く」、国際標準規格ならば「項番や項目の○に基づく」などと指摘する根拠が必要です。

このように根拠を明確にすることにより、監査員、監視員などの指摘内容とそれぞれの担当者によってのバラツキが制御される利点があります。

また、法律、通達、項番などの該当する規格が明確であれば、バラツキの要因は、担当者ごとの規格の解釈によるバラツキとなり、規格の解釈の妥当性や現場の状況による判断の違いなどについて調整すればよいことになり、「監査者」も「被監査者」も感情論抜きで議論ができることになるでしょう。

しかし、このことに対して、監査・監視側から「状況によって難しい」「ケースバイケースが多い」など「監査・監視指導」が困難で、一概に言えないとの意見があるようです。このことは、「監査・監視者」は「被監査・被監視者」に対して、事前に（事故）予測したマニュアルを作成しなさいと言っていることと逆のことではないかと思っています。

すなわち、これまでの多くの「監査・監視指導」経験のなかから、「監査者・監視者」の個人的な経験を「監査・監視指導する組織」としてマニュアル化することが求められるでしょう。そのことによって、状況に対応した「監査・監視指導」ができると考えています。

また、「被監査者（事業者）」も「監査・監視指導内容」の根拠が明確になり、順守しやすい環境が醸成され、さらに、「監査者と被監査者は同等である」との視点から、さらなる議論ができ、かつ、「食の安全・安心」議論が、建前的な議論から現場視点からの実質的な議論へ発展することに期待します。

※ISO：International Organization for Standardization の略。国際標準化機構のこと。工業製品の国際規格を決めている。

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Codex risk management activities on the control of *Vibrio* spp. in Molluscan Shellfish

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The Codex Alimentarius Commission (CAC) is an intergovernmental standard setting body. The standards, recommendations and guidelines elaborated by the CAC are recognized as the international reference for food safety requirements by the WTO Sanitary and Phytosanitary agreement. Within the Codex subsidiary bodies, the Codex Committee on Food Hygiene (CCFH) has a responsibility to address microbiological food safety issues. CCFH at the 32nd session identified *Vibrio parahaemolyticus* in shellfish as one of the priority issues for consideration by the FAO/WHO expert consultations on risk assessment, and agreed to develop a Discussion Paper on Risk Management Strategies for *Vibrio* spp. in seafood at the 34th session.
After receiving encouragement from the Codex Committee on Fish and Fish Products (CCFFP), which has a responsibility to elaborate world wide standards for fish, crustaceans and mollusks, that the CCFH should take the lead this issue, the CCFH agreed to develop risk profile.
At the 39th session, the CCFH decided to initiate a new work to elaborate a Code of Hygienic Practice for *Vibrio* spp. in Seafood. The Code was intended to articulate particular control measures of pathogenic *Vibrio* spp. in seafood in addition to the recommendations of the Recommended International Code of Practice-General Principles of Food Hygiene. The Code focused on the time and temperature control to minimize the growth rate of pathogenic *Vibrio* spp. in seafood, and the prevention of the cross contamination after the heat treatment step. The target microbiological hazards of this Code are pathogenic *V. parahaemolyticus, V. vulnificus* and choleragenic *V. cholerae*.
At 40th session, the CCFH agreed to elaborate a specific Annex for the control of *V. parahaemolyticus* and *V. vulnificus* in molluscan shellfish. In this paper, the role of Joint FAO/WHO Meetings on Microbiological Risk Assessment (JEMRA), CCFFP and CCFH, and the interactions among these bodies will be discussed.

Keywords : Vibrio parahaemolyticus, Vibrio vulnificus , risk assessment, Codex

Introduction

Codex Alimentarius Commission is the international food safety risk management body that has dual mandates;

- (a) protecting the health of the consumers and ensuring fair practices in the food trade;
- (b) promoting coordination of all food standards work undertaken by international governmental and non governmental organizations.(Codex Procedural Manual, 18th ediction)

Most of the development of hygiene related text and guidelines are elaborated through the Codex Committee on Food Hygiene (CCFH) that is one of horizontal committees, and provides basic provisions on food hygiene applicable to all food, and acts as the microbiological risk management body in global level.
CCFH at the 32nd session identified *Vibrio parahaemolyticus* in shellfish as one of the priority issues for consideration by the FAO/WHO expert consultations on risk assessment, and agreed to develop a Discussion Paper on Risk Management Strategies for *Vibrio* spp. in seafood at the 34th session. (Codex 2001, ALINORM 01/13, para. 18,)
After receiving encouragement from the Codex Committee on Fish and Fish Products (CCFFP), which has a responsibility to elaborate world wide standards for fish, crustaceans and mollusks, that the CCFH should take the lead this issue, the CCFH agreed to develop risk profile.
At 39th Session, the CCFH agreed to initiate a new work on risk management on *Vibrio* spp. in seafood in 2007, and Codex has just finalized the elaboration of risk management document on *Vibrio species* control in Seafood based on the application of Good Hygienic Practice. CCFH also developed an Annex on control measures for *Vibrio parahaemolyticus* and *Vibrio vulnificus* in molluscan shellfish. (Codex 2008a)

The purpose of this bivalve Annex is to provide guidance on control measures that minimize the risk arising from the presence of pathogenic *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs, especially minimizing and/or preventing the introduction/contamination and/or the growth of these pathogens, and adequate partial treatment of bivalve molluscs before consumption. Controls for these pathogens are similar but differ to the extent that characteristics of growth and survival differ. The Information in this Annex will be of interest to regulatory authorities, the food industry, consumers, and other interested parties.

1. Scope of the Shellfish Annex

This annex addresses only bivalve molluscs but not all molluscan shellfish. Bivalve molluscs are harvested, handled and consumed differently than most other seafood products and are inherently riskier than other seafood due to their filter feeding activity that concentrates pathogens present in the water.

Four different states of bivalve molluscs are considered:

(i)"live" and (ii) "raw" bivalve molluscs, (iii) raw destined for receiving "post harvest processing", which are not treated before consumption, in Part I

(iv) bivalve molluscs in a "partially treated" state, which are destined to be partially treated before consumption or to be directly consumed with no further treatment, in Part II

(v) bivalve molluscs in a "thoroughly treated" state that are destined for direct consumption, such as canned foods and other sterilized foods, should not be addressed in the annex because they could be adequately covered by the main document and other existing document (i.e. the *Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1- 1969)*).

Post harvest processing is defined as processes (e.g. freezing, high pressure and mild heating) intended to significantly reduce or limit but not completely eliminate *V. parahaemolyticus* and *V. vulnificus* while essentially retaining the sensory characteristics of live bivalve mollusks. After the application of such the process, sensory characteristics of live bivalve molluscs are essentially retained in the bivalve molluscs, despite that they are not alive any more.

Table 1 summarizes the characteristics of the food categories as agreed by the physical working group (CAC 2009, CX/FH 09/41/7)..

Table 1 Status at consumption and control characteristics of two food categories

Characteristics of control by:	Part I Status of food at consumption:			Part II Status of food at consumption:	
	Live	Raw	Raw after post-harvest processing	Partially treated[1] primarily by consumer	Partially treated[1] primarily by business operator
Producer					
Primary production control[2]	+	+	+	+	+
Business operators[3]					
Post-harvest processing[4]	–	–	+	–	–
Time and temperature control	+	+	+	+	+
Adequate partial treatment before consumption[5]	–	–	–	+/–	+
[Proportional time/temperature control][6]					
Consumer					
Additional partial treatment before consumption	–	–	–	+	–

+: conducted, –: not conducted, +/–: conducted or not conducted.

[1] Any treatment intended to significantly reduce or limit but not completely eliminate *Vibrio* spp. in seafood. As a result of partial treatment, the raw sensory characteristics are lost.

[2] Time and temperature control at primary production for live and raw (without post harvest processing) categories are more stringent than other categories to the left.

[3] Establishment including distributors, restaurants, caterers, etc.

[4] For example, quick frozen, high-pressured or mild heat treated oysters, which are retaining sensory characteristic of live oysters.

[5] Conducted due to preference of consumers and wide variations of cooking. Post-harvest processing is excluded.

[6] Equivalent Level of Protection to live and raw categories should be achieved.

Controls in Part I apply to live and raw bivalve molluscs (including those that receive post-harvest processing), while those in Part II apply to bivalve molluscs consumed after partial treatment.

For each category, unique guidance & control criteria to achieve equivalent level of protection are provided,

2. Format and use of the Annex

The format of the Annex follows the basis structure of the *Recommended International Code of Practice - General Principles of Food Hygiene* (Codex2003a, CAC/RCP 1-1969), This Annex should be used in conjunction with the *Recommended International Code of Practice - General Principles of Food Hygiene* (CAC/RCP 1-1969), the *Code of Practice for Fish and Fishery Products* (Codex 2003b, CAC/RCP 52-2003), Hygiene section of the *Standard for Live and Raw Bivalve Molluscs* (Codex 2008b, CODEX STAN 292-2008) and the *Code of Hygienic Practice for Pathogenic Vibrio spp. in Seafood*. The use of this Annex may require modifications and amendments that take into account such factors as regional differences in the prevalence of pathogenic strains of *V. parahaemolyticus* and *V. vulnificus* and the epidemiological data.

3. The target microbiological hazards of this Annex

Target microbiological hazards addressed by the bivalve molluscs Annex are pathogenic *V. parahaemolyticus*, and *V. vulnificus*. It was recognized that there were some case reports of infections caused by other *Vibrio* species (e.g. *Vibrio cholerae* non-O1 / non-O139, *Vibrio alginolyticus*) in certain regions and countries. Due to lack of data for other *Vibrio* species and non-availability of their risk assessments results, the Annex focused on control measures for these two pathogenic *Vibrio* species.

4. Control at Primary Production

Effective control measures for *V. parahaemolyticus* and *V. vulnificus* at primary production typically require an evaluation in terms of the risk associated with

environmental factors in the harvesting area and harvesting practices based on epidemiology and environmental conditions (i.e. water temperature and salinity, air temperature).

Table 2. Predicted temperature-specific V. parahaemolyticus and V. vulnificus growth rates and doubling times in oysters for calculating cumulative growth based on hourly temperature observations (data WHO-FAO 2008).

