state whether each objective has been achieved and if not, why not. This would facilitate a better understanding of the work and why it was developed in such a manner. Descriptions of methods and approaches should not appear in this section.

A concluding paragraph suggesting the steps to be taken following the successful conclusion of this initiative would be beneficial to the end-users, while also stressing that no risk assessment model is complete as long as data gaps exist.

5.2.2 Hazard identification

The final risk assessment document will require an up-to-date review of hazard identification associated with *Campylobacter* spp. and campylobacteriosis in humans and animals.

5.2.3 Exposure assessment:

5.2.3.1 Campylobacter in poultry on the farm and during transportation:

The main features of this module are:
- A model of a single point source of infection in the broiler house. This model is adaptable to investigate alternative sources should data become available.
- A tool for estimating the probability that a random bird will be campylobacter-positive at the point of slaughter.
- A model of the external contamination of birds during catching and transportation.

This part of the model assumes that:

- All strains have the same colonization potential and persistency in the chicken gut: with the current level of knowledge this is the only assumption that can be made, but it may be untrue.
- There are only three transmission scenarios.
- Birds stay in their social clusters during harvesting. However, it is known that birds move during harvesting so the social clusters are unlikely to be maintained.

Data deficiencies and recommendations for model improvements

It is evident that the reduction or elimination of campylobacter colonization in the flock is extremely important. At the farm level there are limited strategies to achieve this aim. Approaches include preventing flock exposure by biosecurity or reducing bird susceptibility to colonization by measures such as vaccination or competitive exclusion treatment (Newell & Wagenaar, 2000[17]). The latter approaches are not yet available commercially and, therefore, biosecurity is the only strategy currently feasible. A module to assess the relative importance of sources of colonization would be extremely useful to risk managers, and such a module has been initiated. However, it was considered by the risk assessment drafting group that there were at this time insufficient data on flock infection sources for the use of such a module.

The part of the model dealing with the effect of catchers contaminating the exterior of birds could, with some modification, be adapted to model the effects of thinning (the early removal of a proportion of the birds) which is considered a major source of broiler colonization.

Using data on between-flock and within-flock prevalence of campylobacter the probability of any random bird being campylobacter-positive can be estimated. The model also demonstrates that the probability of colonization is dependant on age which is consistent with available data (Newell and Wagenaar, 2000[17]). Although the model can indicate the effect of various generic sources on transmission it cannot, at this time, allow an assessment of the sources of exposure to provide targeted strategies for intervention.

5.2.3.2 Processing

The main features of this module are:
- A model which mimics the changes in level of contamination after scalding, defeathering, evisceration and chilling.
- A provision in the model to differentiate the effects of air and water chilling and the use of super-chlorination.
- An estimation of the prevalence of contaminated products
- A provision to model the changes in level of contamination after storage at 4 $^{\circ}$C or frozen at -20 $^{\circ}$C.

This part of the model assumes that:
- Campylobacter do not grow outside the host gut. This is consistent with most scientific evidence (Jacobs-Reitsma, 2000[18]).
- There are a finite number of sites of cross-contamination within a plant. There are however many more sites of potential cross-contamination within a processing plant other than scalding, defeathering and evisceration but these would appear to include the most important sources (Mead 1989[19]).

Data deficiencies and recommendations for model improvements

The poultry production industry has substantially improved hygiene control over the last 30 years. Many of the changes may have effects on the data entered into the model, for example the

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[17] Newell, D. G. & Wagenaar, J. A. 2000. Poultry infections and their control at the farm level. In *Campylobacter, 2nd Ed.*, pp. 497-509. Edited by I. Nachamkin & M. J. Blaser. Washington DC: ASM Press.

[18] Jacobs-Reitsma, W. 2000. Campylobacter in the food supply. In *Campylobacter 2nd Ed.*, pp. 467-481. Edited by I. Nachamkin & M. J. Blaser. Washington DC: ASM Press.

[19] Mead G.C. 1989. Hygiene problems and control of process contamination. In: Processing of poultry (ed Mead G.C.) Chapman Hall, London.pp 183-220.

introduction of multistage scalding and newer evisceration machinery which separates the viscera from the carcass. If there are data or evidence that such changes have a significant effect on campylobacter contamination of carcasses then appropriate changes to the model should be considered.

Overall, the spread of contamination from the gut contents of positive birds will have the greatest effect on the level of contamination on external bird surfaces. However, as the proportion of negative flocks increases, the importance of cross-contamination becomes more apparent. Data is now being collected for the level of contamination on carcasses from campylobacter-negative flocks. This data should be available in the near future, and the expert consultation recommended that at that time it should, if possible, be incorporated into the model.

The model should take account of the published and unpublished data showing that organisms attached to the carcass surface are resistant to environmental effects. (Notermans and Kemplemaker, 1975[20]; G. Mead, personal communication, 2002).

Only freezing and chilled storage were considered in the post-processing part of this module. Recent anecdotal data indicates that modified atmosphere packaging has an impact on levels of campylobacter contamination on poultry products. The expert consultation recommended that when this data comes into the public domain it should, if possible, be incorporated into this module.

There is increasing evidence for variation in the survival of campylobacter strains during processing (Newell *et al*., 2001[21]). This may have a considerable effect on the model especially if the ability of *Campylobacter* spp. to survive is associated with strain virulence. At this time there are no simple methods for assessing the ability to survive but models for this property are available and data is currently being accumulated. Once this data becomes available the expert consultation recommended that it should, if possible, be incorporated into this module.

5.2.3.3 Consumer handling and cooking

The main features of this part of the model are:
- Estimates of the proportion of loosely attached campylobacter cells associated with poultry.
- Estimates of the concentration of campylobacter cells in the drip-fluid from a random wet chilled carcass.
- A model of the effect of temperature, time of exposure and location at a protected site on campylobacter survival.

This part of the model assumes that:
- 10-20% of campylobacter cells are located at protected sites. The expert consultation considered this assumption to be unexpectedly high.

Data deficiencies and recommendations for model improvements

More information is required on consumer practices in domestic kitchens. Information is needed on routes and modes of transmission of campylobacter within the kitchen. Factors affecting campylobacter survival in the kitchen environment are also required.

5.2.4 Hazard characterization

Due to the lack of new data on the dose-response relationship for campylobacter, no further progress can been made in this area. Similarly there is currently a paucity of data on the proportion of campylobacter strains in poultry which are virulent in humans and to which humans are susceptible.

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[20] Notermans S. and Kamplemaker E.H. 1975. Heat destruction of some bacterial strains attached to broiler skin. British Poultry Science, 16: 351-361

[21] Newell, D. G., Shreeve, J. E., Toszeghy, M., Domingue, G., Bull, S., Humphrey, T. & Mead, G. 2001. Changes in the carriage of campylobacter strains by poultry carcasses during processing in abattoirs. *Applied and Environmental Microbiology.* 67, 2636-40.

5.2.5 Risk characterization

As highlighted in section 5.1.3.4 there is a lot of uncertainty associated with the current model, primarily due to the lack of information on the impact of undercooking and cross-contamination on exposure of the consumer to campylobacter. The expert consultation could not foresee that this uncertainty would be significantly reduced in the near future. However, despite this situation, the expert consultation noted that the model could be applied to explore the sensitivity of the risk estimates to a broad range of plausible alternate models and parameters. In addition, as much contextual information as possible on the uncertainty and its implication should be provided for risk managers and also researchers in this area so that consideration can be given as to how this uncertainty can be reduced.

The model remains very useful to study the biological and systematic plausibility of alternate hypotheses regarding these exposure pathways and will contribute to the understanding of the risk posed through these two pathways.

The expert consultation noted that for certain combinations of model parameters a 10-fold reduction in the probability of infection from a single serving had a dramatic effect on the probability of illness (about 4-fold). However, only preliminary results were available as work was ongoing to refine this section. In doing so the expert consultation recommended that the effect combined mitigations (e.g. vaccination and freezing) also be evaluated using the model.

5.2.6 Risk assessment and developing countries

It was noted that the problem of campylobacteriosis in developing countries, at a national public health level, may be considerably different from that in developed countries (Oberhelman and Taylor, 2000[22]). Epidemiological features of campylobacteriosis are distinct and suggest more frequent exposures from various sources. There is substantial and increasing evidence that immunity as a consequence of repeated exposure plays an important role in protection against campylobacteriosis (Cawthraw *et al*., 2000[23]) and this may be particularly relevant in developing countries (Newell & Nachamkin, 1992[24]). It was recommended that such countries take this factor into account when applying the model.

One of the most important issues for developing countries is whether the application of the risk assessment model is appropriate at all. The model may be usefully adapted for the exporting poultry industry in such countries, as it is anticipated that such commercial operations will be similar to those in developed countries. However, for domestic public health purposes, the current risk assessment model would need considerable adaptation. In particular, chicken processing practices may be less standardized and more variable. Moreover, food habits are likely to vary and the immune status of the population is expected to be different.

5.2.7 Data deficiencies

There are numerous data gaps which have precluded the development of a complete risk model. Therefore, the deliberate omission of uncertainty analysis in the model was accepted by the expert consultation.

It was recognized that, for further development of the model, new data are clearly needed and are also required to facilitate selection of appropriate interventions measures by risk managers.

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[22] Oberhelman, R. & Taylor, D. 2000. Campylobacter infections in developing countries. In *Campylobacter 2nd Edition*, pp. 139-154. Edited by I. Nachamkin & M. Blaser. Washington DC: ASM Press.

[23] Cawthraw, S. A., Lind, L., Kaijser, B. & Newell, D. G. 2000. Antibodies, directed towards *Campylobacter jejuni* antigens, in sera from poultry abattoir workers. *Clinical and Experimental Immunology* 122, 55-60.

[24] Newell, D. & Nachamkin, I. 1992. Immune responses directed against *Campylobacter jejuni*. In *Campylobacter jejuni: Current staus and future trends*, pp. 201-206. Edited by I. Nachamkin, M. Blaser & L. Tompkins. Washington DC: ASM Press.

The expert consultation identified the following research priorities, however, it is critical to note that research priorities will vary depending on the specific risk management question the risk assessment is being used to address.

A. Exposure assessment
- Information about the influence of strain-specific variation on campylobacter survival on poultry meat.
- Information on consumer and retail practices in relation to the risk of transfer of campylobacter from poultry products to kitchen surfaces and other foods.
- Data on the routes of campylobacter colonization of broilers at the farm level so that farm interventions can be appropriately targeted.

B. Hazard characterization
- Additional data on dose-response.
- Data on strain variability in relation to virulence and pathogenicity.

5.3 Utility and Applicability

The expert consultation recognized this risk assessment as a resource that can be used by many parties including national authorities. The current document provides a framework for undertaking risk assessment of thermophilic *Campylobacter* spp. in broiler chickens, a modelling approach that can be adapted to various situations and a unique source of data and other relevant information. While it does not overcome the need to establish a risk assessment team at the national level it will significantly reduce the workload of such a team and the time they require to carry out a risk assessment. This exercise has also led to the identification of the types of data that are required for risk assessment. While, it was noted that the collection of such data will depend on the purpose for which the risk assessment is being undertaken, the risk assessment nonetheless highlights a number of areas where data generation activities may need to be focussed.

Furthermore, the model in its current form can be used by risk managers to help them make decisions on the suitability of specific interventions. Given information on the efficacy of an intervention, the relative reduction in the number of possible cases of illness can be assessed. More specific issues in relation to the utility of this work are elaborated on below.

Applicability of the model to different production systems

The model described in the risk assessment has been developed to estimate the risk of thermophilic campylobacter from broiler chickens produced under a specified production system. The model is an example of how a risk assessment could be undertaken and details mathematical models that might be used to describe production practices. In order for the model to be used in different countries it must be modified to reflect prevailing practices.

Utility of the model to risk assessors

The model contains all the elements necessary to enable risk modellers to perform risk assessments for their own production systems. The modular approach used enables modellers to utilize various components of the model individually or collectively. When finally presented the model should be structured in a clear and concise way. This is important if the model is to be used as a tool to aid modellers in conducting risk assessments.

Utility of the model to Risk Managers

Communication is a critical element in ensuring the risk assessment model is of use to risk managers. The model should not be used by risk managers without the aid and advice of competent risk assessors, who can explain the assumptions and uncertainties associated with the model. For example in the current model cooking and cross-contamination are not modelled on 'real' data, because such data are not yet available. The expert consultation noted the limitations placed on the utility of the model by the absence of evidence and lack of consensus among experts on the relative importance of cross-contamination.

5.4 Response to the specific risk management questions posed by the Codex committee on food hygiene

As noted by the previous expert consultation on *Campylobacter* spp. in broiler chickens[25] the risk management questions posed by the CCFH were not tailored to the particular problem of campylobacter in chicken.

At a meeting of a CCFH drafting group[26], established by the committee to develop a discussion paper on risk management strategies for campylobacter in poultry, it was considered that the provision of guidance to the risk managers on the relative efficacy of mitigation strategies would be a useful outcome of the risk assessment. Given this, it was decided that interventions at various points in the overall process would be investigated rather than the investigation of any specific mitigation strategy.

Five scenarios were selected, one dealing with the effect of changing the flock prevalence and four others looking at the effect of reducing campylobacter load, either on the exterior of the chickens at slaughter or in the chickens gut before slaughter. The outcome of these scenarios are presented in the risk characterization and key findings sections (5.1.4.4 & 5.1.5).

Applying the campylobacter risk assessment to particular regions or areas will require the collection and input of data specific for local conditions. Similarly the risk assessment model may need adaptation. The risk assessment model should be used by a risk analysis team including risk assessors. The needs of risk managers must be considered as must the limitations imposed by economic, political, consumer and stakeholder priorities.

5.5 Conclusions and Recommendations

The risk assessment model utilizes a modular approach that is applicable to the entire poultry supply chain. It is flexible to use and capable of dealing with a range of issues relating to campylobacter contamination and its control in poultry from production-to-consumption. The exercise described in the report has covered both the conceptual development of the model and the evaluation of data needed to demonstrate its value. For practical application, however, the model would need to be modified, adapted or even redeveloped to suit the differing circumstances of individual users. The model takes no account of uncertainty because data are lacking on this aspect. In relation to developing countries, the model is particularly relevant to conditions of intensive production and processing that occur where poultry meat is exported. While it could also be adapted to the free range "village chicken" that is a feature of many developing countries, the expert consultation was not aware of any evidence on the risk to public health from this type of bird.

The expert consultation recognized the uncertainties surrounding the relative importance of undercooking and cross-contamination in the kitchen but, from epidemiological evidence, were of the opinion that cross-contamination was the more important factor.

The expert consultation made a number of recommendations aimed at improving the transparency and utility of the risk assessment document:

- The layout of the document should be clarified.
- The major assumptions made in the study should be listed.
- The benefits to the poultry industry from applying the model should be fully explained.
- A simplified description of the model should be made available to risk managers.
- It should be emphasized that the practical application of the model, in whatever form, will require training and guidance of putative users, particularly in developing countries.

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[25] WHO. 2001. Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods; Hazard identification, exposure assessment and hazard characterization of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood, WHO Headquarters, Geneva, Switzerland 213 - 27 July 2001. WHO 2001. http://www.fao.org/es/ESN/food/risk_mra_campylobacter_en.stm / http://www.who.int/fsf/Micro/index.htm

[26] Report of the thirty fourth session of the Codex Committee on Food Hygiene, Bangkok, Thailand, 8 - 13 October 2001 ALINORM 03/13 para. 77. http://www.codexalimentarius.net/reports.asp

- Possible intervention measures should be given in the report to illustrate actions that could be taken by risk managers.
- Technical details given in the background information on production and processing need to be updated.

6 Risk assessment of *Vibrio* spp. in seafood

6.1 Summary of the risk assessments

6.1.1 Introduction: Vibrio *spp. in seafood*

Vibrio spp. are Gram-negative, facultatively anaerobic motile curved rod-shaped bacteria with a single polar flagellum. The genus contains at least twelve species pathogenic to humans, ten of which can cause food-borne illness (Table 6.1). The majority of food-borne illness is caused by *Vibrio cholera*e, *Vibrio parahaemolyticus* or *Vibrio vulnificus* (Oliver and Kaper, 1997[27]; Dalsgaard, 1998[28]). Most countries have guidelines for detecting *V. parahaemolyticus* and *V. cholerae* O1 and O139 in seafood, whereas few have guidelines for *V. vulnificus*. Accordingly, routine microbiological analysis of seafood includes testing for *V. parahaemolyticus* and *V. cholerae* O1 and O139, but seldom for *V. vulnificus*.

Some species are primarily associated with gastrointestinal illness *(V. cholerae* and *V. parahaemolyticu*s) while others can cause non-intestinal illness, such as septicaemia *(V. vulnificu*s). In tropical and temperate regions, disease-causing species of *Vibrio* occur naturally in marine, coastal and estuarine (brackish) environments and are most abundant in estuaries. Pathogenic vibrios, in particular *V. cholerae*, can also be recovered from freshwater reaches of estuaries (Desmarchelier, 1997[29]), where it can also be introduced by faecal contamination. The occurrence of these bacteria does not generally correlate with numbers of faecal coliforms and depuration of shellfish may not reduce their numbers. However, a positive correlation between faecal contamination and levels of *V. cholerae* may be found in areas experiencing cholera outbreaks. A positive correlation between water temperature and the numbers of vibrios has also been shown in several parts of the world. Further, according to data from the United States of America and Denmark, there is a positive correlation between water temperature and both the number of human pathogenic vibrios isolated and the number of reported human infections. This correlation is particularly striking for *V. parahaemolyticus* and *V. vulnificus* (Dalsgaard *et al*., 1996[30]).

The objective of the work was to undertake a risk assessment of *Vibrio* spp. in seafood products that have the most impact on public health and/or international trade. Three species, *V. parahaemolyticus, V. vulnificus* and choleragenic *V. cholerae* (toxigenic *V. cholerae* O1 and O139 that may cause cholera) were identified as being responsible for most illnesses caused by *Vibrio* spp. The approach taken was to quantify those illnesses caused by *Vibrio* spp. in different countries following the consumption of a range of seafoods and this report documents the results of that approach.

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[27] Oliver, J. D., and Kaper, J.B. 1997. *Vibrio* Species. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville, eds. *Food Microbiology: Fundamentals and Frontiers*, p228-264. Washington, D.C., ASM Press.

[28] Dalsgaard, A. 1998. The occurrence of human pathogenic *Vibrio* spp. and *Salmonella* in aquaculture. *International Journal of Food Science and Technology*, 33: 127-138.

[29] Desmarchelier, P.M. 1997. Pathogenic Vibrios. *In* A.D. Hocking, G. Arnold, I. Jenson, K. Newton and P. Sutherland, eds. *Foodborne Microorganisms of Public Health Significance 5th Edition*, p 285 -312. North Sydney, Australian Institute of Food Science and Technology Inc.

[30] Dalsgaard, A. Möller, N.F., Brin, B., Hoei, L. and Larsen, J.L. 1996. Chemical manifestation and epidemiology of *Vibrio vulnificus* in Denmark (summer 1999). European Journal of Clinical Microbiology and Infectious diseases 15. 227 - 232.

6.1.2 Scope

The risk assessment work was undertaken on the following pathogen-commodity combinations:

- *Vibrio parahaemolyticus* in raw oysters consumed in Japan, New Zealand, Canada, Australia and the United States of America.
- *Vibrio parahaemolyticus* in finfish consumed raw.
- *Vibrio parahaemolyticus* in bloody clams consumed in Thailand.
- *Vibrio vulnificus* in raw oysters consumed in the United States of America.
- Choleragenic *Vibrio cholerae* in warm-water shrimp in international trade.

TABLE 6.1: *Vibrio* spp. which cause, or are associated with, human infections (after Dalsgaard, 1998[31])

	Occurrence in human clinical specimens*	
	Intestinal	Non-intestinal
V. cholerae O1 and O139	++++	+
V. cholerae non-O1/non-O139	++	++
V. parahaemolyticus	++++	+
V. fluvialis	++	-
V. furnissii	++	-
V. hollisae	++	-
V. mimicus	++	+
V. metschnikovii	+	+
*V. vulnificus***	+	+++
V. alginolyticus	-	++
V. carchariae	-	+
V. cincinnatiensis	-	+
V. damsela	-	+

*The symbol (+) refers to the relative frequency of each organism in clinical specimens and (-) indicated that the organism was not found

**The ability of *V. vulnificus* to cause gastro-intestinal disease remains to be confirmed

6.1.3 Vibrio parahaemolyticus *in raw oysters consumed in Japan, New Zealand, Canada, Australia and the United States of America*

6.1.3.1 Introduction

FAO and WHO aim to make optimal use of existing risk assessments in their MRA activities. As there have been large outbreaks of illness due *to V. parahaemolyticus* in North America following consumption of raw oysters, the United States Food and Drug Administration (USFDA) commissioned a quantitative risk assessment on the "Public Health Impact of *Vibrio parahaemolyticus* in Raw Molluscan Shellfish" (FDA-VPRA), one output of which was the

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[31] Dalsgaard, A. 1998. The occurrence of human pathogenic *Vibrio* spp. and *Salmonella* in aquaculture. *International Journal of Food Science and Technology*, 33: 127-138.

development of a risk model. A critical component of the model was water temperature. Since high water temperatures are a factor in several countries with significant oyster industries, FAO and WHO decided to undertake a risk assessment on consumption of raw oysters in a number of different countries. As well as generating an estimate of the number of annual illnesses, a further aim was to assess the potential of the model developed in the United States of America to predict oyster-borne *V. parahaemolyticus* illness from oysters grown in different regions and using different production systems.

6.1.3.2 Scope

The risk assessment covers consumption of raw oysters in five countries: New Zealand, Japan, Canada, Australia and the United States of America.

6.1.3.3 Hazard identification

V. parahaemolyticus has been recognized as a major cause of seafood-borne gastroenteritis in Japan (Twedt, 1989[32]; Ministry of Health, Labour and Welfare, Japan, 2000[33]) and other Asian countries. By contrast, in most countries outside of Asia, the reported incidence appears to be low, perhaps reflecting a different mode of seafood consumption. Gastroenteritis caused by this organism is almost exclusively associated with seafood consumed raw or inadequately cooked, or contaminated after cooking. In the United States of America, prior to 1997, illness was most commonly associated with crabs, oysters, shrimp and lobster (Twedt, 1989[32]; Oliver and Kaper, 1997[34]). Four *V. parahaemolyticus* outbreaks associated with the consumption of raw oysters were reported in the United States of America in 1997 and 1998 (DePaola *et al.,* 2000[35]). A new *V. parahaemolyticus* clone of O3:K6 serotype emerged in Calcutta in 1996. It has spread throughout Asia and to the United States of America elevating the status of *V. parahaemolyticus* to pandemic (Matsumoto *et al.*, 2000[36]). 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[37]) and there was also a death in 1992 associated with the consumption of oysters.

6.1.3.4 Hazard characterization

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. It included the elaboration of a dose-response curve. Infection by *V. parahaemolyticus* is characterized by an acute gastroenteritis. Therefore, the end-point of the dose-response curve was defined as gastroenteritis.

A review of the literature was undertaken to identify and characterize the infectivity and genetic factors of *V. parahaemolyticus,* which has both pathogenic and non-pathogenic forms based on the presence of specific virulence genes: *tdh* (thermostable direct haemolysin gene) and *trh* (TDH-related haemolysin gene). Relevant factors with respect to the host and food matrix have been identified and where data are available may be incorporated into the model.

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[32] Twedt, R. M. 1989. *Vibrio parahaemolyticus*. *In* M. P. Doyle, ed. *Foodborne Bacterial Pathogens*, p543-568. New York, Marcel Decker, Inc.

[33] Ministry of Health, Labour and Welfare, Japan 2000. Statistics of Food Poisoning Japan in 2000.

[34] Oliver, J. D., and Kaper, J.B. 1997. *Vibrio* Species. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville, eds. *Food Microbiology: Fundamentals and Frontiers*, p228-264. Washington, D.C., ASM Press.

[35] DePaola, A., C.A. Kaysner, J.C. Bowers, and D.W. Cook. 2000. Environmental investigations of *Vibrio parahaemolyticus* in oysters following outbreaks in Washington, Texas, and New York (1997, 1998*). Applied and Environmental Microbiology*, 66: 4649-4654.

[36] Matsumoto, C., J. Okuda, M. Ishibashi, M. Iwanaga, P. Garg, T. Rammamurthy, H. Wong, A. DePaola, Y.B. Kim, M.J. Albert, and M. Nishibuchi. 2000. Pandemic spread of an O3:K6 clone of *Vibrio parahaemolyticus* and emergence of related strains evidenced by arbitrarily primed PCR and toxRS sequence analysis. *Journal of Clinical Microbiology*, 38: 578-585.

[37] Kraa, E. 1995. Surveillance and epidemiology of food-borne illness in NSW, Australia. *Food Australia*, 47(9): 418- 423.

The determination of the dose-response relationship was based on the best available data. Human volunteer studies were available for the construction of the dose-response curve for *V. parahaemolyticus*, however, these studies characterize the dose-response relationship for *V. parahaemolyticus* administered with a pH-neutralizing buffer rather than with a food matrix. The data were analysed using curve-fitting routines to find a best fit for the Beta-Poisson dose-response curve. Because of the limited amount of data available from human volunteer studies the resulting dose-response relationship is uncertain. This uncertainty was accounted for by representing the dose-response relationship in the form of a family of plausible data-derived dose-response curves determined using resampling techniques. Figure 6.1 shows the most probable dose-response curve for *V. parahaemolyticus*; however, the family of curves representing uncertainty that surrounds the curve is not shown.
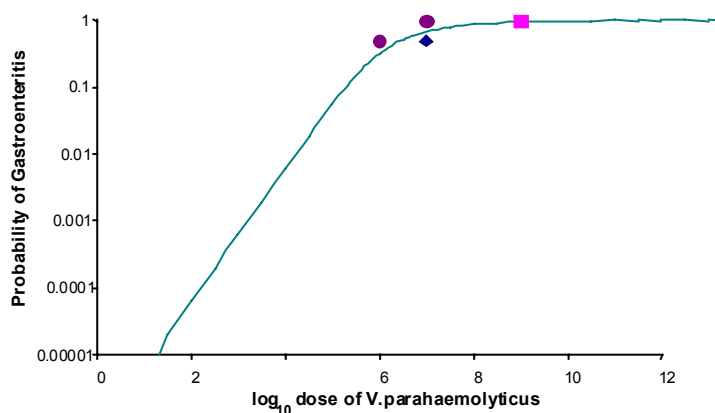
FIGURE 6.1: Beta-Poisson dose-response curve for *V. parahaemolyticus (*endpoint modelled is gastrointestinal illness*)*

⸺Beta-Poisson

♦ Sanyal and Sen (1974)[38]

■ Aiso and Fujiwara (1963)[39]

● Takikawa (1958)[40]

6.1.3.5 Exposure assessment

In the United States of America, during 1997 and 1998, there were more than 700 cases of illness due to *V. parahaemolyticus*, the majority of which were associated with the consumption of raw oysters. In two of the 1998 outbreaks a serotype of *V. parahaemolyticus* previously reported only in Asia, O3:K6, emerged as a principal cause of illness for the first time. It was suggested that warmer than usual water temperatures were responsible for the outbreaks.