Oyster Temperature (°C)	*V. parahaemolyticus*		*V. vulnificus*	
	Growth rate[1] (logs/hr)	Doubling time (hrs)	Growth rate[2][1][2] (logs/hr)	Doubling time (hrs)
10	0.008	35.8	0	
11	0.013	24.0	0	
12	0.017	17.3	0	
13	0.023	13.0	0	
14	0.030	10.1	0.011	27.4
15	0.037	8.11	0.022	13.7
16	0.045	6.64	0.033	9.12
17	0.054	5.54	0.044	6.84
18	0.064	4.69	0.055	5.47
19	0.075	4.02	0.066	4.56
20	0.086	3.49	0.077	3.91
21	0.099	3.06	0.088	3.42
22	0.112	2.70	0.099	3.04
23	0.126	2.40	0.110	2.74
24	0.140	2.15	0.121	2.49
25	0.156	1.93	0.132	2.28
26	0.172	1.75	0.143	2.11
27	0.189	1.59	0.154	1.95
28	0.207	1.45	0.165	1.82
29	0.226	1.33	0.176	1.71
30	0.246	1.23	0.187	1.61
31	0.266	1.13	0.198	1.52
32	0.287	1.05	0.209	1.44
33	0.309	0.97	0.220	1.37
34	0.332	0.91	0.231	1.30
35	0.356	0.85	0.242	1.24

[1] Square root of growth rate (in logs/hr) = 0.0202*Temperature – 0.1103, if Temperature > 10°C
[2] Growth rate (in logs/hr) = 0.011*(Temperature -13) if Temperature > 13°C The information in this table is based on growth rates of natural *V. parahaemolyticus* and *V. vulnificus* populations in *Crassostrea virginica* as described in the *V. parahaemolyticus* and *V. vulnificus* Risk Assessments and is based on environmental conditions in *Vibrio* populations occurring in the US. These growth rates may be different in other species of bivalve molluscs, and countries should consider using local species to confirm growth rates.

For estimating risk, it is important to understand that *V. parahaemolyticus* grows faster at colder temperatures than *V. vulnificus* (growth rates for *V. parahaemolyticus* and *V. vulnificus* in the oyster, *Crassostrea virginica* are provided in Table 2 (CAC 2009, CX/FH 09/41/7)..).Predictive tools using these environmental monitoring parameters and growth rates as inputs have been developed based on the FAO/WHO risk assessments and are available to estimate corresponding *V. parahaemolyticus* and *V. vulnificus* levels and risk.

Monitoring of bivalve molluscs at harvest for the levels of total *V. vulnificus* and total and pathogenic *V. parahaemolyticus* should be conducted to determine the regional and seasonal variation. Prevalence of pathogenic strains of *V. parahaemolyticus* and *V. vulnificus* and the epidemiological data, including the susceptibility of the population, should be considered. This information and factors articulated below are useful for model inputs and evaluation of model outputs and application of appropriate controls.

- ✓ levels of *V. parahaemolyticus* and/or *V. vulnificus*, or environmental parameters that exceed testing/monitoring criteria that are based on risk assessment, if applicable.
- ✓ An unusual increase of *Vibrio* spp. illnesses.

5. Factors to be considered in determining the need for controls in a given harvest area

The Competent Agency having a jurisdiction should consider the following factors in determining the needs of control at harvest area;

- ✓ The number of sporadic illnesses and outbreaks of *V. parahaemolyticus* and *V. vulnificus* associated with bivalve molluscs - harvested from a distinct hydrographic area, and whether these illnesses are indicative of an annual reoccurrence;
- ✓ Water temperatures representative of harvesting conditions. Water temperatures below 15°C (McLaughlin et al, 2005) for *V. parahaemolyticus* and below 20°C for *V. vulnificus* have generally not been historically associated with illnesses;
- ✓ Time period to first refrigeration and post-harvest air temperatures above the minimum growth temperatures for *V. parahaemolyticus* (approximately 10°C) and *V. vulnificus* (approximately 13°C), which may increase risk regardless of harvest water temperature;
- ✓ Harvest practices that allow radiant solar heating to raise temperatures of bivalve molluscs to temperatures above ambient air temperatures prior to harvest (i.e. intertidal harvest) and exposure time;
- ✓ Salinity ranges and optima are different for *V. parahaemolyticus* and *V. vulnificus*. Environmental and epidemiological data indicate low *V.*

parahaemolyticus and *V. vulnificus* levels and few cases of illnesses are associated with bivalve molluscs when salinity exceeds 35 ppt (g/l) and 30 ppt (g/l), respectively.

6. Hygienic production of food sources at Primary Production

Pre-harvest and harvest measures should be applied as necessary such as:

- ✓ Restrict harvest or otherwise prevent use of product for raw consumption (e.g. close area to harvest or divert product for further processing).
- ✓ Where possible, sink bivalve molluscs below the thermocline where the growth of pathogenic *Vibrio* spp. will not occur
- ✓ Relay bivalve molluscs to areas where risk is sufficiently reduced (e.g. relay bivalve molluscs with *V. vulnificus* to high salinity offshore waters)

7. Combination of measures to control *Vibrio* spp. in bivalve molluscs consumed in partially treated state

Combination of control measures in Part II is applied for bivalve mollusks destined for partial treatment, in view of preventing cross-contamination and ensuring sufficient risk reduction throughout the entire food chain. The combination of measures of the treatment and those described in Primary Production Section of Part II should achieve at least an equivalent level of protection to the level of protection applied for raw or live bivalve mollusks. Bivalve molluscs destined for partial treatment should be handled separately from those to be consumed live, untreated raw or after post-harvest processing. Partial treatment was to reduce the level of vibrios, but was not to eliminate them completely, unlike sterilization. It was highlighted that any measure or practice to reduce or limit, but not to eliminate *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs should be adequately validated to ensure that the control measures were effective and such validated control measures should be implemented under an HACCP system.

Conclusion

Foodborne V. *parahaemolyticus* illness occurs from various seafood, however, in most countries, bivalve such as oyster is the predominant implicated seafood due to the filter feeding characteristics. This risk is controllable by applying combination of control measures along with food chain. Risk assessment and other scientific information should be used to make a better and transparent decision making on the selection of the risk management option(s).

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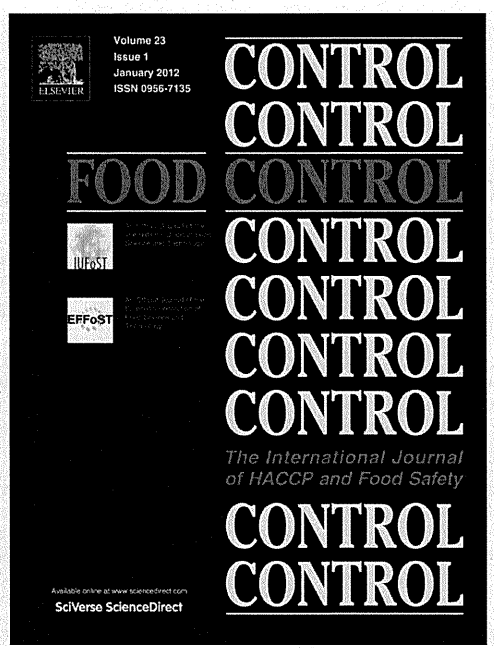

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Semi-quantitative study to evaluate the performance of a HACCP-based food safety management system in Japanese milk processing plants

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ABSTRACT

This study aimed to gain an insight in the performance of Hazard Analysis and Critical Control Points (HACCP)-based food safety management systems (FSMS) implemented in Japanese milk processing plants. Since 1995, Japan has a comprehensive approval system for food manufacturing establishments by evaluating the development and implementation of GHP and HACCP by the food manufacturing companies/operators. An FSMS-diagnostic instrument was applied to assess the level of the core control and assurance activities in the FSMS and to judge the risk level of the context wherein the companies operate. The data were collected in 13 dairy companies (mostly located around Tokyo area) and involved in-depth interviews performed (by the National Institute of Public Health) with responsible quality assurance persons of respective companies.

The results revealed that the microbial food safety output was higher for companies with national HACCP approval. They have more advanced FSMS in combination with a less risky context. All Japanese companies scored high on technology-dependent activities (i.e. preventive measures and intervention processes), but less in managerial activities as monitoring and typical quality assurance activities as validation and verification of the FSMS. Japan has a detailed vertical legislation, leading to a "hazard-based" and "legislation-based" FSMS compared to a "science-, or risk-based" FSMS common in Europe.

1. Introduction

Hazard Analysis and Critical Control Points (HACCP) has long been internationally recognized and accepted as the system for effective food safety management (CAC, 2003). It is a systematic preventive approach to food safety (FS) for identifying potential contamination and subsequently evaluating that the process is in control of those points or steps of the agri-food chain critical to FS. However, its success and effectiveness in preventing food borne diseases and reducing FS risks to an acceptable level depend on its correct implementation and application (FAO and WHO, 2006, pp. 53–55; Lawley, 2007; Kök, 2009). The use of hygienically designed equipment and prerequisite programs (PRPs) as Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP) and sanitation standard operational procedures, need to be there prior to HACCP implementation (EHEDG, 1997; Jacxsens, Devlieghere, & Uyttendaele, 2009; Kök, 2009; Panisello & Quantick, 2001; Roberto, Brandão, & da Silva, 2006; Walker, Pritchard, & Forsythe, 2003). However, governments and food companies have interpreted HACCP differently (Ropkins and Beck, 2000). It can be i) legislation-based, the law prescribes which "control measures" and "intervention processes" need to be implemented; ii) hazard-based, the sector (guideline-driven) prescribes the HACCP principles and PRPs; or iii) science or risk-based, all elements of the FSMS are scientifically underpinned, tailored and supported by scientific evidence for the individual company's specific situation or a risk analysis is applied (Buchanan & Whiting, 1998; Orriss and Whitehead, 2000; Sperber, 2005; Unnevehr & Jensen, 1999).

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In Japan, the revision of Food Sanitation Law (Law No. 233, 1947) came into force in 1998. By this amendment, a voluntary HACCP and GHP based approval system was introduced to milk and milk products, meat products, then expanded to surimi-based products, low acid canned food, and soft drink producing establishments (FAO and WHO, 2006, pp. 53–55; JETRO, 2006). Since microbiological criteria for final product and standards for processing and storage are articulated in the Ministerial Ordinance on Milk and Milk Products Concerning Compositional Standards etc. (MHLW Ordinance No.52, 1951) (JETRO, 2008), the number of food borne outbreaks associated with milk consumption is very limited in Japan. In 2010, 156 approvals have been given by the Ministry of Health, Labour and Welfare (MHLW) of Japan to multiple milk processing establishments, while 497 milk processing establishments were registered. It is estimated that 60% of milk plants which process more than 2 tons of raw milk gained approval (Japan Dairy Association, 2005). The objective of this study is to investigate how the implementation of the voluntary HACCP and GHP based approval system affected the FSMS implemented in Japanese milk processing plants by an independent analysis and to gain an insight into the actual performance of current FSMS in the Japanese milk industry.

The FSMS-diagnostic instrument (FSMS-DI) was applied in 13 Japanese milk processing companies with the focus on the milk process and not the performance of the milk products itself (e.g. by microbial analysis). The FSMS-DI includes tools to diagnose the level of core control (Luning, Bango, Kussaga, Rovira, &. Marcelis, 2008), assurance activities (Luning et al., 2009), the risk level of the context wherein the systems operate (Luning, Marcelis, et al., 2010) and the microbial food safety output (Jacxsens et al., 2010). The results of this semi-quantitative study are related in terms of annual sales and having HACCP approval by the Japanese government (MHLW) of these 13 milk companies.

2. Materials and methods

2.1. Characterization of the Japanese milk processing companies

The assessment has been carried out in 13 milk companies (located mostly around Tokyo area, Japan), which were randomly selected on their annual sales and willingness to participate. The companies were grouped as biggest (more than 100,000,000,000 Japanese Yen, $n = 3$), big (between 10,000,000,000 and 100,000,000,000 Japanese Yen $n = 4$), medium (between 1,000,000,000 and 10,000,000,000 Japanese Yen, $n = 1$) and small (less than 1,000,000,000 Japanese Yen, $n = 5$) (Table 1). The companies represent the typical types of businesses within the Japanese dairy sector.

All 13 companies produce milk, using Ultra High Temperature (UHT) and/or Long Temperature Long Time (LTLT) pasteurization. In addition, all thirteen companies produce milk products, e.g. reduced milk fat drink, fermented milk products; but the focus for all companies in this study was on the processing of the milk as representative processing unit, meaning that the processing of milk is present in each company, results in the same outcome and is the least complex to compare.

With the exception of three small companies and one medium company (J, K, L and M), all had HACCP approval by the MHLW (Table 1).

2.2. Food safety management system – diagnostic instrument

The FSMS-DI enables a systematic assessment of the effectiveness of the companies specific FSMS, separate from the implemented (private) QA standards. It comprises a list of 58 indicators (Table 2–5) with corresponding grids including concise descriptions of different situations, to assess: a) riskiness of the context characteristics wherein the company operates, b) levels of core control and core assurance activities of the FSMS, and c) microbial food safety output levels.

The instrument is systematically assessing context factors (i.e. product, process, organisational, and chain environmental characteristics (Table 2)), which cannot (easily) be changed on the short term, but which influence the microbiological food safety output of the system. A high-risk context corresponds to more vulnerability (to safety problems), ambiguity (lack of insight in underlying mechanisms), and uncertainty (lack of information) inherent to the context characteristics, which requires an advanced FSMS (Luning, Jacxsens, et al., 2010). For each 'context' indicator, three different situations have been described representing respectively a low (score 1), moderate (score 2) and high-risk situation (score 3) for decision-making in the FSMS activities (Luning, Marcelis, et al., 2010). For the context factors product and process characteristics, the low, moderate, and high-risk situation descriptions represent, respectively low, potential, and high chance on contamination, growth, or survival of pathogens, and other undesired microorganisms. The descriptions for low, moderate, and high-risk situations for organisational characteristics correspond with, respectively, supportive, constrained (restricted), and lack of administrative conditions for appropriate decision-making. For the chain characteristics, the low, moderate, and high-risk situation description correspond with low, restricted, and high dependability on other chain actors.

Core control measures and assurance activities are crucial for prevention and reduction of microbial contamination along the chain, and assure that safety requirements will be met. Indicators for the core control activities (Table 3) are addressing major technology-dependent and managerial activities in design and operation of preventive measures, intervention processes, monitoring systems and operation control measures and core assurance activities (Table 4) as defining system set-up, validation, verification, and documentation and record keeping. For each 'activity' indicator four different levels have been described, which represent low (score 0), basic (score 1), average (score 2), and advanced level (score 3). The basic level (score 1) for control activities is typified by use of own experience, general knowledge, ad-hoc analysis, incomplete, not standardised, unstable, regularly problems. For assurance, the basic level is typified by problem driven, only checking, scarcely reported, no independent positions. The average

Table 1
Characterization of the 13 Japanese milk companies.

Company number	Total annual sales	HACCP Approval by Ministry of Health, Labour and Welfare	Total number of employees (% temporary)
A	B+[a]	Yes	325 (53.5)
B	B[b]	Yes	220 (59.0)
C	B+	Yes	163 (3.1)
D	B+	Yes	259 (32.8)
E	B	Yes	97 (12.4)
F	B	Yes	72 (50.0)
G	M[c]	Yes	95 (10.5)
H	B	Yes	100 (11.0)
I	M	Yes	128 (37.5)
J	S[d]	No	8 (37.5)
K	S	No	10 (60.0)
L	S	No	18 (5.6)
M	M	No	74 (37.8)

[a] B+ = Biggest.
[b] B = Big.
[c] M = Medium.
[d] S = Small.

Table 2
The frequency of the individual scores for the context factors ($n = 13$). (Score 1, 2, and 3 represent respectively low, moderate and high-risk context).

Context factors	Indicators	Frequencies		
		1	2	3
Product characteristics	Risk of raw materials	0	9	4
	Risk of product(s) (groups)	0	1	12
	Safety contribution of packaging concept	0	13	0
Process characteristics	Extent of intervention steps	0	13	0
	Production process changes	10	3	0
	Rate of product/process design changes	13	0	0
Organisational characteristics	Technological staff	11	2	0
	Variability of workforce	13	0	0
	Operator competences	6	6	1
	Management commitment	8	3	2
	Employee involvement	9	4	0
	Formalisation	9	3	1
	Information systems	4	7	2
Chain environmental characteristics	Safety contribution in chain	0	0	13
	Supplier relationships	5	7	1
	Customer relationships	5	6	2
	Requirements of stakeholders	11	2	0

level (score 2) for control activities is characterised by being based on expert (supplier) knowledge, use of (sector, governmental) guidelines, best practices, standardised, sometimes problems. For assurance, this average level corresponds with active, additional analysis, regular reporting, and experts support. The advanced level (score 3) means that the control or assurance activity is characterised by use of specific information, scientific knowledge, critical analysis, procedural methods, systematic activities, and independent positions (Luning et al., 2008; Luning, Jacxsens, et al., 2010; Luning et al., 2009).

The seven food safety performance indicators (FSPI) (Table 5) give a first insight in the microbial food safety (FS) output of the FSMS without the requirement of doing actual microbiological analyses. These indicators represent four levels: not applied (no FSMS evaluation and or the specific food safety performance is not known) (score 0), poor (score 1), moderate (score 2) and good food safety output (score 3). A good food safety output is determined by aspects like a structured sampling plan, a systematic evaluation of the FSMS using specific criteria and having no food safety problems due to (a) problem(s) in the FSMS (Jacxsens et al., 2010).

The data were collected in each milk processing plant by the FSMS-DI, which involved in-depth interviews with responsible quality assurance persons of respective companies. The interviews were performed by the National Institute of Public Health.

Data processing was performed using Microsoft Office Excel. For a first and overall impression, overall scores for context, FSMS performance and FS output were calculated based upon taking all scores of the indicators for respectively context, FSMS performance (core control and assurance) and FS output divided by the total number of the respective indicators. These mean scores have further been transformed to overall assigned scores. If a mean score for FSMS (control or assurance activities) was between 0 and 0.2

Table 3
Frequency of the individual scores to compare control activities (preventive measures design, intervention processes design, monitoring system design and the operation) for the 13 milk companies. (Score 0, 1, 2, and 3 represent low, basic, average and advanced level).

Core control activities	Indicators	Frequencies			
		0	1	2	3
Preventive	Hygienic design of equipment and facilities	0	4	0	9
	Cooling facilities	0	0	0	13
	Sanitation programs	0	0	2	11
	Personal hygiene requirements	0	0	1	12
	Raw material control	0	0	0	13
	Product specific preventive measures	0	0	4	9
Intervention	Intervention equipment	0	2	1	10
	Packaging intervention equipment	0	0	1	12
	Maintenance and calibration program for (intervention) equipment	0	2	2	9
	Intervention methods	0	1	3	9
Monitoring	Analysis of CCP/CPs	2	0	10	1
	Standards and tolerances design	0	0	4	9
	Analytical methods to assess pathogen levels	0	13	0	0
	Measuring equipment to monitor process/product status	0	0	2	11
	Calibration program for measuring and analytical equipment	1	2	3	7
	Sampling design (for microbial assessment) and measuring plan	0	0	0	13
	Corrective actions	3	1	1	8
Operation	Actual availability of procedures	1	0	10	2
	Actual compliance to procedures	1	0	2	10
	Actual hygienic performance of equipment and facilities	0	0	0	13
	Actual cooling capacity	0	0	0	13
	Actual process capability of physical intervention processes	0	0	0	13
	Actual process capability of packaging intervention	0	1	2	10
	Actual performance of measuring equipment	0	0	0	13
	Actual performance of analytical equipment	0	0	1	12

Table 4
The frequency and the individual scores for the assessment of assurance activities to compare the core assurance activities of the 13 milk companies (Score 0, 1, 2, and 3 represent low, basic, average, and advanced level).