Temperature profiles in the oyster industries of Japan, New Zealand, Australia, Canada and the United States of America were obtained, together with consumption levels of oysters and bacterial levels of *V. parahaemolyticus* in the oysters. The objectives were to quantify the exposure of consumers to pathogenic *V. parahaemolyticus* from consumption of raw oysters in these countries.

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[38] Sanyal, S.C., and Sen P.C. 1974. Human volunteer study on the pathogenicity of *Vibrio parahaemolyticus*. *In* T. Fujino, G. Sakaguchi, R. Sakazaki, and Y. Takeda. eds. *International Symposium on Vibrio parahaemolyticus*. p. 227-230. Tokyo, Saikon Publishing Company.

[39] Aiso K & Fujiwara K. 1963. Feeding tests of the pathogenic halophilic bacteria. *Annual Research Report Institute of Food Microbiology Chiba University*, 15:34-38.

[40] Takikawa I. 1958. Studies on pathogenic halophilic bacteria. *Yokohama Medical Bulletin*, 9:313-322.

The FDA-VPRA model was used as the base to accommodate data inputs from other countries. This model incorporates all phases in the harvest - post-harvest – consumption continuum in three modules (Figures 6.2-6.4).

Figure 6.2 shows a conceptual model for the harvest module. Water temperature is the driving input with regard to the initial numbers of *V. parahaemolyticus* in oysters. In the way the analysis is constructed regional and seasonal temperature variations allow for a multi-year analysis that can account for long-term temperature trends. Water salinity is shown in dotted lines to indicate that for some model applications salinity may be another important input.
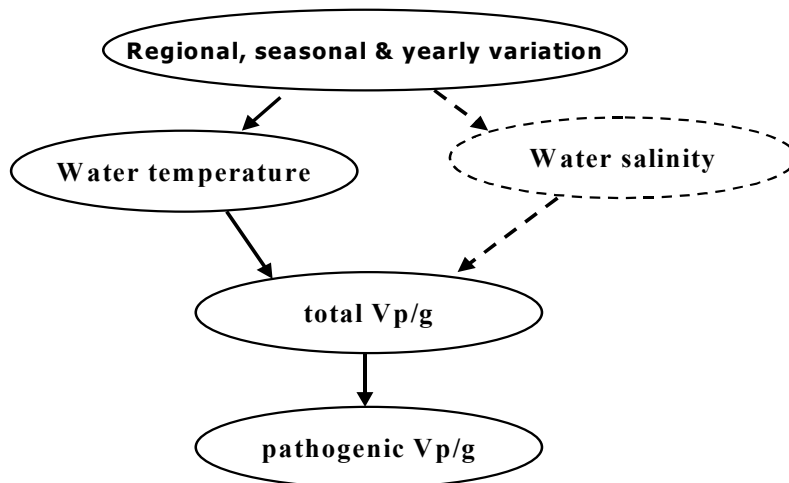
FIGURE 6.2: Harvest module for exposure assessment of *V. parahaemolyticus* in oysters.
(Vp = *Vibrio parahaemolyticus*)
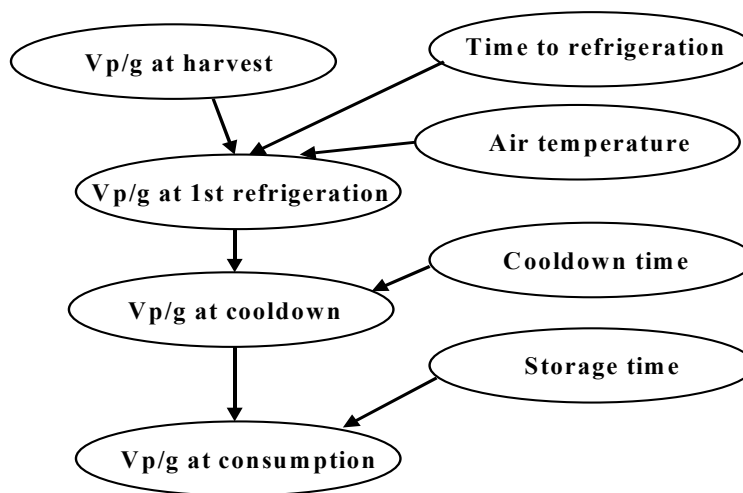
FIGURE 6.3: Post-harvest module for exposure assessment of *V. parahaemolyticus* in oysters.
(Vp = pathogenic *Vibrio parahaemolyticus*)

Figure 6.3 shows the conceptual model for post-harvesting practices. The post-harvest module determines the role of post-harvest processing and handling on the numbers of pathogenic

V. parahaemolyticus at consumption. The bubble denoting "V.p/g at harvest" is the output of the harvest model shown in Figure 6.2. Inputs on the time the oysters are out of the water and the air temperature are used to predict growth of *V. parahaemolyticus* in the oysters. Growth continues as the oysters are cooled but at a different rate. *V. parahaemolyticus* levels decrease during storage and the storage time is therefore an input time that affects *V. parahaemolyticus* numbers.

Figure 6.4 represents the consumption module. The bubble denoting "path Vp/g (numbers) at consumption" is the output of the post-harvest module. This number is multiplied by the number of oysters per serving and the weight of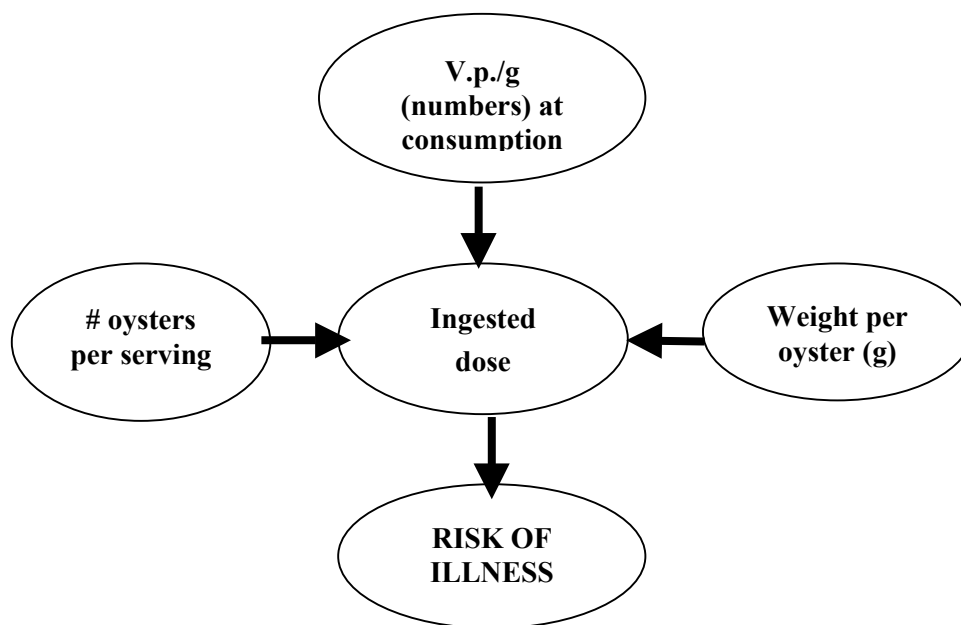 the oysters to yield the ingested dose. This ingested dose is used in the dose-response to calculate the risk of illness associated with the consumption of one oyster meal.

FIGURE 6.4: Consumption module for exposure assessment of *V. parahaemolyticus* in oysters (Vp = pathogenic *Vibrio parahaemolyticus*)

6.1.3.6 Risk characterization

The data from the five countries were analysed for incorporation into the risk assessment model. The risk assessment model was modified to allow for a monthly analysis of data from Japan, Australia, and New Zealand. The analysis for Canada and the United States of America was done on a seasonal basis. Using the Japanese data only one simulation, consisting of 100 000 iterations, was undertaken, as multiple year temperature data were not available. Thirteen simulations, consisting of 10 000 iterations were undertaken for Australia reflecting the availability of 13 years of data. As only one year's data were available for New Zealand, one simulation, consisting of 100 000 iterations, was undertaken. For Canada 1 000 simulations, consisting of 10 000 iterations, based on United States of America Pacific Northwest data, was undertaken. The analysis for the United States of America consisted of 10 000 iterations for seasons in four regions.

6.1.3.7 Key findings

Introduction

The complete data sets required to test the applicability of the model to harvesting waters of countries other than the United States of America were not available. In particular *tdh*[+] and *trh*[+] data were lacking and in such cases United States of America data was used as a surrogate to allow for testing of the model.

Japan

Based on the available data set[41], the preliminary predictions of illness are shown in Table 6.2. The model predicted low levels of illness for November to April. The model was not run for the months of May to October as oysters for raw consumption are not harvested during this period[42].

It was difficult to compare this with epidemiological data for *V. parahaemolyticus*-related oyster illnesses in Japan for a number of reasons. The Japanese surveillance system focuses mainly on outbreaks of food-borne disease and therefore the number of laboratory confirmed reported illnesses may not include sporadic cases or diffuse outbreaks and the extent of under-reporting is not known (K Osaka, personal communication, 2002). In addition the food source of the illness may not always be identified. However, in cases where oysters have been identified as the food source causing illness, large variability in the annual number of *V. parahaemolyticus*-related oyster illnesses has been noted over the last five years[43]. It is also worth noting that the model estimation is based on data (e.g. air and water temperature, salinity) available from only one of the major harvesting areas and therefore does not necessarily capture the situation in the different oyster growing areas in Japan.

TABLE 6.2: Preliminary predictions of *V. parahaemolyticus* illness in Japan associated with oyster consumption

	First quarter (Jan-Mar)	Second quarter (Apr-Jun)	Third quarter (Jul-Sep)	Fourth quarter (Oct-Dec)	Total
Number of predicted illnesses	4	1 (April only[42])	0[42]	196[42] (Nov-Dec only)	201

Australia

Based on the available data set, the preliminary predictions of illness are shown in Table 6.3. The model predicted more illnesses than the number of reported cases (J. Sumner, personal communication, 2002). The application of United States of America surrogate data to a different species of oyster, specifically the Sydney rock oyster, may have a role in the overestimation of risk.

TABLE 6.3: Preliminary predictions of *V. parahaemolyticus* illness in Australia associated with oyster consumption

	First quarter (Jan-Mar)	Second quarter (Apr-Jun)	Third quarter (Jul-Sep)	Fourth quarter (Oct-Dec)	Total
Number of predicted illnesses	157	28	10	33	228

New Zealand

The model predicted more illnesses than the number of reported cases (D.J. McCoubrey, personal communication, 2002) (Table 6.4). As extensive use of surrogate data from the United States

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[41] Japanese water temperature is available at http://www.hiroins-net.ne.jp/suisansc/suion.html; Air temperature data came from the Japanese Meteorological Agency; Ogawa, H. Tokunou, H., Kishmoto, T., Fukuda, S., Umemura, K. & Takata, M. (1989) Ecology of *V. parahaemolyticus* in Hiroshima Bay. Hiroshima. Journal of Veterinary Medicine No. 4. (in Japanese); Consumption data came from "Family income and expenditure survey (2000) (The Japan Institute of Labour) and "The national nutrition survey" (1995) (Japanese Ministry of Health and Welfare)

[42] Oysters for raw consumption are not harvested in Japan from May to October in the area where the data was collected because Microbiological criteria (MPN of coliform group in harvesting seawater, the total plate count numbers, MPN of coliform groups and MPN of total *Vibrio parahaemolyticus* in oyster) are exceeding the standards set by the Ministry of Health, Labour and Welfare.(Personal communication, Ken Osaka)

[43] Anonymous. 1999. Report of Food Poisoning , Japan 1997-2001, Ministry of Health, Labour and Welfare, Japan.

of America was necessary as inputs for some of the parameter required to run the model, the true risk may be much lower than that predicted.

TABLE 6.4: Preliminary predictions of *V. parahaemolyticus* illness in New Zealand associated with oyster consumption

	First quarter (Jan-Mar)	Second quarter (Apr-Jun)	Third quarter (Jul-Sep)	Fourth quarter (Oct-Dec)	Total
Number of predicted illnesses	13	17	0	5	35

Canada

The preliminary results (Table 6.5) indicate that model predicted cases of illness that are relatively close to the number of reported cases [44][45]). The proximity of the Canadian harvesting waters to one of the regions of the United States of America that was modelled allows greater confidence in these predictions. It should be noted that the model did not consider the mitigation to cool oysters immediately after harvest that was introduced in the Canadian oyster industry in 2000, as the data used was collected prior to the implementation of this measure.

TABLE 6.5: Preliminary predictions of *V. parahaemolyticus* illness in Canada associated with oyster consumption

	First quarter (Jan-Mar)	Second quarter (Apr-Jun)	Third quarter (Jul-Sep)	Fourth quarter (Oct-Dec)	Total
Number of predicted illnesses	0	1	7	0	8

United States of America

The predicted numbers of illness for the United States of America as shown in Table 6.6. In this case the dose-response relationship was adjusted to take into account the estimation that the actual number of cases of *V. parahaemolyticus* illness in the United States of America exceeds the reported number of cases by a factor of 20 to 1 (Mead *et al,* 1999[46]). However, it was acknowledged that the predicted number of illnesses associated with oyster consumption is probably still an overestimation as the study of Mead *et al* (1999)[46] that estimated the degree of under-reporting used statistics on the annual incidence of *V. parahaemolyticus* illness and not only those for which oyster was the vehicle of transmission. Evidence for validation of the model comes from the observed agreement between model predictions of *V. parahaemolyticus* numbers with observed harvesting and retail numbers of *V. parahaemolyticus*.

Table 6.5: Preliminary predictions of *V. parahaemolyticus* illness in the United States of America associated with oyster consumption

	First quarter (Jan-Mar)	Second quarter (Apr-Jun)	Third quarter (Jul-Sep)	Fourth quarter (Oct-Dec)	Total
Number of predicted illnesses	40	1587	3881	376	5884

[44] Cato, J.C. 1998. Economic values associated with seafood safety and implementation of seafood, Hazard Analysis Critical Control Point (HACCP) programmes. *FAO Fisheries Technical Paper.* No. 381. Rome, FAO. 1998.