Core assurance activities	Indicators	Frequencies				HACCP approval									No HACCP approval			
						B+[a]	B[b]	B+	B+	B	B	M[c]	B	M	S[d]	S	S	M
		0	1	2	3	A	B	C	D	E	F	G	H	I	J	K	L	M
Setting system requirements	Translation of stakeholder requirements into own FSMS	4	3	3	3	3	2	2	3	3	2	1	0	1	0	0	0	1
	Systematic use of feedback information to modify FSMS	1	2	3	7	3	3	3	3	3	2	3	2	3	0	1	2	1
Validation	Validation of preventive measures	0	3	3	7	3	3	3	3	3	3	1	3	2	1	2	2	1
	Validation of intervention measures	0	3	3	7	3	3	3	3	3	3	1	3	1	2	1	2	2
	Validation of monitoring system	0	6	2	5	3	3	2	1	3	3	1	3	1	1	1	2	1
Verification	Verification of people related performance	1	3	9	0	2	2	2	2	2	2	2	2	2	1	1	1	0
	Verification of equipment and methods related performance	1	1	10	1	2	2	2	2	2	3	2	2	2	1	2	2	0
Support of food assurance	Documentation	1	0	9	3	3	3	3	2	2	2	2	2	2	2	2	2	0
	Record keeping system	0	1	9	3	3	3	3	2	2	2	2	2	2	2	2	2	1

[a] B+ = Biggest.
[b] B = Big.
[c] M = medium.
[d] S = Small.

then assigned score 0, if between 0.3 and 1.2 (assigned score 1), if between 1.3 and 1.7 (1_2), if between 1.8 and 2.2 (2), if between 2.3 and 2.7 (2_3), and if mean score was between 2.8 and 3.0 then assigned score 3. For context factors and FS output, if the mean score was between 1 and 1.2 then assigned score 1, if between 1.3 and 1.7 (assigned score 1_2), if between 1.8 and 2.2 (2), if between 2.3 and 2.7 (2_3), and if between 2.8 and 3.0 then assigned score 3 (Luning, Jacxsens, et al., 2010) for context; Jacxsens et al., 2010 for FS output). For detailed analysis also the frequency of the scores are given to perceive any differences between companies and to have insight on possibilities to improve their FSMS. Companies were seen as being different from each other, when the frequency was distributed over all scores, meaning for this study no score had a frequency of nine or more.

3. Results and discussion

The FSMS-DI was developed as a self-assessment tool for companies to perform an analysis of their FSMS (e.g. Jacxsens, Kussaga, et al., 2009; Luning, Marcelis, et al., 2010; Sampers et al., 2010), but it can also be used by competent authorities to assess the FSMS performance in a sector (by comparing companies against each other) and to identify sector-specific bottlenecks in the FSMS as applied in this study.

An overall picture of the performance of the FSMS in the Japanese milk companies is illustrated in Table 6. Companies were all operating in a moderate (assigned score 2) to low-risk context (assigned score 1_2), but showed differences in FSMS performance, and FS output. The FSMS performance of the 13 companies ranged from basic (assigned score 1_2) to advanced (assigned score 3), whereas their FS output ranged from <2_3 (companies K, L and M) to ≥2_3. Companies without HACCP approval by MHLW (i.e. companies J, K, L and M), with the exception of company J, showed a lower performance of FSMS (Table 6).

Three out of four companies without HACCP approval by MHLW are small companies (Table 6). This situation could indicate a possible source of food safety problems. Small businesses have been mentioned as important locations in the transmission of food borne illness (Walker et al., 2003). Many small and medium enterprises (SMEs) face more hurdles when implementing quality assurance standards and guidelines and maintaining their system (Holy von, 2004; Jacxsens et al., in press; Sugimura and Iizawa, 2003). It is recognized that larger companies generally have better food safety management systems in place (Kök, 2009).

Table 5
Frequency of the individual scores to compare the food safety output for the 13 milk companies (Score 0, 1, 2, and 3 represent not applied, poor, moderate and good level).

Food safety performance indicators	Frequencies			
	0	1	2	3
Food Safety Management System evaluation	4	5	1	3
Seriousness of the remarks	3	0	1	9
Microbiological food safety complaints	0	0	1	12
Hygiene related complaints	0	0	8	5
Product sampling	0	0	1	12
Judgement criteria	0	1	1	11
Hygiene and pathogen non-conformities	0	0	3	10

3.1. Context situation

First the riskiness of the context situation, shown in Table 2, was examined as a more risky context demands a more advanced FSMS in order to be able to produce food products with a good food safety level (Jacxsens, Kussaga, et al., 2009; Luning, Marcelis, et al., 2010). The thirteen milk companies have similar product and process characteristics (Table 2), because they produce the same products under similar processing conditions. It concerned moderate risk products due to restricted critical (heat inactivating) treatments to reduce pathogens.

Differences between the companies were noticed in their organisational characteristics indicating that the companies provide different types of organisational support (Table 2). The small and medium companies seemed less well organised,

Table 6
Assigned scores for context, Food Safety Management System (FSMS) performance and Food Safety (FS) output for 13 Japanese milk companies. (Context 1 → 3: low to high-risk, FSMS performance 1 → 3: basic to advanced level, FS output 1 → 3: poor to good food safety output).

Context	FSMS	FS	Companies
1_2	3	3	A, B
1_2	2_3	3	C
1_2	2_3	2_3	D, E, F, G
2	2_3	2_3	H, I
2	2	2_3	J
2	2	2	K, L
2	1_2	2	M

probably due to the lack of financial and human resources, training and motivation (Celaya et al., 2007; Holy von, 2004). Adequate resources should be provided by a committed management in order to acquire all basic prerequisite programs (Panisello and Quantick, 2001).

Eight companies scored 1 (low-risk) regarding management commitment (Table 2). On the other hand mainly the smaller businesses (companies I, J, K, L and M) scored 2 or 3 (moderate – high-risk) (Table 2). Low management commitment increases the chance of inappropriate operation on food safety issues in control and assurance activities, negatively affecting the performance.

Companies C, F, H, I, K and L scored 2 for riskiness due to operator competences, company J scored 3, and the others scored 1 (low-risk), this appears independent of the volume of annual sales and setting high requirements on operators (situation 1). According to Ko (2010), operators must attain a high degree of food safety knowledge, as well as the ability to effectively apply such knowledge in food handling practices to achieve consistent safe food handling practices. However, owners of small, independent businesses commonly have little or no food knowledge or followed only basic hygiene training (Baş, Yüksel, & Çavuşoğlu, 2007). Lack of competent operators, require stricter operator control and more robust procedures. Companies I, K, L, M scored 2 or 3 for formalisation, which means that they have restricted (or absence) use of procedures and organised consultative structures which increases the chance on unexpected decision-making behaviour at safety tasks (Luning, Marcelis, et al., 2010). Companies L and M scored 3, and companies C to D and H to K scored 2 to for information systems, which means that they only have a standard information system for bookkeeping or an information system linked to production. Lack of adequate information systems hinder appropriate decision-making in food safety tasks (McMeekin et al., 2006), and put more requirements on verification activities (Luning, Jacxsens, et al., 2010). Lack of adequate information systems has been addressed to lack of time and lack of understanding of how to set-up a documented (information) system (Holt and Henson, 2000).

The companies also differ in riskiness in chain environment characteristics, as they have different relationships with their suppliers, customers, and other stakeholders. The majority of companies scored 3 (which means they had no ability or the power to set requirements on suppliers' specifications or their food safety management system) or 2 (they could only set specifications) (Table 2). The high scores for customer relationship (no influence on customer use of their products) were mainly found for the small businesses, while supplier relationship characteristics were more scattered for the big and medium businesses. A high-risk situation for supplier and customer relations requires more systematic and advanced control of supplied raw materials and final products (Table 3). Nevertheless control on appropriate product use of their customers (Bruhn and Schutz, 1998), next to setting supplier requirements and ensuring contract specification compliance, auditing potential suppliers and monitoring them (Holleran, Bredahl, & Zaibet, 1999) can decrease the requirements on the own FSMS and increase FS.

In general the smaller companies do not operate in a more risky context than the medium and big ones.

3.2. Core control activities

Similar results are found over the 13 companies for the core control activities (Table 3). Mainly level 3 is seen meaning that the control activities (e.g. temperature control, cleaning and disinfection, …) are typified by being science-based, tailored, tested, complete, accredited, stable performance, and full awareness (people aspects) (Luning et al., 2008). This situation can be explained by the fact they are strictly following the Japanese hygiene legislation, which is detailed and put strict demands on good practices (GMP, PRP) and control activities. All companies scored 3 for preventive control activities as 'cooling facilities' and 'raw material control' (Table 3), as the Milk Ordinance articulated a storage requirement of final product and microbiological and other criteria for raw milk (JETRO, 2008). For the monitoring activities 'Standards and tolerances design', 'Measuring equipment to monitor process/product status' and 'Sampling design and measuring plan', high levels were found (2–3) as in Japan the methodology of analysis is prescribed by the legislation (Forsythe and Hayes, 1998). This explains also the low level (1) for 'Analytical methods to assess pathogen levels' and the lower levels found for 'Calibration'. Microbiological criteria for final product and analytical methods were articulated in the Ministerial Ordinance on Milk and Milk Products Concerning Compositional Standards etc. (MHLW Ordinance No.52, 1951) (JETRO, 2008). All companies shall implement these regulatory requirements, and the harmonization of test methods with internationally recognized methods in Japan is behind compared to other industrial countries, this is one of the reasons why test methods used in these milk plants were not internationally standardised methods (level 1) e.g. ISO methods.

A few differences were noticed in control activities. Four companies (D, I, J and M) scored 1 for the preventive control measure 'hygienic design of equipment and facilities' (Table 3), irrespective of the annual sales or having a HACCP approval. Although, various preventive and control strategies like hygienic plant lay-out and design of equipment, choice of materials, correct use and selection of detergents and disinfectants coupled with physical methods need to be suitably applied for quality and safety of foods (EHEDG, 1997; Kumar and Anand, 1998; Graham, 2009). The scores 1 and 2 for the intervention measures were only found for the companies without HACCP approval, while the others had mainly score 3 (Table 3).

Table 3 shows that the four companies without HACCP approval (J, K, L and M) did not have a complete and differentiated description of 'corrective actions' (levels 0 or 1) necessary for a continuous improvement of the effectiveness of the FSMS (Table 3) (NACMCF, 1992; Holleran et al., 1999). As already indicated these companies (K, L and M) had a low level of formalisation (high-risk context, score 1). Company M even had no procedures in place (score 0) and had no knowledge of compliance to procedures (score 0) (Tables 2 and 3).

Obviously, no analysis of critical control points (CCPs) was executed nor by company J and L themselves nor by external experts (Table 3). This is due to the fact that flow diagrams of all milk plants were almost the same; raw milk receipt - raw milk test - cool down - clarifier - holding tanks - primary heat - homogenizer - UHT- cool down - holding – packaging - final products testing - cold storage - chilled distribution. Selecting CCP (UHT heat treatment) is, therefore, self-evident. Some companies replied that they just used Codex decision tree and/or HACCP manual developed by Japan Dairy Association. In addition, the UHT process is well documented and has a long history of operation. Most companies did not, therefore, perform scientific validation studies (e.g. microbiological testing, challenge test) and just follow scientific literatures and/or industry guidelines values for the Critical Limit (120–130 °C for 2 s). This approach is included as one of validation methods in Codex validation document (CAC, 2008). Tools such as microbiological challenge testing, storage testing, and predictive modelling, however, can give information of what happens actually during food processing, distribution and subsequently handling (CAC, 2008; Notermans & in 't Veld, 1994).