[45] Anonymous, 1997, Canada Communicable Disease Report - Volume 23-19, October 1, 1997.

[46] Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. and Tauxe R.V. 1999. Food-Related Illness and Death in the United States *Emerging Infectious Diseases*, 5 (5), 607-625.

6.1.3.8 Limitations and Caveats

It was difficult to critically evaluate the performance of the model in harvesting waters outside of the United States of America. In many cases the raw data on which to adapt the model to local conditions were not available because:

- The data had not been accumulated because of expense or lack of need for the data.
- The data were in summary form and therefore not amenable to reanalysis.
- The data were difficult to retrieve from stored printed form and to convert into electronic format.
- The methodology used to generate the data in countries other than the United States of America was not comparable to that used in generating the data that served as a basis for the establishment of the model parameters.

Where limited data were available, judgement was needed on how to adapt this data for incorporation into the model. However, there is currently no guidance on this issue or even whether adaptation of data is desirable.

Validation of model predictions by epidemiological observations was complicated by the fact that the relationship between observed and predicted illness is generally unknown. In the United States of America the ratio of predicted to observed illness has been estimated to be 20 to 1 (Mead *et al*, 1999[47]). This relationship has not been estimated for other countries and where it may differ from that in the United States of America.

Limited data has the effect of reducing the variance of the model's prediction of risk. The reduced variance of predictions may be misinterpreted as greater confidence in a predicted risk than a predicted risk with wider variance that is based upon more extensive data.

The species of oyster may have a profound effect on the model and further research is needed to develop the oyster-*V. parahaemolyticus* ecology knowledge base.

Accurate model predictions may require adapting the model to parameters that are critical to harvesting areas and are different from those in the United States of America where the model was developed. For example, salinity may be a critical element in the control of *V. parahaemolyticus* in New Zealand and Australia. The model will be elaborated to test whether the addition of this parameter can improve model predictions.

The use of surrogate data, particularly in relation to the occurrence of *tdh+* and *trh+* strains, may limit the utility of the model in predicting illnesses from *V. parahaemolyticus* contaminated oysters harvested from waters other than those of the United States of America. Obtaining these data may be difficult, especially when few illnesses associated with oysters from certain harvesting areas lead to the fact that data (required by the model) have not being collected.

6.1.3.9 Data gaps

The risk assessment identified a number of data gaps which limited in particular the application of the model developed in the United States of America to oysters harvested in different regions of the world. Some of the main data and knowledge gaps include:

- Multi-year temperature data for seawater and air at harvesting areas.
- Data for determining the temperature to *V. parahaemolyticus* numbers relationship in some harvesting areas.
- Characterization of harvesting activities in some areas.
- Information on the role of oyster ecology in altering the model parameters.
- Data for tdh^+ and trh^+ prevalence of the total *V. parahaemolyticus* in some national harvesting waters.
- Dose-response information, the lack of which results in uncertainty in the dose-response relationship and adds substantial variance to predictions of illness.

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[47] Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. and Tauxe R.V. 1999. Food-Related Illness and Death in the United States *Emerging Infectious Diseases*, 5 (5), 607-625

- Methodology for estimating the ratio of reported to total cases of illness. Appropriate methodology needs to be officially developed and applied in countries that wish to compare the number of illnesses predicted by risk assessment to the number of reported & recorded cases of illness.
- Data sources that can indicate whether or not the model is succeeding. Validation of the risk assessment model should be attempted at as many intermediate stages as is practical.

6.1.4 Vibrio parahaemolyticus *in bloody clams*

6.1.4.1 Introduction

V. parahaemolyticus has been recognized as a major cause of food-borne gastroenteritis in Japan and other Asian countries. However, the data available on *V. parahaemolyticus* and seafood, other than oysters, that was also suitable for the quantitative risk assessment were very limited world-wide.

A small-scale study was undertaken, based on data collected in the Songkla Province of southern Thailand. A joint Thai-Japanese team carried out the study on the prevalence and concentration of *V. parahaemolyticus* in non-oyster seafood. All strains of *V. parahaemolyticus* and pathogenic strains which have the *tdh* and / or the *trh* gene, and thus have the potential to produce TDH and /or TRH, were enumerated in the data collection process. No foodborne disease surveillance data for this area were available. However, the preliminary study showed that the strains isolated from clinical specimens in this area were identical, in terms of serotype and molecular genetics, with the strains isolated from the shellfish harvested in the area rather than other seafood such as fish and shrimps. Therefore, a popular bivalve in Thailand, the bloody clam (*Anadara granosa)*, was chosen as the target seafood in this risk assessment. This shellfish is also traded in the Southeast Asian region.

6.1.4.2 Scope

Using state of the art techniques, the data necessary for developing the quantitative risk assessment were collected and a model was elaborated in a developing country situation where there was a lack of quantitative data.

6.1.4.3 Hazard identification

V. parahaemolyticus is considered to be an important cause of seafood-borne disease in Thailand. A survey of clinical specimens obtained from patients with diarrhoea resulted in the isolation of 294 pathogenic strains from 317 cases that were confirmed positive for *V. parahaemolyticus* (Table 6.6). Several seafood items were also tested for pathogenic strains of *V. parahaemolyticus* and in this preliminary study shellfish were the most commonly contaminated among the samples tested (Table 6.6). The profile of strains (serotype and possession of *tdh* / *trh* gene) isolated from clinical samples were consistent with that of the strains isolated from shellfish (Table 6.2). Therefore, shellfish were considered as an important source of *V. parahaemolyticus* infection.

6.1.4.4 Hazard characterization

The dose-response model used in the hazard characterization of *V. parahaemolyticus* in oysters (see section 6.1.3.4) was also used in the hazard characterization of *V. parahaemolyticus* in bloody clams.

6.1.4.5 Exposure assessment

The exposure assessment was divided into four stages; harvest, retail, cooking and consumption as shown in Figure 6.5.

Data on the prevalence and numbers of *V. parahaemolyticus* in clams was collected at each step of the exposure pathway. A single lot of the clams was taken from a boat shortly after landing at

the harvest site. Following initial sampling ("Harvest" stage), the remaining clams were transported to the local market area, which was located close to the laboratory. A sample of clams were examined at this point to represent the "Retail" stage. Thereafter, the clams were maintained outside of the laboratory for a period of time to simulate the transportation step; these were subsequently examined in the laboratory. Typically, the clams are cooked in the home by boiling briefly (in some cases with insufficient heating). The "Cook (Boiling)" stage was simulated in the laboratory and the clams were tested thereafter. To obtain consumption data, local people were interviewed on the frequency and quantity of bloody clams consumed.

TABLE 6.6: Results of the study on isolation of *V. parahaemolyticus* from seafood and the most common strain profiles of isolates from clinical specimens and seafood

	Isolation of pathogenic strains of *V. parahaemolyticus*	O3:K6 *tdh+, trh-*	O1:K25 *tdh+, trh-*
Seafood samples*			
Shellfish(bivalves)	13/268 (4.4%)	8(62%)	2(15%)
Shrimp	0/50	0	0
Crab	0/9	0	0-
Fish	0/100	0-	0-
Clinical samples**	294/11 375 (2.6%)	192 (65%)	22(7.5%)

*Samples were examined over a four year period from 1998 to 2001. During the first year of the study period pathogenic *V. parahaemolyticus* were only isolated from shellfish. Therefore, during the subsequent years of the survey, efforts focussed mainly on detection of pathogenic *V. parahaemolyticus* in shellfish samples.

** *V. parahaemolyticus* was isolated from 317 diarrhoea specimens out of a total of 11 375 samples that were examined during a survey sporadic cases of illness with diarrhoea in 1999. Specimens came from different patients in two big hospitals in the province. Of the 317 cases confirmed positive for *V. parahaemolyticus*, 294 of these were confirmed to be pathogenic strains of *V. parahaemolyticus*.
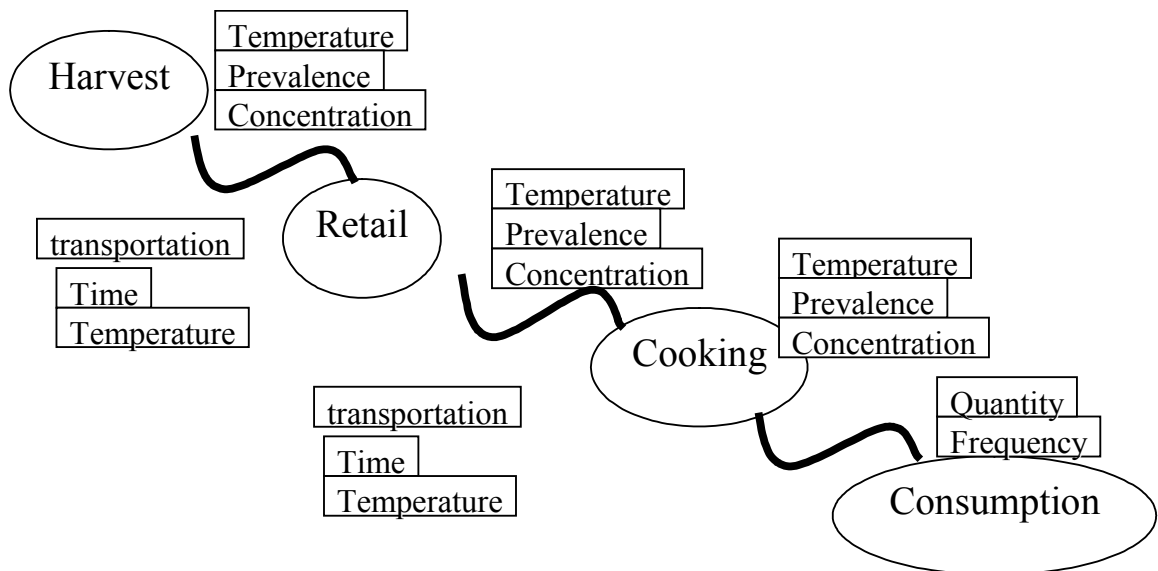
FIGURE 6.5: Schematic representation of the exposure model developed for the risk assessment of *V. parahaemolyticus* in bloody clams.

The laboratory analysis undertaken included *V. parahaemolyticus toxR* gene sequencing to identify *V. parahaemolyticus* and group-specific PCR (GS-PCR) to detect pandemic strains carrying the *tdh* gene. The prevalence of total and *tdh*[+] or *trh*[+] *V. parahaemolyticus* strains was examined at harvest and retail, and after cooking.

The total numbers of *V. parahaemolyticus* from culture and PCR methods were assumed to have a lognormal distribution. The prevalence of total *V. parahaemolyticus* after boiling was estimated using the laboratory generated data. The prevalence of *tdh*[+] and *trh*[+] strains that possibly remain in clams after boiling was estimated by assuming that the ratio of the prevalence of total and virulent strains before heating was maintained after boiling. The same assumption was made with regard to numbers.

Comparison was made between the predicted and observed values of total *V. parahaemolyticus* numbers during transportation from harvest to the retail stage, in order to determine whether the increase in numbers could be analysed or predicted using an equation developed in the FDA-VPRA.

Although bloody clams are a popular seafood in this region there were no available data on their consumption. Therefore a small preliminary consumption survey was undertaken. Fourteen people (students and workers) at the university were selected for interview because of their accessibility. They were interviewed on how frequently they ate clams at home and how many they ate at one meal.

6.1.4.6 Risk characterization

The output of the exposure assessment feeds into the hazard characterization to produce the risk characterization output. The probability of getting ill following consumption of a single serving of clams was estimated for a defined population (i.e. people who were interviewed) by using the "dose" calculated in the exposure assessment and the dose-response equation. The probability of getting ill per year was further estimated by multiplying the frequency of clam consumption per year. The consumption data for bloody clams were used for estimating the risk of ingesting pathogenic strains of *V. parahaemolyticus*.

6.1.4.7 Key findings

1. The total number of *V. parahaemolyticus* was estimated as 6.5 /clam, with a standard deviation of 2.2 /clam, at harvest, and 7.8 /clam, with a standard deviation of 2.0 /clam, at retail.
2. After boiling, *V. parahaemolyticus* was detected in only one and two out of 32 samples by PCR and culture methods, respectively. Pathogenic strains were not isolated from any of the boiled samples.
3. Using the data generated from culture methods, the mean probability of illness per year due to clam consumption was estimated to be 9.18E-10 per person (approx. 1 person per 1 000 000 000 people becomes ill per year) and maximum probability was 9.34E-6 (approx. 1 person per 100 000 people becomes ill per year).
4. The observed growth rate of *V. parahaemolyticus* in bloody clams was found to be half the rate of growth predicted by the FDA-VPRA *V. parahaemolyticus* growth rate model in oysters.
5. Although time and resources were limited and there was a lack of quantitative data, this study indicated that, even when such obstacles exist, progress can still be made on data generation and risk assessment modelling.

6.1.4.8 Limitations and Caveats

The link between human illness and consumption of bloody clams was based on detection of strains of equivalent serotype and molecular genetics in both clinical samples and bivalve samples. There were no data from outbreak investigations or case control studies of sporadic cases to confirm this link or to prove that illness was indeed caused by foodborne transmission. Additional data is required to strengthen this linkage and this should ideally be included in the risk profile that is undertaken before the risk assessment is commissioned.

The results are restricted to a single food item, and the sample size may not be sufficiently large. Therefore, the data presented in table 6.6 should be interpreted with caution. Furthermore, the study on survival of *V. parahaemolyticus* from harvest to consumption was carried out for only a three month period in one specific area in Thailand. More data are needed for other months and other areas.

Because the cooking (by boiling) module was developed based on experimental data using fixed time / temperature values within a very limited range, scenario analysis with different time / temperature combinations are impossible. Also the consumption survey was carried out on a small group of people working within the same environment and therefore may not be representative of the region as a whole.

The cross-contamination model was not applied in this risk-assessment, because of lack of data and appropriate models for cross-contamination. Due to insufficient epidemiological data, model validation could not be undertaken.

6.1.4.9 Gaps in the data

To improve the risk assessment, the following data will be needed.
- Quantitative data on *V. parahaemolyticus* in bloody clams and other shellfish in various combinations of water temperature and salinity.
- The proportion of virulent strains in various shellfish, areas and seasons.
- The differences in sensitivity between virulent strain and non-virulent strain to heating and other mitigation steps.
- Data from a case control study or an outbreak investigation to strengthen the linkage between consumption of bloody clams and *V. parahaemolyticus* illness in humans.

6.1.5 Vibrio parahaemolyticus *in finfish*

6.1.5.1 Introduction

V. parahaemolyticus is a leading cause of seafood-borne illness in Japan and other Asian countries Several reports exist on the high prevalence of the organism in a variety of seafoods, in particular finfish, lobster and shrimp. Outbreaks due to *V. parahaemolyticus* associated with fish and shellfish other than oysters have been reported in some countries including the United States of America, Thailand, China (Taiwan) and Spain. With the globalization of Japanese cuisine and the increased practice of eating raw fish and shellfish, there is an increased possibility of *V. parahaemolyticus* infection as a result of consumption of these foods. A risk assessment of *V. parahaemolyticus* in finfish could provide useful information for reducing this risk.

An exposure assessment document was prepared and presented to an expert consultation[48] in 2001. Although the drafting group had decided not to include this part in the final report due to the lack of quantitative data, it was noted that, although not a complete quantitative risk assessment, it still includes information that may be important for many countries and therefore should be recorded and available in the public domain.

This work could currently be described as a qualitative (descriptive) risk assessment. An effort to collect quantitative data on total *V. parahaemolyticus* in finfish, as well as data on virulent strains, is not yet completed. However, should it be possible to collect the necessary quantitative data a revised document, incorporating such data will be prepared.

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[48] WHO. 2001. Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods: Hazard identification, exposure assessment and hazard characterization of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood, WHO Headquarters, Geneva, Switzerland 23 - 27 July 2001. WHO 2001. http://www.fao.org/es/ESN/food/risk_mra_campylobacter_en.stm / http://www.who.int/fsf/Micro/index.htm

6.1.5.2 Scope

This work focused on describing the possible contamination of finfish by *V. parahaemolyticus* from harvest to consumption.

6.1.5.3 Hazard identification

Published data on the prevalence and concentration of *V. parahaemolyticus* in finfish and other seafood were collected and collated. Literature reviews were also conducted through Medline and other resources on the world wide web.

6.1.5.4 Hazard characterization

The dose-response model used in the hazard characterization of *V. parahaemolyticus* in oysters (see section 6.1.3.4) was also considered to be applicable in the case of *V. parahaemolyticus* in finfish.

6.1.5.5 Exposure assessment

The pathway from pre-harvest to consumption was divided into four stages; pre-harvest, harvest, post-harvest and consumption. It includes a descriptive explanation of the possible risks of *V. parahaemolyticus* contamination at each stage. The possibility of proliferation / reduction of *V. parahaemolyticus* in each stage was considered through a qualitative description of the data collected.

6.1.5.6 Risk characterization

Because insufficient data were available to bring the assessment forward, no further work was undertaken.

6.1.5.7 Key findings

1. The prevalence and numbers of *V. parahaemolyticus* in seawater are influenced by seawater temperature and salinity. However, there may be other influencing factors such as plankton and tides.
2. Many species of finfish could be contaminated with *V. parahaemolyticus* though the prevalence and number of *V. parahaemolyticus* present vary with species. Differences in prevalence and numbers seemed to be associated with the species and their habitat (e.g. coastal or deep-sea).
3. Coastal seawaters used at landing docks and at markets were shown to be highly contaminated with *V. parahaemolyticus*. Therefore, the post-harvest stage may be of particular importance with regard to contamination of finfish.
4. This conceptual modelling approach could be appropriate for determining the potential effectiveness of mitigation strategies such as the use of chlorinated water and thermal processing.
5. The fluctuation of time and temperature during transportation and storage may be less important for finfish than raw oysters as *V. parahaemolyticus* was shown not to proliferate significantly on finfish samples up four hours at $25^{o}C$.
6. Washing the visceral cavity after evisceration of the intestine reduced the numbers of *V. parahaemolyticus* on the fish fillet compared to eviscerated fish in which the visceral cavity had not been washed.
7. Food preparation in the home, including the time prior to washing out the visceral cavity, was identified as an important step in relation to cross-contamination and reducing the numbers of *V. parahaemolyticus*.

6.1.5.8 Limitations and Caveats

This is a qualitative (descriptive) risk assessment and quantitative data on the prevalence and concentration of *V. parahaemolyticus* in targeted seafoods are needed to undertake quantitative risk assessment.

6.1.5.9 Gaps in the data

The lack of quantitative data prevented the completion of this risk assessment. Primarily data in the following areas are needed.

- Number and proportion of pathogenic *V. parahaemolyticus* cells in various species of finfish.
- Frequency of consumption and quantity of raw fish consumed.
- Transportation practices (time and temperature).

6.1.6 Vibrio vulnificus *in raw oysters*

6.1.6.1 Introduction

The general approach to undertaking this assessment and many of the parameters were adopted from the FDA-VPRA and the FAO/WHO *V. parahaemolyticus* risk assessment, which are the only available quantitative risk assessments for a *Vibrio* spp. in raw oysters. Due to the lack of appropriate data from outside of the United States of America for many of the model inputs this assessment relies almost totally on data from this country. The approach for determining dose-response used exposure and illness frequency. For this reason some elements of hazard characterization included in the exposure assessment.

The choice of the United States of America data was intended only to provide an example on how to apply the exposure model to a different national situation. This model could be further tested and modified when appropriate data from other countries or situations become available.

6.1.6.2 Scope

The main objective of this risk assessment was to determine the usefulness of adapting the FDA-VPRA model to assess the risk from *V. vulnificus* associated with the consumption of raw oysters. In addition it aims to identify the most appropriate data as well as the data gaps and limitations for modelling *V. vulnificus* in oysters, conduct a risk characterization of *V. vulnificus* in raw oysters using available data and evaluate targeted mitigation levels aimed at reducing the risk of *V. vulnificus* illness

6.1.6.3 Hazard Identification

V. vulnificus has been associated with primary septicaemia in individuals with chronic pre-existing conditions, following consumption of raw bivalves. This is a serious, often fatal, disease. In the United States of America, it carries the highest death rate of any food-borne disease agent (Mead *et al*, 1999[49]). To date, *V. vulnificus* seafood-associated disease has almost exclusively been associated with oysters (Dalsgaard *et al*, 2001[50]; Oliver and Kaper, 1997[51]). In addition to the primary septicaemia that follows ingestion, *V. vulnificus* is known to infect wounds of otherwise healthy individuals, although the majority of patients with serious wound infections have an underlying disease. Such wound infections occur most often as a result of contamination of pre-existing wounds with seawater or after contact with fish or shellfish. *V. vulnificus* has in a few cases been isolated from patients with gastrointestinal disease, however, its role as a primary cause of gastrointestinal disease remains to be determined. Recently, cases of primary septicaemia associated with *V. vulnificus* infections seem to have been related to consumption of a variety of raw seafood products in Korea and Japan (S. Yamamoto, personal communication, 2001).

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[49] Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. and Tauxe R.V. 1999. Food-Related Illness and Death in the United States *Emerging Infectious Diseases*, 5 (5), 607-625

[50] Dalsgaard, A., Hoei, L., Linkous, D. and Oliver, J.D. 2001. *Vibrio vulnificus*. In Y.H. Hui, M.D. Person and J. R. Gorham (eds). Foodborne disease handbook, vol 1 Bacterial Pathogens, New York, Marcel Dekker Inc.

[51] Oliver, J. D., and Kaper, J.B. 1997. *Vibrio* Species. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville, eds. *Food Microbiology: Fundamentals and Frontiers*, p228-264. Washington, D.C., ASM Press.

6.1.6.4   Exposure assessment

A schematic representation of a conceptual model of the *V. vulnificus* risk assessment model showing integration of all the modules is outlined in Figure 6.6. This includes the exposure assessment modules for harvest, post-harvest and consumption that were derived from the FDA-VPRA. The exposure assessment examined the appropriateness of transferring inputs from the *V. parahaemolyticus* risk assessment to that of *V. vulnificus*. Where this was not possible alternate approaches were developed. The predicted exposure was validated with data from a survey of *V. vulnificus* numbers in raw oysters at retail.

6.1.6.5   Hazard characterization

V. vulnificus can occasionally cause mild gastroenteritis in healthy individuals, but for specific subpopulations *V. vulnificus* can cause a serious septicaemia that frequently leads to death in susceptible people. Therefore, the endpoint for the dose-response curve is defined as septicaemia. There was not adequate information to differentiate between virulent and avirulent strains of *V. vulnificus*. Therefore, all *V. vulnificus* strains were considered to be equally pathogenic.

While data from human volunteer studies were available for the construction of dose-response curves for *V. parahaemolyticus* and *V. cholerae* O1, no such data were available for *V. vulnificus*. Therefore, an alternate approach is being attempted. The dose-response relationship can be estimated by fitting a Beta-Poisson model using monthly data on the numbers of *V. vulnificus* in United States of America Gulf of Mexico oysters and the estimated consumption of raw oysters together with the monthly reported cases of *V. vulnificus*-associated septicaemia in that country. With further research, this risk relationship will be applied in the *V. vulnificus* risk assessment and validated. Preliminary results from this work were used in the risk characterization. However as this is a new approach to developing a dose-response relationship it is currently being fine-tuned and therefore the dose-response curve is not shown here.

6.1.6.6   Risk characterization

The risk characterization linked the exposure assessment and the dose-response to predict *V. vulnificus* illness rates. The predictions were compared to the observed illness rates. The model was then used to evaluate targeted mitigation levels for risk reduction.

6.1.6.7   Key findings

1. The FDA-VPRA provided a useful framework to model the risk of *V. vulnificus* septicaemia from consumption of raw oysters.

2. The model predictions of *V. vulnificus* exposure were validated using independent data from a survey of raw Gulf Coast oysters from the United States of America. Averaging water and air temperature over a ten year period, as was done in the *V. parahaemolyticus* risk assessment, could cause substantial deviations from observed levels in unusual climatic conditions such as the La Niña that occurred in 1998. However, there was good agreement between observed and predicted numbers of *V. vulnificus* using observed temperatures during this period as shown in Figure 6.7.

3. Preliminary results for the dose-response model indicated that closer agreement between predicted and reported illness rates can be obtained by either eliminating data associated with unusual climatic conditions or by using temperature, exposure and illness data individually for each month of each year available (1995 to 2001) without averaging.

4. An aggregate population dose-response could be approximated using available data on the differences in exposure to *V. vulnificus* from consumption of United States of America Gulf Coast oysters and reported illness frequency during warm and cold months.

5. The approach for determining dose-response circumvents the lack of data on frequency of virulent strains in raw oysters and uncertainty concerning the susceptible population by assuming that these do not vary from month to month.

6. Preliminary results giving the predicted illness rate using the averaging of years approach produced good agreement with observed illness rates except during the winter.

7. Preliminary evaluations of mitigations aimed at reducing *V. vulnificus* numbers in oysters to 3, 30 and 300 CFU/g indicate 60% to almost total reduction in predicted numbers of illness per year in the United States of America. Although some further refining of the modelling is required it appeared that this approach was appropriate for determining the potential effectiveness of specific mitigations to reduce *V. vulnificus* illness associated with consumption of raw United States of America Gulf Coast oysters.
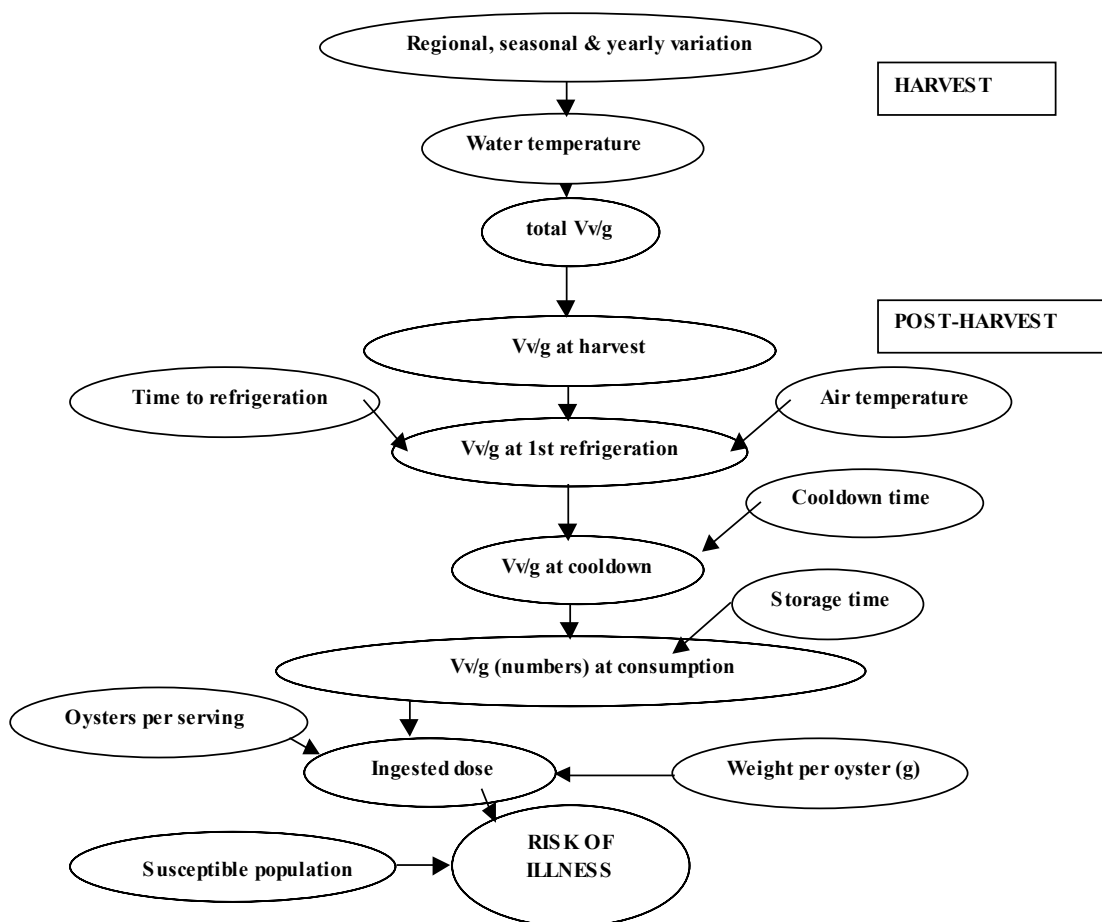
FIGURE 6.6: Schematic diagram of the *V. vulnificus* conceptual risk assessment model showing integration of all the modules.