The information obtained, can be the basis for setting safety criteria at CCPs in the food processing operation, which still must be verified under field conditions (Jacxsens, Kussaga, et al., 2009; Notermans & in't Veld, 1994). Smaller companies could introduce a HACCP plan fully based on a HACCP guide drawn by the sector, as adoption of the HACCP system can be expensive and a serious burden for smaller milk factories due to lack of time, lack of finances or experience (Sugimura and Iizawa, 2003; Walker et al., 2003; Holy von, 2004). Certain minimum requirements need to be determined to implement HACCP by the small business. As seen in this study these involve the concepts of process description, process monitoring, corrective action and record keeping (Holy von, 2004).

3.3. Core assurance activities

A more scattered picture is found for the core assurance activities (Table 4). In fact, not all assurance activities are conducted at the most advanced level (3), especially not with the companies without HACCP approval (J, K, L and M). In fact the, the small and medium companies scored the low level (score 0) and basic level (score 1). Companies A to F and H, being the big and biggest companies, have their assurance activities already at level 2 or 3 (Table 4). Assurance activities, such as 'translation of stakeholder requirements into own FSMS' are not required by the Japanese hygiene legislation or sector guidelines. These guidelines are mostly focused on control activities and not on assurance (JETRO, 2008). For six companies (D, G, I, J, K, L and M), the validation of CCP and monitoring system was based on historical and/or commonly available knowledge, executed by own people on ad-hoc basis; findings (not) scarcely described (Table 4). A more scientific evidence-based, systematic, and independent validation would improve the monitoring activities of the companies J, K, L and M (Table 3). Although the validation of preventive measures also scored 1 (Table 4), their preventive control measures (Table 3) were of advanced level (score 3), as these are fully described in the Japanese hygiene legislation and probably therefore not validated.

Verification of procedures and compliance were for most companies (except for companies J, K, L and M) based on analysing procedures (both content and presence) and records, with defined frequency, by independent internal staff and internal report (level 2).

In the beginning of the HACCP implementation in the Japanese milk industry, generic HACCP plans were utilized as a basis to develop factory or line specific HACCP plans. The results of this study indicated that this process is not good enough in some plants, and more factory specific validation study and verification activities should be performed. The approval criteria of the HACCP and GHP based approval system by the MHLW is based on Codex General Principles on Food Hygiene and Codex HACCP 7 principles (MHLW, 1996), and does not include management and quality assurance parts of FSMS, which may be why the results indicated weakness in these areas.

Assurance activities are more long term based (Sporleder & Goldsmith, 2001) and companies encounter these as difficult to implement as well as time-consuming (Jacxsens et al., in press). However, it should be an objective to enforce a sustainable FSMS, as validation and verification, sampling plans and documentation are important to assure food safety (Luning et al., 2009; Taylor & Kane, 2005).

All companies (except company M) showed an advanced level for documentation and record keeping (Table 4). Documentation supports the food processors to ensure the safety of products for sale (Cullor, 1997 and Stefan, 1997) and can help validating their system (Ilyukhin, Haley, & Singh, 2001).

3.4. Food safety output

The Food Safety (FS) output is measured via the food safety performance indicators (Table 5). Results from Table 5 show that the FSMS of most companies are not evaluated or only via inspection by the competent authorities. Only the FSMS of one small company is also evaluated via a third party audit (level 2). Three big companies (A, B and C) scored 3, which means that more than one audit is performed by accredited party(s) or in combination with an inspection of the national food safety agency (Jacxsens et al., 2010). Only one company (B) has a certification (ISO9001) (ISO, 2008) for the whole food chain.

A few companies have hygiene complaints (level 2), which can be dedicated to one specific problem in the functioning of the FSMS, so improvement should be made.

Most other food safety performance indicators scored 3, as only the milk production was highlighted in this study. This part of the process (UHT and LTLT milk process) is an overall acknowledged, well established and validated heat treatment. The next steps, the production of milk products, are less well generally established and need to be scientifically underpinned and tailored. The lack of assurance activities such as verification and validation for the own company specific situation will probably have a bigger effect on the food safety output of the milk products.

4. Conclusion

Food safety output is not only affected by the food safety management system but also depends on the riskiness of the context characteristics. A more risky context requires a more advanced FSMS to achieve a good food safety output. The Japanese milk companies operate in a moderate to high-risk context which put requirements on the level of their control and assurance activities. Obviously, all companies scored high on technology-dependent control activities (preventive measures and intervention processes), but less in more managerial quality assurance activities. Japan has a detailed vertical legislation, leading to a "hazard-based" and "legislation-based" FSMS and not a "risk-based" FSMS like is common in Europe (European Food Hygiene Regulations 852/2004, 853/2004). The Japanese companies implement the legal requirements, which strictly prescribe control measures and intervention steps for ensuring safety of milk and milk products. This is indeed reflected in the high scores for the corresponding control activities for all companies. However, the lower scores for other control activities as CCP determination, microbiological analyses, sampling, maintenance and calibration, indicate that they do not yet tailor these activities for company specific characteristics. This is certainly lacking for the companies without national HACCP approval which are mainly smaller companies.

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Quantitative Risk Assessment of *Vibrio parahaemolyticus* in Finfish: A Model of Raw Horse Mackerel Consumption in Japan

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The aim of this study was to evaluate the effects of implemented control measures to reduce illness induced by *Vibrio parahaemolyticus* (*V. parahaemolyticus*) in horse mackerel (*Trachurus japonicus*), seafood that is commonly consumed raw in Japan. On the basis of currently available experimental and survey data, we constructed a quantitative risk model of *V. parahaemolyticus* in horse mackerel from harvest to consumption. In particular, the following factors were evaluated: bacterial growth at all stages, effects of washing the fish body and storage water, and bacterial transfer from the fish surface, gills, and intestine to fillets during preparation. New parameters of the beta-Poisson dose-response model were determined from all human feeding trials, some of which have been used for risk assessment by the U.S. Food and Drug Administration (USFDA). The probability of illness caused by *V. parahaemolyticus* was estimated using both the USFDA dose-response parameters and our parameters for each selected pathway of scenario alternatives: washing whole fish at landing, storage in contaminated water, high temperature during transportation, and washing fish during preparation. The last scenario (washing fish during preparation) was the most effective for reducing the risk of illness by about a factor of 10 compared to no washing at this stage. Risk of illness increased by 50% by exposure to increased temperature during transportation, according to our assumptions of duration and temperature. The other two scenarios did not significantly affect risk. The choice of dose-response parameters was not critical for evaluation of control measures.

KEY WORDS: Dose-response model; food-borne microbial disease; quantitative risk assessment; raw fish; *Vibrio parahaemolyticus*

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1. INTRODUCTION

Vibrio parahaemolyticus (*V. parahaemolyticus*) is a bacterium that is found in marine life and water during the warm season. The consumption of food contaminated with *V. parahaemolyticus* strains carrying either the *tdh* or *trh* gene, or both, are thought to cause human gastroenteritis. It was reported that about 12,000 patients were infected with *V. parahaemolyticus* in Japan during 1998, but these annual cases have recently decreased to 1,000–3,000.[1]

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Hara-Kudo *et al.*[2] reported the prevalence of *tdh*-positive *V. parahaemolyticus* in horse mackerel, shellfish, and sea urchins collected from various areas of Japan between June and October in 2001. They detected *V. parahaemolyticus* in 165 of 173 samples, of which 16 were *tdh*-positive; the density[9] of *V. parahaemolyticus* was in the range of < 3 to 94 MPN/10 g. The proportion of *tdh*-positive isolates in the total *V. parahaemolyticus* samples ranged from <3/46,000 (0.007%) to 93/4,300 (2%) in the *tdh*-positive samples.

In the United States of America, the United Kingdom, and other countries, *V. parahaemolyticus* infection is usually associated with shrimp, lobster, crab, and shellfish such as oyster.[3] The joint Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) expert meetings on microbiological risk assessment (JEMRA) selected *V. parahaemolyticus* in heat-treated bloody clam for quantitative risk assessments (RA), and a report on this RA has been published.[4] In Japan, however, other types of seafood are also important as sources of food-borne illness caused by *V. parahaemolyticus* because the Japanese population consumes a large quantity of finfish. Raw finfish is consumed as *sashimi*, *tataki*, and *sushi*, all year around in Japan, including the warm season. By contrast, raw oysters are mainly consumed in the cold or moderate season. Other uncooked seafood such as sea urchin and salted squid or finfish guts are popular and sometimes cause outbreaks of gastroenteritis.[5] In addition, other uncooked foodstuffs can become contaminated by *V. parahaemolyticus* from seafood through cross-contamination during preparation, and thus cause food poisoning.[5]

JEMRA also selected *V. parahaemolyticus* in raw finfish for quantitative RA, and horse mackerel (*Trachurus japonicus; ma-aji* in Japanese), which is one of the most popular types of finfish consumed in Japan, was selected as being typical of all finfish eaten raw.[6] Horse mackerel is harvested from the seas all around Japan, except in the northernmost region. According to an official survey in 2006,[7] the average Japanese household purchases 38 kg of raw finfish and shellfish per annum including 1.7 kg of horse mackerel and 2.2 kg of *sashimi*.[10] The proportion of horse mackerel in raw fish bought by a household varies from 0.4% in Hokkaido to 2% in Tohoku and 9% in Kyushu. The difference in consumption by district reflects the larger quantity of horse mackerel harvested in the southwest of Japan.

Horse mackerel is thought to comprise coastal and offshore groups. The former fetches a higher price in markets and is preferred for raw consumption. The high horse mackerel harvest season is summer in many areas, although it is consumed all year round. A preference for the coastal group, heavy consumption during the summer, and harvesting from warm seas confer a high potential for horse mackerel to be a source of *V. parahaemolyticus* infection.

This study is based on the fundamental structure developed for the FAO/WHO finfish RA, in which most of the authors of this study were involved, but reexamines risk reductions associated with different mitigation scenarios. In addition, new data were collected from an extensive survey of the literature, mainly published by Japanese local public health laboratories, and incorporated into our model.