6.1.6.8 Limitations and Caveats

This risk assessment framework and in particular the dose-response relationship developed using data for consumption of raw Gulf Coast oyster in the United States of America could be applied to oysters and other molluscan shellfish species in other regions of the United States of America and perhaps other countries. However, the use of data from this model as surrogate data for other regions should be carefully considered, especially in conditions such as temperature and salinity that are substantially different from those used to construct this model, and with shellfish species, culture conditions or industry practices that are different than the submerged bottom culture that is typical of the United States of America Gulf Coast. A modified temperature vs. *V. vulnificus* relationship for high salinity (30-35 ppt) may be more appropriate for many parts of the world where shellfish production is predominantly taking place in highly saline waters as the current model does not incorporate salinity and overestimates *V. vulnificus* densities at high salinities. The model framework

is quite flexible and most model inputs could be easily adapted to fit specific situations if appropriate data were available.

The dose-response approach used in this assessment was a curve-fitting of the data to a beta-poisson model. While the beta-poisson model was selected any other empirical model that fits the data could be used. Extrapolation of the beta-poisson parameters used in this analysis beyond the range of normal consumption would be inappropriate.

The application of this model to predict the risk of *V. vulnificus* illnesses from seafoods other than molluscan shellfish was limited as the ecology of this bacterium differs considerably as do industry practices and consumer handling. However, the dose-response relationship could be useful in determining the risk of other seafoods if *V. vulnificus* levels in these products were known at the point consumption. The accuracy of these assessments would depend on the extent of matrix effects on the dose-response.

The model does not account for variation of strain virulence. Regional or seasonal variation in *V. vulnificus* virulence could alter the current dose-response and affect illness estimates.

This assessment was based on the distribution of at risk individuals[52] in the population of the United States of America and this parameter would need to be redefined on a country by country basis depending on the size and characterization of the at risk population.
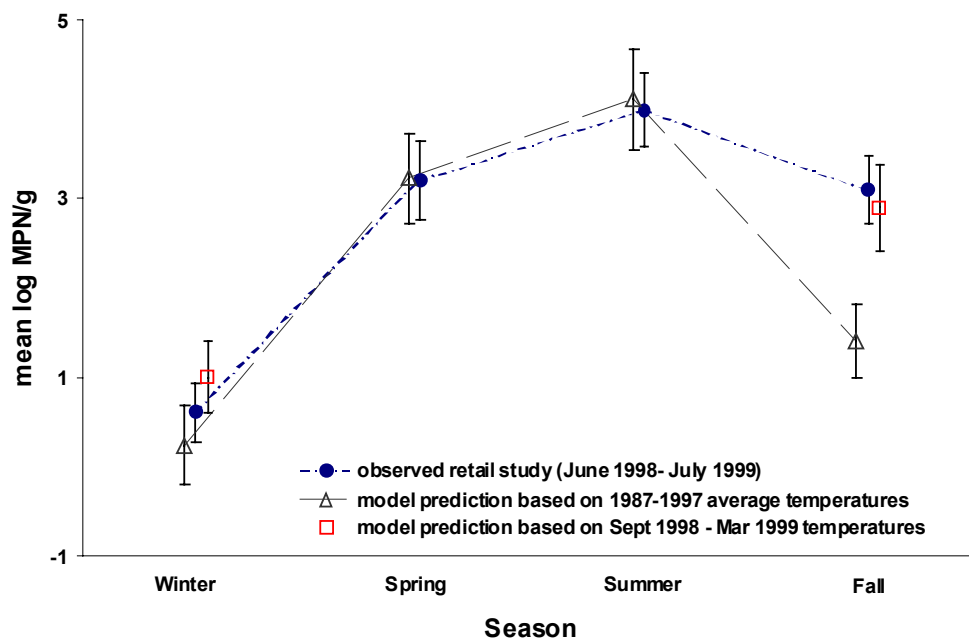
FIGURE 6.7: Predicted and observed numbers of *V. vulnificus* per gram oyster on a seasonal basis.

Since the dose-response data was generated in part using monthly illness rates in the United States of America, this data, in its current format, cannot be used to validate the model. However, illness data from that country may be useful for validation of the risk characterization in a different format (i.e. retrospective analysis of annual illness rates before and after specific mitigations). In 1997 the Interstate Seafood Sanitation Conference (ISSC) in the United States of America adopted a time-

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[52] Individuals with predisposing conditions which include insulin-dependant diabetes, liver disease (cirrhosis), gastric acidity, cancer, hepatitis B & C, kidney disease, haemochromatosis, AIDS, being immunocompromised due to treatment/surgery, asthma, rheumatoid arthritis, psoriatic arthritis, lupus, polymylagia rheumatica, giant cell arthritis and being a transplant recipient.

temperature matrix for reducing the time to first refrigeration of oysters from 20 to 10h under certain circumstances. The model could be used to analyse the effect on exposure and predicted illness and these could be compared to illness rates before and after adoption of the time-temperature matrix.

6.1.6.9 Gaps in the data

In the course of this work the following data gaps were identified.

- There was sufficient exposure data available for modelling the risk of *V. vulnificus* illness from consumption of raw Gulf Coast oysters in the United States of America using the proposed approach but this data especially numbers of *V. vulnificus* at harvest is also lacking in most other countries.
- There is a lack of reliable markers for virulence determination of *V. vulnificus,* thus requiring the assumption that all strains are virulent.
- The incidence of specific risk factors in the population consuming a seafood of interest and exposure associated with this seafood are the primary data needed for applying this model to other countries.
- Validation of the model in a given region or country would require epidemiological data on the incidence of primary septicaemia caused by *V. vulnificus* on a monthly basis.

6.1.7 Choleragenic Vibrio cholerae *O1 and O139 in warm-water shrimps for export*

6.1.7.1 Introduction

The justification for undertaking a risk assessment of this product-pathogen combination was that shrimp is an important commodity in international trade and is occasionally suspected to be involved in transmission of cholera, although there is little or no evidence that imported shrimp are actually the vehicle of transmission. The total world shrimp production in 1999 was about four million tons, of which 1.3 million tons were traded internationally with three quarters of this originating from developing countries (FAO, 1999[53]). Shrimp exports are negatively affected, particularly when there are cases of cholera in shrimp producing countries.

A risk assessment of choleragenic *V. cholerae* O1 and O139 in shrimp for domestic use had also been initiated. This was discontinued as shrimp consumed domestically does not appear to be an important vehicle for transmission of cholera. Also, major difficulties and uncertainties exist in defining handling and storage practices; possible routes of faecal cross-contamination and consumption practices of domestic shrimp.

6.1.7.2 Scope

To assess the health risk of cholera associated with the consumption of imported warm-water shrimp.

6.1.7.3 Hazard identification

Toxigenic *V. cholerae* O1 and O139 are the causative agents of cholera, a water- and food-borne disease with epidemic and pandemic potential. Non-O1/non-O139 strains may also be pathogenic but are not associated with epidemic disease. Non-O1/non-O139 strains are generally nontoxigenic, usually cause a milder form of gastroenteritis than O1 and O139 strains, and are usually associated with sporadic cases and small outbreaks rather than epidemics (Desmarchelier, 1997[54]).

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[53] FAO (1999) World production of fish, crustaceans and molluscs reached 126.2 million tonnes in 1999, an increase of 7.2 % above the 1998 level. http://www.fao.org/fi/trends/worldprod99e.asp .

[54] Desmarchelier, P.M. 1997. Pathogenic Vibrios. *In* A.D. Hocking, G. Arnold, I. Jenson, K. Newton and P. Sutherland, eds. *Foodborne Microorganisms of Public Health Significance 5th Edition*, p 285 -312. North Sydney, Australian Institute of Food Science and Technology Inc.

Outbreaks of cholera have been associated with consumption of seafood including oysters, crabs and shrimp (Oliver and Kaper, 1997[55]). The largest outbreak was a pandemic in South America in the early 1990s when *V. cholerae* O1 caused more than 400,000 cases and 4,000 deaths, in Peru (Wolfe, 1992[56]). Contaminated water used to prepare food, including the popular, lightly marinated fish *ceviche*, was associated with the outbreak.

Cholera occurs in areas with inadequate sanitary conditions and infrastructure and is associated with faecal contamination of water and foods. *V. cholerae* is widely distributed in coastal and estuarine environments all over the world and there exists over 170 serotypes of *V. cholerae*.

According to WHO definitions[57], only serotypes O1 and O139 are the causes of cholera. Ability to produce cholera toxin (CT) is the determining virulence factor for causing cholera. However, environmental strains of *V. cholerae* O1 have often been shown to be non-toxigenic. Though some strains of non-O1/O139 *V. cholerae* may also cause gastroenteritis, the disease is of a mild to moderate severity[58]. Choleragenic *V. cholerae* is susceptible to inactivation by cooking. Most of the risk associated with choleragenic *V. cholerae* comes from the consumption of raw seafood or from cross-contamination of the foods by food handlers or contaminated water.

Accordingly, this risk assessment considers only choleragenic *V. cholerae* O1 and O139.

6.1.7.4 Hazard characterization

V. cholerae O1 and O139 have both pathogenic and non-pathogenic forms based on the presence of specific virulence genes, *ctx* (cholera toxin gene). Infection by choleragenic *V. cholerae* O1 and O139 is characterized by an acute gastroenteritis. Therefore, the end-point of the dose-response curve was defined as gastroenteritis.

Human volunteer studies were available for the construction of the dose-response curves for *V. cholerae* O1. Reasonable Beta-Poisson dose-response parameters were obtained from data sets; however, the human volunteer studies characterize dose-response relationships for pathogens administered with a pH-neutralizing buffer rather than for pathogens administered with a food matrix. In normochlorohydric adult volunteers, doses of up to 10^{11} choleragenic *V. cholerae* given without buffer or food did not reliably cause illness, whereas doses of 10^4 to 10^8 organisms given with NaHCO$_3$ (sodium bicarbonate) resulted in diarrhoea in 90% of individuals. Dose-response curves (Figure 6.8) show that a high dose of *V. cholerae* O1 (10^6) was normally needed to cause illness when *V. cholerae* are consumed in food. In populations not exposed to choleragenic *V. cholerae* all age groups are equally susceptible. Immunity seems to be serotype specific.

6.1.7.5 Exposure assessment

This risk assessment includes aquacultured and wild-caught warm-water shrimp. Choleragenic *V. cholerae* O1 and O139 generally occur in waters with salinity's between 0.2 to 20 ppt. Therefore, water and shrimp from offshore waters have not been found to contain choleragenic *V. cholerae*. Thus, it is assumed that any presence of choleragenic *V. cholerae* in offshore wild caught shrimp is caused by post-harvest cross-contamination. Though the presence of choleragenic *V. cholerae* in aquaculture environments is very rare, the model assumes that such choleragenic *V. cholerae* strains could be present in shrimp at levels similar to those found in coastal waters of cholera endemic countries.

The model developed was based on shrimp handling, processing and storage practices in units approved for export of shrimp (Figure 6.9). Such approval is based on sanitary requirements as described in Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP). Shrimp

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[55] Oliver, J. D., and Kaper, J.B. 1997. *Vibrio* Species. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville, eds. *Food Microbiology: Fundamentals and Frontiers*, p228-264. Washington, D.C., ASM Press.

[56] Wolfe, M. 1992. The effects of cholera on the importation of foods: Peru- a case study. *PHLS Microbiology Digest*, 9: 42-44.

[57] WHO Fact Sheet N107

[58] Morris, J.G. Jn. 1990. Non-O group 1 *Vibrio cholerae*: a look at the epidemiology of an occasional pathogen. *Epidemiological Review*, 12: 179-191.

intended for export are generally iced immediately after harvest and transported in ice to certified processing units that meet GHP/GMP requirements. However, a worst-case scenario of shrimp being processed in non-approved units was also considered.