The present model simulates the density of *V. parahaemolyticus* in whole horse mackerel and in fillets, from fishing to consumption, assuming initial densities of *V. parahaemolyticus* on the surface, in the gills, and in the intestine of the fish at harvest. The number of pathogenic *V. parahaemolyticus* ingested at one meal is simulated and the probability of becoming ill after eating raw horse mackerel is estimated for each scenario. Probability of illness was estimated using the parameters of the beta-Poisson dose-response model that were determined from all reported human feeding trials, including data that were not used by the U.S. Food and Drug Administration (USFDA) RA,[8] and was compared with the probability estimated using the dose-response model determined in the USFDA RA.

2. MODEL

2.1. Horse Mackerel from Harvest to Consumption

Horse mackerel for raw consumption is caught from the seas around Japan, cooled immediately on fishing boats, and stored at ≤5 °C to maintain

[9] The number of organisms in unit mass, unit volume (both for gills, intestine, whole fish, and fillet), or unit area (for surface) is referred to as density in this article. This may be termed concentration elsewhere. Only the number of organisms in a unit volume of liquid is termed concentration here. The number is given as most probable number or colony forming unit (cfu) in the literature, depending on the measurement method. We made no distinction and used them interchangeably.

[10] Raw fish purchased by households is not necessarily consumed raw. Some, but not all, purchased *sashimi* is likely to be horse mackerel.

freshness. The fish are landed, transported to markets, retailers, and finally to households.[11] During this time, some of the fish are washed with clean water at the port or market (collectively called at landing). Some are stored in pasteurized seawater and others are stored in water that may be contaminated with *V. parahaemolyticus*, and some fish may be exposed to high (room) temperatures during transportation. Fillets are cut from whole horse mackerel either at the retailers or within households. Raw foods such as *sashimi* and *tataki* are prepared from fillets and are consumed after a short unrefrigerated period.

2.2. Model Outline

V. parahaemolyticus populating the surface, gills, and intestine of horse mackerel before preparation is transferred to fillets during preparation. The densities of *V. parahaemolyticus* on the body surface and in the gills and intestine are used to model the effects of washing the whole fish body and the level of contamination associated with different microbiologic levels of storage water. The density in fillets after preparation was estimated from the whole fish body density before preparation, which was calculated from the densities in parts of the fish using proportional constants determined from available experimental data. The entire duration from harvest to consumption was divided in terms of *V. parahaemolyticus* growth estimates into three periods: (1) a period from harvest to preparation (low temperature), (2) an optional, high-temperature period during transportation, and (3) a period from preparation to consumption (possibly room temperature).

We assumed that the proportion of pathogenic *V. parahaemolyticus* strains remained constant from harvest to consumption. The probability of illness due to *V. parahaemolyticus* infection after consumption of a simulated meal of raw horse mackerel was calculated using the beta-Poisson dose-response model.

The RA model is described in detail below and in Appendix A. Fig. 1 shows the outline, and Table I shows the variables and input parameters.

2.3. Initial Densities of Total *V. parahaemolyticus* and Proportion of Pathogenic Strains

V. parahaemolyticus is thought to populate the surface, gills, and intestine of horse mackerel. Only the density of this organism in the gills is usually reported, and we found only one report that included density on the surface and in the gills and intestines of 22 contaminated horse mackerel among 27 purchased just after landing in Niigata Prefecture, Japan between July and October of 1992.[9] The initial densities of *V. parahaemolyticus* in these parts were fixed in our baseline model to these reported values.[9] These data are obtained for horse mackerel just after landing, but we used them as the initial values at harvest as the growth of *V. parahaemolyticus* from harvest to market is negligible under typical storage conditions at low temperatures maintained with ice.

The environmental proportion of pathogenic strains in total *V. parahaemolyticus* is only a few percent at most and varies greatly among samples, as reported by Hara-Kudo *et al.*[2] We used a fixed value of 1% as the proportion in our baseline model, and then estimated the effect of its variation (Section 3.2).

2.4. Growth of *V. parahaemolyticus*

It was assumed that there was no difference in proliferation between strains with or without pathogenic genes as there are no comprehensive data to show that a difference exists. All *V. parahaemolyticus* strains were assumed to proliferate at the same temperature-dependent rate k until maximal density $\rho_{\max}$ is reached.[12] Temperature was assumed to be constant in each of the three (two if the optional high-temperature period does not exist) growth periods for a single fish, and a value for temperature for each period was generated in iterations of simulations according to the distribution shown in Table I. The time lag after a change in condition was not taken into account and steady state was assumed for k in each period. Hence, the density at time t after the start of each period is given as $\rho(t) = \min(\rho(0)e^{kt}, \rho_{\max})$.

The growth rate k of *V. parahaemolyticus* is given by Miles *et al.*[10] as a function of temperature T (in K) and water activity a_w in the temperature range of 281.8–328.5 K as:

$$\sqrt{k} = 0.035634(T - 278.5) \times \{1 - \exp[0.3403(T - 319.6)]\} \times \sqrt{(a_w - 0.921)\{1 - \exp[263.64(a_w - 0.998)]\}}.$$

In our model, a_w is fixed at the optimal value of 0.985. This formula has been used to assess the risk of

[11] Although household consumption is modeled, the model can be used to reflect consumption in other locations such as restaurants.

[12] Not realized in our model under the assumed conditions.

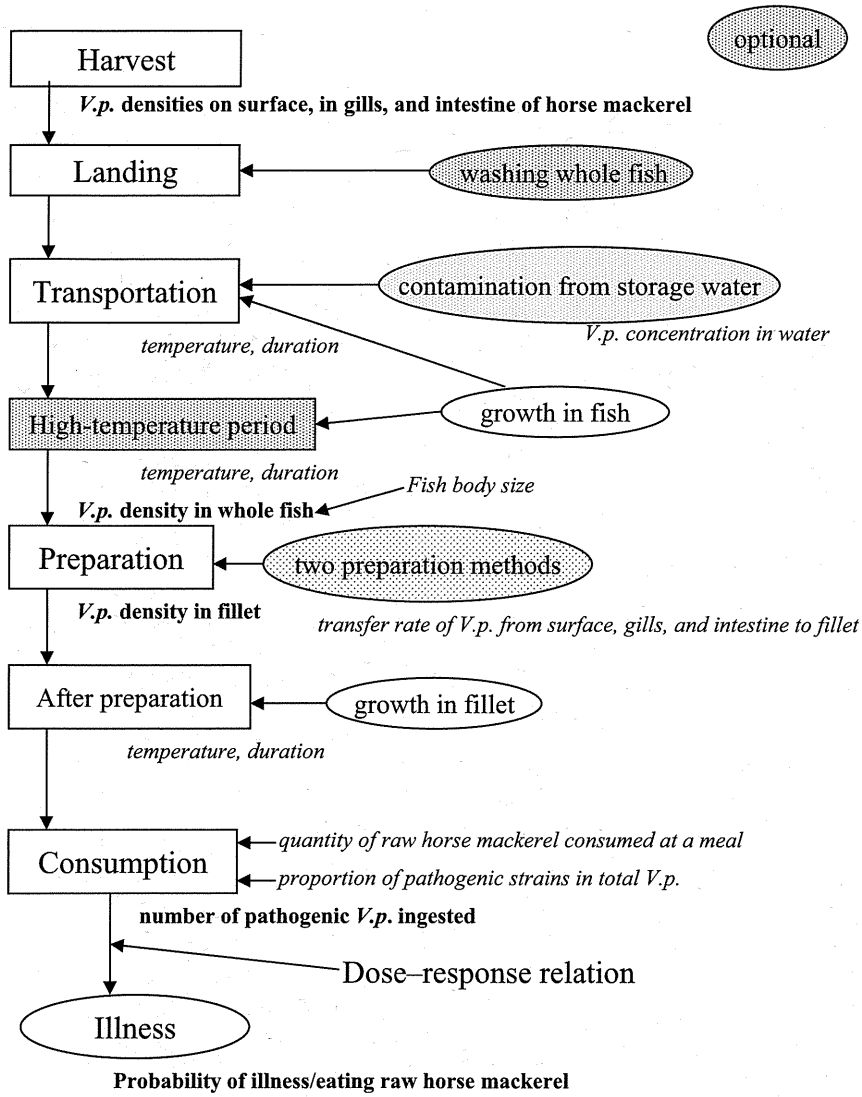

Fig. 1. Risk assessment model of pathogenic *V. parahaemolyticus* for raw horse mackerel consumption. Simulated variables are expressed in bold, and input parameters are expressed in italic.

V. parahaemolyticus in raw oysters by the USFDA[8] with an adjustment factor; the growth rate given by this formula was multiplied by the adjustment factor for raw oysters. We applied the adjustment factor as described below.

The growth of *V. parahaemolyticus* in horse mackerel has been studied by several Japanese investigators (including Horie *et al.*,[11] Takehara *et al.*,[12] and Watanabe *et al.*[13]). Fig. 2 shows the growth rates estimated from their data, the range of growth rates used by the USFDA for raw oysters, and the rates derived from the formula of Miles *et al.*[10]

As the growth rate given by the formula of Miles *et al.* can be made compatible with the data on horse mackerel by a multiplication factor, we adopted this formula with pre- and postpreparation adjustment factors. Before preparation: the inverse of the adjustment factor is given by a triangular distribution (most likely = 4, minimum = 2, maximum = 5). The parameters were given by the USFDA RA, but the minimum value was changed from 3 to 2, allowing more growth than in oysters to reproduce the data on horse mackerel above 10°C (Fig. 2). After preparation: the adjustment factor is given by the normal distribution with the mean of 0.422 and the standard deviation of 0.075, which were determined from data for horse mackerel flesh by Horie *et al.*[11]

The density of *V. parahaemolyticus* usually decreases at temperatures below 10°C; for example, Watanabe *et al.*[13] reported a decrease on fillets at 5°C, although Takehara *et al.*[12] found that the organism proliferated on the surface of horse mackerel

Table I. Variables and Parameters of Model

Variables and Parameters		Distribution	Value			Data Source
			Mean	Sigma		
			Likely	Min	Max	
Density of *V.p.* at harvest	Surface	Fixed	5.10 cfu/cm^2			Ohno *et al.*[9]
	Gill	Fixed	660 cfu/g			
	Intestine	Fixed	1300 cfu/g			
Upper limit of density of *V.p.*	Surface	Fixed	10^5 cfu/cm^2			
	Gill	Fixed	10^7 cfu/g			
	Intestine	Fixed	10^9 cfu/g			
Reduction of *V.p.* by washing whole fish at landing	Surface	Fixed	0.0432			Watanabe *et al.*[13]
	Gill	Fixed	0.6787			
Transfer of *V.p.* from contaminated storage water to fish (ln) (mL/cm^2)	Surface	Normal	4.3257	0.2771		Kumagai *et al.*[14]
	Gill	Normal	1.3565	0.3621		
Concentration of *V.p.* in contaminated storage water ($\log_{10}$) (cfu/100 mL)		Normal	2.1298	1.1246		Reports from local gov. labs[6,15–19]
Growth rate	Base	Mathematical model				Miles *et al.*[10]
Growth rate adjustment factor	Before preparation	Triangular[a]	4	2	5	USFDA oyster RA[8]
	Fillet	Normal	0.422	0.075		Horie *et al.*[11]
Transportation period	Duration	PERT	36 h	6 h	60 h	
	Temperature	PERT	6 °C	3 °C	9 °C	
High-temperature period before preparation	Duration	PERT	1.5 h	0.5 h	3 h	
	Temperature	PERT	18 °C	10 °C	25 °C	
Transfer of *V.p.* to fillet during preparation ($\log_{10}$)	Washing	Normal	−1.9921	0.4545		Watanabe *et al.*[13]
	No washing	Normal	−0.8449	0.4897		
After-preparation period	Duration	PERT	0.5 h	0	4 h	
	Temperature	PERT	22 °C	10 °C	35 °C	
Proportion of pathogenic strains		Fixed	1%			
Quantity of horse mackerel eaten at a meal		Raw data	70 g	2.5 g	250 g	National survey[6]
Horse mackerel size	Body weight	Fixed	80 g			Kumagai *et al.*[14]
	Gill weight	Fixed	0.7 g			
	Intestine weight	Fixed	5.6 g			
	Surface area	Fixed	96 cm^2			

[a]The inverse of the adjustment factor is given by a triangular distribution. The minimum value was changed from 3 (the USFDA oyster RA) to 2.

Notes: Mean and sigma (standard deviation) are given for normal distributions. Likely, minimum, and maximum are given for PERT and triangular distributions and raw data. PERT is a beta distribution scaled to the range specified by min and max.

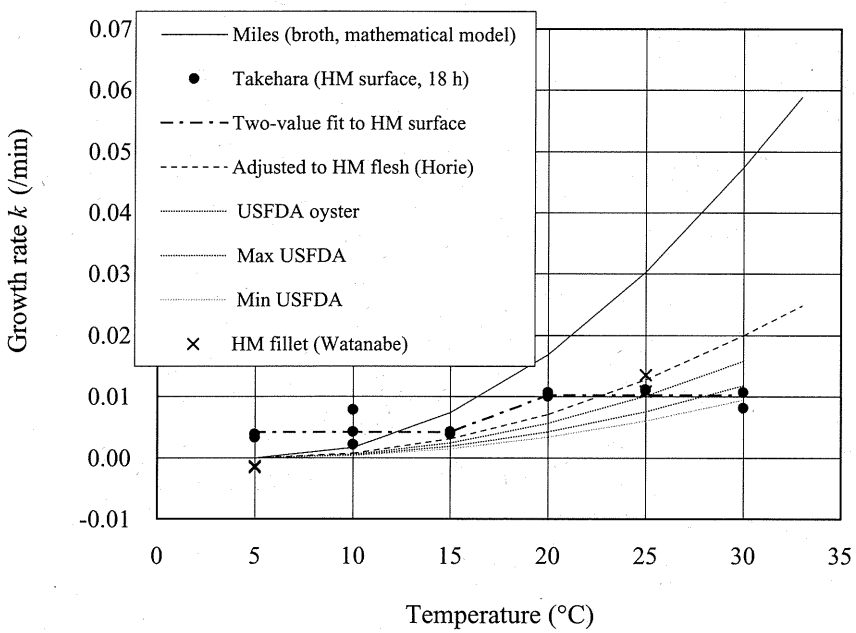

Fig. 2. Growth rate of *V. parahaemolyticus* in horse mackerel, broth, and oyster. Temperature dependence of the growth rate computed using the mathematical model of Miles *et al.*,[10] its adjustment using the USFDA RA of raw oysters,[8] and data from horse mackerel (Horie *et al.*,[11] Takehara *et al.*,[12] and Watanabe *et al.*[13]) are shown.

		Before Washing		After Washing		After/Before	
		Mean	Sigma	Mean	Sigma	Mean	Sigma
Surface	cfu/cm^2	389.1		16.8		0.0432	
	$\log_{10}$	2.590	0.345	1.225	0.285	−1.365	0.448
Gills	cfu/g	4636.3		3146.8		0.6787	
	$\log_{10}$	3.666	0.766	3.498	0.263	−0.168	0.810
Intestine	cfu/g	6032.3		10363.8		1.718	
	$\log_{10}$	3.780	0.378	4.016	0.411	0.235	0.558

Table II. Effect of Whole Fish Washing

Notes: From the laboratory simulation by Watanabe *et al.*[13] They immersed five horse mackerel in water contaminated with 2.5×10^5 cfu/mL *V. parahaemolyticus,* and then simulated washing by processing three times for one minute with tap water (no further information given).

at 5°C. We used the formula of Miles *et al.* with adjustment for temperatures above 278.5 K (5.35°C), and assumed no change in density (neither growth nor decrease) below this temperature, as widely accepted.[13]

2.5. Washing Whole Fish with Clean Water

This option is recommended at landing possibly to decrease the risk of food poisoning associated with *V. parahaemolyticus*. Watanabe *et al.*[13] measured *V. parahaemolyticus* density on the surface and in the gills and intestines of artificially contaminated horse mackerel before and after washing with tap water. We used the geometric mean of five samples and their ratios of before and after washing (summarized in Table II) to estimate the reduction in *V. parahaemolyticus* density on the surface and in gills. No difference was found for the intestine and we assumed no change.

2.6. Contamination of Whole Fish from Storage Water

We examined the effect of contamination from water in which the fish were stored between harvest and preparation.

[13] The usual recommendation is that oysters intended for raw consumption should be stored below 5°C on the grounds that *V. parahaemolyticus* does not grow below this temperature.

Table III. Transfer of *V. parahaemolyticus* from Contaminated Water to Fish Surface

Duration (min)	A: Concentration in Water (cfu/100 mL)	B: Density After Immersion (cfu/cm^2)	B/A (100 mL/cm^2)	Reference
1	4×10^6	78	2×10^{-5}	Kumagai *et al.*[14]
10	9.3×10^7	10^2 –10^3	$(0.1–1) \times 10^{-5}$	Ohno *et al.*[9]

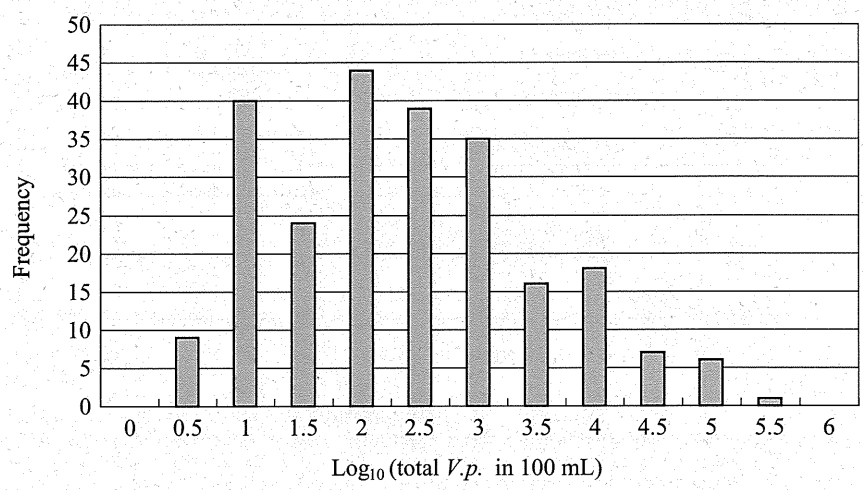

Fig. 3. Concentration of *V. parahaemolyticus* in water used at fish market or during transportation of fish in Japan.[6,15–19]

Table III shows the effects of contaminated water determined by Kumagai *et al.*[14] and Ohno *et al.*[9] These data were obtained by simulation experiments in which horse mackerel were immersed in highly contaminated saline for short periods in the laboratories. Although these conditions are not typical of storage in practice, we used the data from these experiments because of the difficulty in estimating the effect of contaminated water in natural conditions. Because an effect of storage duration on the contamination of fish was not apparent from Table III, we did not include time dependence and assumed a steady state of fish contamination. We adopted the data of Kumagai *et al.*,[14] who also examined gills and intestines, and we computed the increase in density on the surface and in gills, assuming proportionality to the *V. parahaemolyticus* concentration in water. The proportional constants were generated by lognormal distributions with parameters determined from the data obtained by Kumagai *et al.*[14] (Table I). We assumed no effect on bacterial levels in the intestine.

The concentration of *V. parahaemolyticus* in storage water is given as a lognormal distribution (Fig. 3) determined from samples collected between June and October in Japan, excluding in Hokkaido and Tohoku where only small quantities of horse mackerel are harvested.[6,15–19]

2.7. Preparation

Watanabe *et al.*[13] simulated the preparation procedure of horse mackerel fillets. They contaminated the fish with *V. parahaemolyticus* by injecting 1 mL bacterial solution containing 4.3×10^5 organisms of *V. parahaemolyticus* into the visceral cavity and dipping the whole fish in the same bacterial solution for 10 minutes. The fish were stored in a refrigerator for 90 minutes, and then either washed or not before cutting and filleting. Table IV summarizes their results.