The major factors influencing the numbers of choleragenic *V. cholerae* in shrimp are time and temperature during handling, processing and storage. In the absence of available data it was necessary to make assumptions on distributions of time and temperature under such conditions. Adequate data were available on the effect of washing, freezing and cooking on the numbers of choleragenic *V. cholerae* in shrimp. In particular, the duration of frozen storage before consumption will cause a significant reduction in numbers of choleragenic *V. cholerae*. Limited information was available on the levels of faecal cross-contamination during handling and multiplication of choleragenic *V. cholerae* in raw shrimp. The model takes into account the pronounced reduction in the levels of choleragenic *V. cholerae* that would occur during cooking of shrimp either before export or before consumption. It also assesses the risk if shrimp were consumed raw or inadequately cooked in the importing country.
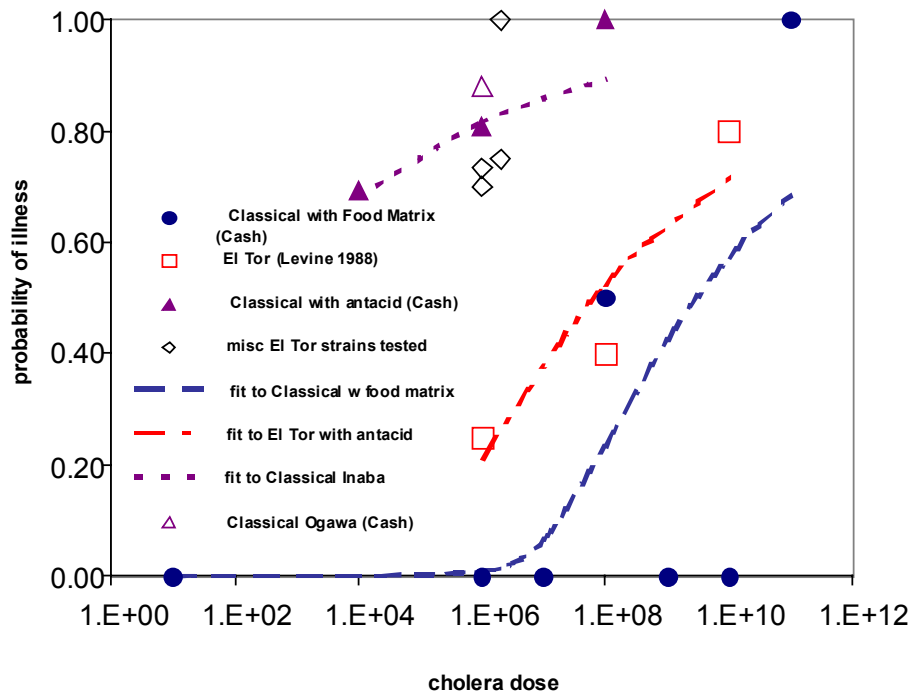
FIGURE 6.8: Beta poisson does-response curve for *Vibrio cholerae*

6.1.7.6 Risk characterization

The risk characterization was undertaken by combining the dose-response model with the estimated exposure to choleragenic *V. cholerae* via shrimp. Based on the available data, including additional information that was identified by the expert consultation, but not yet considered in the risk assessment, it will be feasible to progress with a semi-quantitative risk assessment. Further, epidemiological data on cholera cases reported to the WHO from major countries importing warm-water shrimp is available and this, together with data on shrimp imports and consumption, will be collected to validate the model.

6.1.7.7 Key findings

1. Following an extensive literature review it was noted that while *V. cholerae* is widely distributed in the environment, only strains producing cholera toxin and belonging to serotypes O1 and O139 are causative agents of cholera.

2. Contamination of shrimp, either wild-caught offshore or aquacultured, with choleragenic *V. cholerae* could happen during handling and processing, but there is very little opportunity for the multiplication of *V. cholerae* in shrimp processed in units meeting GMP/GHP requirements.

3. Major log-reductions in numbers of choleragenic organisms occur during washing, freezing and cooking.

4. Dose-response curves (Figure 6.8) show that a high dose of choleragenic *V. cholerae* O1 (10^6) is normally needed to cause illness when choleragenic *V. cholerae* O1 are consumed in food.

5. The qualitative (descriptive) risk assessment showed that there was not a public health problem associated with the consumption of imported warm-water shrimp.
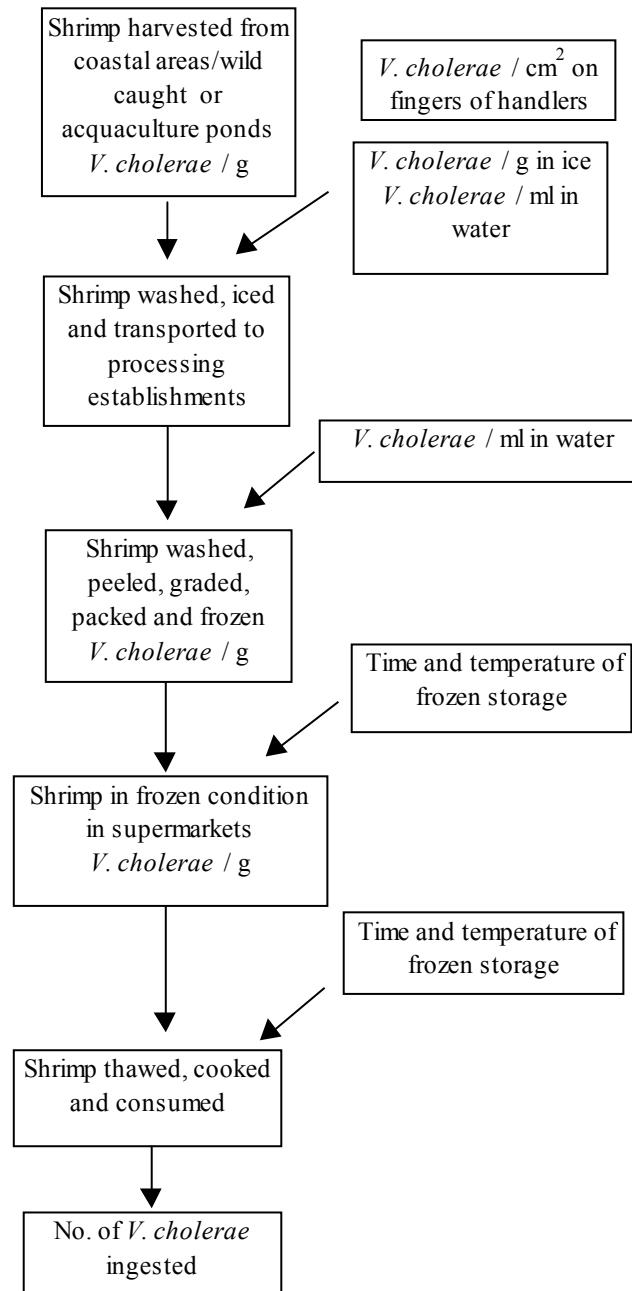
FIGURE 6.9: Conceptual model for risk assessment of choleragenic *V. cholerae* in warm water shrimp for export.

6.1.7.8   Limitations and caveats

There was limited or negative data available on the level of choleragenic *V. cholerae* O1 and O139 in shrimp at harvest. Estimations were based on reported levels found in waters.

Only one 20-year old reference was available on the lack of multiplication of choleragenic *V. cholerae* in raw shrimp.

There was no data on the level of choleragenic *V. cholerae* that may be transmitted by shrimp handlers, e.g. on fingers. Therefore, an assumption had to be made on transmission of *V. cholerae* by faecal cross-contamination.

The dose-response data for choleragenic *V. cholerae* O1 consumed with food were available for the classical biotype but not for the El Tor biotype. The El Tor biotype is the more common form and dose-response information on this form when administered with food rather than acid neutralizing vehicles is desirable.

6.1.7.9   Gaps in the data

The following data gaps were identified in the course of the work.
- Data on the levels of choleragenic *V. cholerae* O1 and O139 in natural waters and aquaculture environments.
- Data on the multiplication of choleragenic *V. cholerae* in cooked and raw shrimp.
- Data on levels of faecal cross contamination during handling of shrimp.
- Data to clarify the dose-response when the El Tor form is ingested in food.

6.2   Review of the Risk Assessments

The expert consultation undertook a technical review of the draft risk assessment document entitled "A Draft Risk Assessment of *Vibrio* spp. in Seafood." The expert consultation evaluated the risk characterization, as well as the underlying data, assumptions, and associated uncertainty and variability. The expert consultation recognized the extensive work undertaken by the drafting group, provided additional references to meet some of the specific data needs of the risk assessment and made recommendations on how to improve the document.

6.2.1   Introduction: Vibrio *spp. in seafood*

The expert consultation noted that it was desirable to state that the pathogenicity of *V. parahaemolyticus* is associated with TDH and/or TRH production. With regard to *V. cholerae*, it is necessary to state clearly that epidemic cholera is only associated with cholera toxin-producing strains of serogroups O1 and O139. It was recognized that not all professionals may be aware that virulence of *V. parahaemolyticus*, and O1 and O139 *V. cholerae* is associated with certain toxin-encoding genes, and that diagnostic tests can be used to separate these strains from others.

The expert consultation expressed concern that testing of seafood for *Vibrio* spp. was sometimes based on inappropriate markers (e.g. genus, species and/or non-consideration of pathogenic factors) that do not reflect the potential to cause human illness. It was agreed that the risk assessment should include a section outlining the *Vibrio* spp., types and virulence factors that may be included in the examination of different types of seafood in order to protect public health.

6.2.2 Vibrio parahaemolyticus in raw oysters consumed in Japan, New Zealand, Canada, Australia and the United States of America oysters

The expert consultation identified that the risk assessment should include further consideration of the oyster industry practices in different parts of the world as these could have a significant effect on the appropriateness of the present model. In particular, this applied to the uncommon use of refrigerated transport and storage in many countries. The caveats that applied to the assessment due to these differences should be specified. It was determined that modelling should also consider salinity as a parameter, since in some areas of the world, salinity remains high throughout the year and exerts an effect in addition to that of seawater temperature. This might be addressed by having separate models for areas of relatively constant high salinity; relatively constant low salinity; and varying salinity. It was also necessary to consider that different oyster species might behave differently with regard to both the concentration of *V. parahaemolyticus* in the harvest area and to the growth of the organism during periods of temperature abuse or cool-down. Several of these considerations could have led to the model over-predicting the incidence of illness due to *V. parahaemolyticus* in oysters in Australia and New Zealand.

It was highlighted that the model did not currently incorporate consideration of TRH-producing strains of *V. parahaemolyticus*, nor did it presently encompass possible variability in the prevalence of TDH-producing strains seen in other countries. The model could be amended in the future to address these aspects.

The risk assessment should include fuller consideration of uncertainty and variability and it would be useful to include an outline of the use of the @Risk model. It would be necessary to specify as fully as possible the data needed, as well as any key caveats, if the model were to be applied or modified to be used in other regions and for other species.

6.2.3 Vibrio parahaemolyticus in bloody clams

The expert consultation congratulated the drafting group and the associated researchers for undertaking a valuable targeted risk assessment in a very short period of 5 months, going from literature review and capture of novel data to modelling and drafting of the assessment. The process itself could form an example for future targeted assessments.

The expert consultation recommended that the drafting group revise the assessment itself to ensure clarity with regard to the data and methods used (both microbiological and modelling) and to ensure that the figures and other outputs were sufficiently explained in the text. There was the potential to include more about the uncertainty and variability in the model and to look in more detail at the modelling aspects of the mitigation process (boiling). These recommendations did not detract from the quality of the work that has already been done.

6.2.4 Vibrio parahaemolyticus in finfish

An exposure assessment had been prepared previously and presented to the first expert consultation in Geneva in 2001[59]. FAO and WHO, in conjunction with the drafting group, subsequently decided that there were insufficient data to take the assessment forward at the present time and therefore no further work had been undertaken. The expert consultation recognized the useful content included in the exposure assessment and determined that it should be included in the final report on the *Vibrio* risk assessments and could then form the basis for possible future work and as support to FAO/WHO member countries and Codex.

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[59] WHO. 2001. Report of the Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods; Hazard identification, exposure assessment and hazard characterization of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood. WHO Headquarters, Geneva, Switzerland 23 - 27 July 2001. WHO 2001. http://www.fao.org/es/ESN/food/risk_mra_campylobacter_en.stm / http://www.who.int/fsf/Micro/index.htm

6.2.5 Vibrio vulnificus *in raw oysters*

The expert consultation noted the success in extending the use of the *V. parahaemolyticus* risk assessment and model to another species. It was discussed that the models are currently based on United States of America Gulf Coast data sets, and that the illness data used for validation are from the same country. The development of an additional salinity-temperature model will assist in extending this tool to other environments where high salinity may be a factor in limiting exposure. The stipulations noted for the *V. parahaemolyticus* assessment also apply to this assessment and appropriate data needs and caveats for application to other regions also need to be emphasized. The assessment should also include further discussion as to how the tool can be used in regions where shellfish -associated *V. vulnificus* infection may be of importance.

6.2.6 *Choleragenic* Vibrio cholerae *O1 and O139 in warm-water shrimp for export*

According to WHO definitions[60], only serotypes O1 and O139 are the causes of cholera and the ability to produce cholera toxin (CT) is the determining virulence factor. However, environmental strains of *V. cholerae* O1 have often been shown to be non-toxigenic, therefore, according to the expert consultation, seafood products should only be analysed for cholera toxin - producing *V. cholerae* O1 and O139.

The adaptation of the *V. parahaemolyticus* model in oysters (based on temperature-*V. parahaemolyticus* numbers predictor) to a *V. cholerae* O1/O139 choleragenic model in warm-water shrimp was recognized as difficult because of the absence of a good predictor for *V. cholerae* numbers in shrimp, and it would therefore require a large amount of work to develop a completely new model.

The export of warm-water shrimp constitutes an important aspect of world trade. It was generally agreed that the available qualitative (descriptive) risk assessment showed that there was not a public health problem associated with the consumption of imported warm-water shrimp; however a semi-quantitative risk assessment should be undertaken with the available data in order to assist risk managers to better understand this.

It was decided not to proceed with a risk assessment of shrimp consumed by domestic markets in tropical countries. Since shrimp are consumed cooked, any illness will be the result of cross-contamination and this is not specific to any single food commodity.

6.3 *Utility and applicability*

The expert consultation emphasized the need for the risk assessments to be distributed as widely as possible and in forms that are appropriate to the target groups. As well as the planned Executive Summary and full Technical Report, an Interpretative Summary should be produced that explains the use and limitations of these risk-based tools without great emphasis on details of the models. FAO and WHO should consider the publication of entire consultation reports in respected, widely read journals. Some issues that need to be considered here are FAO and WHO policies on copyright, the need for peer review of already heavily reviewed documents and the timing of publication. In addition, the drafting group should be encouraged to submit relevant aspects of the work for publication in peer-reviewed journals to reach a greater audience. It will be important to consider publication of elements prior to publication of the full report in order to contribute to general development of microbiological risk assessment for foods.

It will be necessary for presentations to be made to relevant stakeholders, including Codex, in order to ensure that the relevant aspects were fully emphasized. Provision of appropriate PowerPoint presentations by FAO and WHO would assist in this. It was also perceived that the results of the work would be of value to the seafood industry and thus simplified summaries of the assessments should be submitted to trade periodicals. The benefits of these risk assessments will be realized through the use of trainers who are skilled in communicating with diverse types of audiences. FAO and WHO should

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[60] WHO Fact Sheet N107

make the software models available together with guidance on their use. They should also provide technical assistance to developing countries wishing to extend the assessments to local needs.

These risk assessments could be used to support relevant risk management decisions. The output from the assessments should also be used to formulate and target research needs, e.g. to fill identified data gaps.

6.4 Response to the specific questions posed by the Codex Committee on Fish and Fishery Products

A number of questions were posed to the expert consultation in relation to management strategies for food-borne illness due to *V. parahaemolyticus* and *V. vulnificus*. These were posed to the members of the consultation in their roles as experts in the microbiology of vibrios and/or seafood technology. The four questions posed by the CCFFP and the response from the expert consultation are outlined below.

Question 1: Whether the following pre-harvest control measures (testing/monitoring the following parameters and consequential closure of the harvesting area) are effective in the control of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in bivalve molluscs:

- Testing of bivalve mollusc meat for *Vibrio parahaemolyticus* and *Vibrio vulnificus*

- Temperature monitoring of the growing water

- Water testing for *Vibrio parahaemolyticus* and *Vibrio vulnificus*

- Salinity monitoring

Response from the consultation: The concentrations of *V. parahaemolyticus* and *V. vulnificus* in shellfish may be measured directly or predicted by monitoring temperature and salinity. There will not necessarily be a direct relationship between these surrogate variables and the measured concentrations of pathogenic vibrios for a particular area as there is uncertainty and variability in the current models. The predictive abilities of the models would be improved by incorporating local data and considering additional factors such as hydrodynamic effects and sunlight. The effectiveness of these measures in controlling illness would depend on the instigation of an appropriate mitigation (or multiple mitigations) and this is not confined to closure of a harvesting area.

The current models do not include modules relating to the concentration of the two pathogens in seawater and thus the utility of measuring this cannot be estimated. If the appropriate data were gathered then the models could be extended accordingly.

Question 2 Are the following post-harvest treatment technologies, alone or in combination, effective in the reduction or elimination of *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs:

- hydrostatic pressure
- rapid cooling
- irradiation
- mild heat treatment (pasteurization)
- freezing and thawing
- depuration

Response from the consultation: These may all have the effect of reducing the numbers of pathogenic vibrios but the effectiveness will vary according to the conditions of use, and there may be a need to balance between obtaining the maximum possible reduction in bacterial content and retaining consumer-acceptance of either the product or the process. Reports on the effectiveness of depuration vary greatly and this may again depend on the conditions of use - some reports indicate that proliferation of vibrios may occur during this process. The general opinion of the expert consultation is shown on a qualitative/semi-quantitative basis in table 6.7.

The current models could be adapted to enable estimates to be obtained of the effectiveness of the mitigations in reducing illness. With regard to the mitigation of the closure of harvesting areas,

estimates could also be obtained of the proportion of harvest lost by application of a particular scenario.

Some of the listed mitigations are also used in combination, e.g. hydrostatic pressure and freezing; depuration and hydrostatic pressure or pasteurization.

TABLE 6.7: The comparative effectiveness of a number of mitigation strategies in reducing *Vibrio* spp..

Mitigation	Comparative effectiveness in reducing *Vibrio* spp.
Hydrostatic pressure	+++
Rapid cooling	+/++
Irradiation	+++
Pasteurization	+++
Freezing and thawing	++
Depuration	+/-
Relay at high salinity for 2 weeks (for V. vulnificus)	++
Commercial heat-treatment	+++

-	no effect
+	some reduction
++	moderate reduction
+++	significant reduction

References and further reading relating to inactivation strategies

Calik, H., Morrissey, M.T., Reno, P.W. & An H. 2002. Effect of high pressure processing on *Vibrio parahaemolyticus* strains in oysters. *Journal of Food Science*, 67: 1506-1510.

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Eyles, M. J., and G. R. Davey. 1984. Microbiology of commercial depuration of the Sydney rock oyster, *Crassostrea commercialis*. *Journal of Food Protection,* 47:703-706.

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Motes, M.L. and DePaola, A. 1996. Offshore suspension relaying to reduce levels of *Vibrio vulnificus* in oysters (*Crassostrea virginica*). *Applied and Environmental Microbiology,* 62, 3875-3877.

Richards, G. P. 1988. Microbial purification of shellfish: A review of depuration and relaying. J. Food Protect. 51:218-251.

Son, N. T., and G. H. Fleet. 1980. Behaviour of pathogenic bacteria in the oyster, *Crassostrea commercialis*, during depuration, re-laying, and storage. *Applied and Environmental Microbiology,* 40:994-1002.

Question 3 For *Vibrio parahaemolyticus* - Are food-borne illnesses caused by the heat resistant toxin produced by the pathogen or by the pathogen itself?

Response from the consultation: The illness is caused by the toxin but only if this is produced in the intestine following colonization by a strain producing TDH, TRH or both toxins.

Question 4: What is the availability of methods of analysis for *Vibrio parahaemolyticus* toxin gene (*tdh*)?

Response from the consultation: Both *tdh* and *trh* genes can be detected using PCR with relevant primers and by membrane filtration-hybridization methods with non-isotopic oligonucleotide or PCR-generated probes. For quantification, PCR methods can be applied in an MPN format whereas membrane filter-hybridization can be used for direct colony enumeration. PCR and colony hybridization procedures are also available for the thermolabile haemolysin gene (*tlh*) for determining *V. parahaemolyticus* species. As with conventional methods, there is scope for standardization and/or the determination of the relative performance of current methods.

6.5 Response to the needs of the Codex Committee on Food Hygiene

A CCFH drafting group has prepared a document titled "Discussion Paper on Risk Management Strategies for *Vibrio* spp. in Seafood." In that paper, some risk assessment needs and questions for risk assessors were identified, which include an evaluation of the impact of several potential interventions on the risk of *V. parahaemolyticus* infection. The current risk assessment of *Vibrio* spp. in seafood is addressing many of these potential interventions through the models that have been developed including the influence of temperature on growth and the impact of various target reduction levels of *Vibrio* spp. in oysters on the risk of illness. The effects of various mitigation strategies are also described in section 6.4.

6.6 Conclusions and recommendations

The drafting group has progressed towards improving the risk assessments since the last expert consultation[61]. The majority of the previous recommendations have been addressed in the current draft report.

The vibrio risk assessments should include clear advice as to the species, serogroups, serotypes and genotypes that should be considered as being of public health significance with respect to the trade and consumption of seafood. Interpretative summaries of the risk assessments should be produced in order to maximise the understanding of the work by other professionals. Where appropriate, these summaries should have limited descriptions of the mathematical modelling in order to reach a wider audience.

It was recommended that the risk assessment on "*Vibrio cholerae* in shrimp" be developed further on semi-quantitative basis only and the opinions of risk managers should be sought before any work is undertaken towards a fully quantitative risk assessment.

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[61] WHO 2001. Report of the Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods; Hazard identification, exposure assessment and hazard characterization of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood. WHO Headquarters, Geneva, Switzerland 23 - 27 July 2001. WHO 2001. http://www.fao.org/es/ESN/food/risk_mra_campylobacter_en.stm / http://www.who.int/fsf/Micro/index.htm

The exposure assessment on "*Vibrio parahaemolyticus* in finfish" should be included in the final report on the vibrio risk assessments as this contains information that may be useful to a number of countries.

The procedures used to undertake the risk assessment on bloody clams should be recognized as a way to expedite the development of pathogen-commodity risk model. The draft of the risk assessment itself should be revised to ensure greater transparency of the modelling approaches.

The risk assessment on "*Vibrio parahaemolyticus* in oysters" should include further consideration of the oyster industry around the world (practices, species effects, harvest water salinity, etc). Where the model cannot be modified appropriately, the potential constraints and/or the additional data needs should be clearly identified. It should identify the potential shortcomings of the assumption which precluded the consideration of TRH-producing strains as pathogenic and also the possible consequences of the high prevalence of TDH-producing strains in some parts of the world. Further consideration should be given to uncertainty and variability, and it should also include an description of the Excel-based model in order to assist understanding its potential applications and limitations.

The risk assessment on "*Vibrio vulnificus* in oysters" should include consideration of the potential constraints and/or the additional data needs to extend it to other geographical areas outside of the United States of America. It should identify more clearly those areas of the world and the seafood products that were currently known to be associated with food-borne *V. vulnificus* infection.

In order to undertake further risk assessments it will be necessary to obtain additional information on the proportion of strains of each *Vibrio* spp that possess pathogenic traits. Virulence factors of *V. parahaemolyticus* (TDH and TRH) and *V. cholerae* O1 and O139 (CT) should be identified where appropriate so as to specify the strains that are relevant to human illness. The expert consultation believed that additional information on dose-response relationships with respect to food contaminated with *Vibrio* spp. could be obtained by investigation of outbreaks.

7 Conclusions

The assessments presented at this expert consultation were in varying degrees of completion. The risk assessments for *Campylobacter* spp. in broiler chickens, *V. vulnificus* and *V. parahaemolyticus* in oysters, are most suited at the present time for use by risk managers to help them make informed risk management decisions. The models use a modular approach which lends flexibility for their use in assessing production systems not specifically covered in the current models. The utilization of the models was demonstrated by a number of examples showing how certain mitigation strategies may lead to a reduction in the number of cases of disease. The models are still evolving and once fully completed will become a useful tool for risk managers.

Since the last consultation, the hazard characterization and exposure assessment were merged to produce a preliminary risk characterization. The risk characterization can provide risk managers with the ability to gain insight into the relative effectiveness of various mitigation strategies. Future activities will focus on ensuring the full potential of the risk assessment is achieved. In addition, a full exploration of the model will be undertaken to fully understand the scope and limitations

8 Recommendations

The expert consultation recommended that FAO and WHO should:

- ensure that for future risk assessment activities, a clear-cut statement of purpose be provided by the risk managers at the start of risk assessment activities, followed by an active and continuous interaction between risk managers and risk assessors. It is important that risk managers are involved in the design of the risk assessment and assist in making explicit the scope of the assessment. This will ensure that the outcomes will be of maximum use to all parties.

- highlight the need to collect quantitative data to all steps of the risk assessments, so as to provide appropriate risk management conclusions.

- provide assistance to developing countries to gather quantitative information for risk assessment. Appropriate resources should be provided to developing countries to train and undertake risk assessments and those wishing to extend the risk assessments to local needs.

- encourage microbiologists to develop and employ techniques to differentiate pathogenic and non-pathogenic strains.

- encourage governments that have set or intend to set targets for food-borne diseases addressed in this report to use the models presented to inform their decisions in setting appropriate food safety objectives and intervention strategies.

- commission a document to assist a wider readership in understanding this report. The recommended document would describe for non-specialists appropriate information on;

    i) shellfish and poultry e.g. production methods, processing, economics and legislation.

    ii) *Vibrio* spp. and *Campylobacter* spp. (surveillance, epidemiology and microbiology).

    iii) risk assessment (its approach, components and how it can answer risk managers' questions).

- facilitate hands-on demonstrations for identifying and collecting relevant data for risk assessment for specific commodity/pathogen combinations including risk characterization using various modelling software.

- develop new ways of communicating the concepts of risk assessments to users.

- promote utilization of the valuable framework models that have now been established to undertake further risk assessments. It would be relatively easy to use modules from these risk assessments to develop a risk assessment of *Salmonella* in shrimps. The experts consider this would be a valuable risk assessment for future consideration.

- consider, for future risk assessment activities, the involvement of scientific experts to serve as a standing advisory resource group to the risk assessment drafting group, for the duration of a specific work assignment. This would be a significant complementary activity to the more formal in-person experts meetings and would certainly take greater advantage of the intellectual resources of the expert community through their knowledge of and access to relevant data, references and network of colleagues.

- encourage the submission of appropriate aspects of the work to peer-reviewed technical publications and the seafood and poultry trade press.

- make the software models available in a format usable by other professionals and provide appropriate guidance on their use.

Annex 1: List of participants

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Annex 2: List of working documents

Two working papers were prepared for, and presented during the expert consultation. These served as the basis for the discussions, which led to the development of the report and the recommendations. These documents were prepared for FAO and WHO by a number of expert drafting groups. The full text of these documents will be made available on the FAO and WHO webpages; http://www.fao.org/es/esn/food/risk_mra_en.stm and http://www.who.int/fsf/Micro/index.htm.

Paper no.	Title	Authors
		(in alphabetical order)
MRA 02/01	A draft risk assessment of *Campylobacter* spp. in broiler chickens	***Steve Anderson***, Food and Drug Administration, United States of America
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MRA 02/02	A draft risk assessment of *Vibrio* spp. in seafoods	***John Bowers***, Food and Drug Administration, United States
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