The mean density of total *V. parahaemolyticus* in fillet ρ_{fillet} is given as the product of the density in the total fish body before preparation ρ_{total} and the proportional constant $b_{\text{preparation}}$ calculated from the data in Table IV as:

$$\rho_{\text{fillet}} = b_{\text{preparation}} \rho_{\text{total}}.$$

Here, ρ_{total} is given by the density of total *V. parahaemolyticus* in each part (σ_{surface}, ρ_{gill}, and $\rho_{\text{intestine}}$) and quantities describing fish size (surface area S, gill weight m_{gill}, intestine weight $m_{\text{intestine}}$, and weight of a fish M) as:

$$\rho_{\text{total}} = (\sigma_{\text{surface}} S + \rho_{\text{gill}} m_{\text{gill}} + \rho_{\text{intestine}} m_{\text{intestine}})/M.$$

The density of *V. parahaemolyticus* ρ_{fillet} increases depending on the duration of and the

Table IV. Transfer of *V. parahaemolyticus* to Fillets During Preparation

			Density of *Vibrio parahaemolyticus*							Log_{10}(ratio)	
			1	2	3	4	5	Mean	Sigma	Mean	Sigma
Prestudy condition	Whole body	cfu/g	1,500	2,400	930	2,400	930				
		log_{10}	3.176	3.380	2.968	3.380	2.968	3.175	0.206		
Prepared without washing visceral cavity	Fillet	cfu/g	750	240	240	43	240				
		log_{10}	2.875	2.380	2.380	1.633	2.380	2.330	0.444	−0.845	0.490
Prepared with washing visceral cavity	Fillet	cfu/g	23	43	4	9	23				
		log_{10}	1.362	1.633	0.602	0.954	1.362	1.183	0.405	−1.992	0.454

Notes: From the laboratory simulation by Watanabe *et al.*[13] They contaminated horse mackerel with *V.p.* by injecting 1 mL of bacterial solution (which contains 4.3×10^5 organisms of *V. p.*) into the visceral cavity and dipping the whole fish in the same bacterial solution for 10 minutes, before storing in a refrigerator for 90 minutes. Then they prepared fillets either with washing inside the visceral cavity or without washing. Densities of *V.p.* in whole fish before preparation and in fillets were measured for five samples. Log_{10}(ratio) is given by $\log_{10}(\rho_{\text{fillet}}) - \log_{10}(\rho_{\text{wholebody}})$. The proporional constant $b_{\text{preparation}}$ (in Appendix A) is given by $10^{\log(\text{ratio})}$.

temperature during the storage period between preparation and consumption.

2.8. Consumption

The quantity of raw horse mackerel consumed at a meal was simulated by resampling from the Japanese consumption data collected on a single day in November 1995, when 59 of 14,240 individuals consumed raw horse mackerel.[6] The serving size ranged from 2.5 to 250 g (average, 73 g). Although these data were obtained by a survey conducted on a single day, we included them in our model by sampling randomly from the data of 59 individuals to estimate the number of pathogenic *V. parahaemolyticus* ingested from a single serving.

The number of pathogenic *V. parahaemolyticus* ingested with raw horse mackerel at one serving is given as the product of the density of *V. parahaemolyticus* in a fillet after growth, the proportion of pathogenic strains, and the quantity of raw horse mackerel consumed in one meal.

2.9. Dose-Response Model

The beta-Poisson dose-response model was applied to approximate the probability of illness P_{ill} as:

$$P_{\text{ill}} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha},$$

where D is the mean number of pathogenic *V. parahaemolyticus* ingested in a single meal and α and β describe the distribution of probability of illness caused by a microorganism.

The dose-response relationship was determined from human feeding trial data[20–22] utilized in the USFDA *V. parahaemolyticus* RA in raw oysters.[8] Three human feeding trials were completed before 1975, and the results are summarized in Appendix B. The USFDA RA used data from 20 of 27 individuals as shown in Table A3 and determined the beta-Poisson, probit, and Gompertz parameters of dose-response models. These models reproduced the human feeding trial results equally well, but the USFDA RA selected the beta-Poisson model because it was the only one that satisfied the mechanistic criteria identified by FAO/WHO.[23]

The reason for the exclusion of the data of seven individuals from the determination of parameters was not clearly explained in the USFDA RA report. We attempted to evaluate the effect of the exclusion. Takikawa[20] gave only dilution of bacterial culture media instead of dose of bacteria in his findings on human feeding trials. We assumed an undiluted concentration of 10^9 cfu/mL as a typical maximum concentration in culture media for his data. The criterion for illness was not given in Takikawa's report, and we applied two new case criteria of (1) the development of diarrhea and the presence of pathogenic bacteria in feces (bP1) and (2) three or more episodes of diarrhea (bP3). We followed the procedure of Haas *et al.*[24] to determine parameters for these criteria. The dose-response results using these parameters together with those of the USFDA model are shown in Table V and Fig. 4. To estimate statistical uncertainty, the USFDA provided 21 sets of parameters determined from data generated by bootstrapping. The resulting dose-response curves are also included

Table V. Comparison of Dose-Response Relations

Models	α	β	P_{ill}/Dose at Low Dose	ID1	ID10	ID50	ID90	Likelihood
FDAmean			5.99×10^{-7}					
FDA1	1.47×10^{6}	3.53×10^{14}	4.16×10^{-9}	2.41×10^{6}	2.53×10^{7}	1.66×10^{8}	3.53×10^{8}	3.4×10^{-4}
FDA2	3.89	2.28×10^{8}	1.71×10^{-8}	5.90×10^{5}	6.26×10^{6}	4.45×10^{7}	1.84×10^{8}	6.9×10^{-4}
FDA3	1.26×10^{7}	7.20×10^{14}	1.75×10^{-8}	5.74×10^{5}	6.02×10^{6}	3.96×10^{7}	1.32×10^{8}	4.1×10^{-3}
FDA4	636.53	1.65×10^{10}	3.86×10^{-8}	2.61×10^{5}	2.73×10^{6}	1.80×10^{7}	5.98×10^{7}	2.1×10^{-2}
FDA5	1.31	2.93×10^{7}	4.47×10^{-8}	2.26×10^{5}	2.45×10^{6}	2.04×10^{7}	1.41×10^{8}	8.2×10^{-3}
FDA6	35.81	5.42×10^{8}	6.61×10^{-8}	1.52×10^{5}	1.60×10^{6}	1.06×10^{7}	3.60×10^{7}	5.5×10^{-2}
FDA7	20.84	1.99×10^{8}	1.05×10^{-7}	9.60×10^{4}	1.01×10^{6}	6.73×10^{6}	2.32×10^{7}	8.2×10^{-2}
FDA8	0.52	3.61×10^{6}	1.44×10^{-7}	7.05×10^{4}	8.11×10^{5}	1.01×10^{7}	2.99×10^{8}	4.1×10^{-2}
FDA9	14.87	8.78×10^{7}	1.69×10^{-7}	5.94×10^{4}	6.24×10^{5}	4.19×10^{6}	1.47×10^{7}	6.6×10^{-2}
FDA10	0.47	1.50×10^{6}	3.13×10^{-7}	3.24×10^{4}	3.77×10^{5}	5.06×10^{6}	2.00×10^{8}	1.1×10^{-1}
FDA11	10.58	2.99×10^{7}	3.54×10^{-7}	2.84×10^{4}	2.99×10^{5}	2.02×10^{6}	7.27×10^{6}	2.2×10^{-2}
FDA12	0.60	1.31×10^{6}	4.58×10^{-7}	2.21×10^{4}	2.51×10^{5}	2.85×10^{6}	5.95×10^{7}	1.6×10^{-1}
FDA13	1.00	1.80×10^{6}	5.56×10^{-7}	1.82×10^{4}	2.00×10^{5}	1.80×10^{6}	1.62×10^{7}	1.3×10^{-1}
FDA14	0.15	2.33×10^{5}	6.44×10^{-7}	1.61×10^{4}	2.37×10^{5}	2.34×107	1.08×10^{12}	3.4×10^{-4}
FDA15	8.59	1.30×10^{7}	6.61×10^{-7}	1.52×10^{4}	1.60×10^{5}	1.09×106	4.00×10^{6}	4.4×10^{-2}
FDA16	0.19	2.29×10^{5}	8.30×10^{-7}	1.24×10^{4}	1.70×10^{5}	8.57×106	4.20×10^{10}	4.1×10^{-3}
FDA17	0.25	2.36×10^{5}	1.06×10^{-6}	9.68×10^{3}	1.24×10^{5}	3.54×106	2.36×10^{9}	2.1×10^{-2}
FDA18	0.32	2.57×10^{5}	1.25×10^{-6}	8.20×10^{3}	1.00×10^{5}	1.99×106	3.42×10^{8}	5.5×10^{-2}
FDA19	0.43	3.04×10^{5}	1.41×10^{-6}	7.19×10^{3}	8.44×10^{4}	1.22×106	6.40×10^{7}	8.2×10^{-2}
FDA20	6.92	4.49×10^{6}	1.54×10^{-6}	6.53×10^{3}	6.89×10^{4}	4.73×105	1.77×10^{6}	2.2×10^{-2}
FDA21	0.69	4.34×10^{5}	1.59×10^{-6}	6.37×10^{3}	7.16×10^{4}	7.51×105	1.18×10^{7}	6.6×10^{-2}
bP1	0.1713	1.182×10^{5}	1.45×10^{-6}	7.14×10^{3}	1.00×10^{5}	6.64×10^{6}	8.14×10^{10}	
bP3	0.3363	7.355×10^{6}	4.57×10^{-8}	2.23×10^{5}	2.71×10^{6}	5.04×10^{7}	6.91×10^{9}	

Notes: Doses are in cfu. Probability of illness P_{ill} is given by $1 - (1 + dose/\beta)^{-\alpha}$ (the beta-Poisson model). FDA determined dose-response parameters from feeding test data of 20 subjects (FDA12) and data generated by bootstrapping (FDA1–11, 13–21).[8] FDAmean is the mean of FDA1–21 with weights of likelihood. bP1 and bP3 are determined from data of 27 subjects with different criteria of illness. bP1: diarrhea and detection of *V.p.* in feces; bP3: more than three episodes of diarrhea. ID1, ID10, ID50, ID90 are doses at which the mean probabilities of illness are 1, 10, 50, and 90%, respectively.

in Fig. 4. Dose-response relationships obtained from case criteria bP1 and bP3 fell within the range of uncertainties associated with the USFDA's parameter sets. The dose-response curves from bP1 and bP3 gave a higher and lower probability of illness at a low dose, respectively, but both gave a lower probability of illness at a very high dose within the range of uncertainty.

3. RESULTS

3.1. Risk Estimate and Evaluation of Mitigation Measures

Fig. 5 shows the results of the Monte Carlo simulation performed using @RISK4.5 (Palisade Corporation, Ithaka, NY) with 10,000 iterations for each scenario. Averages of five simulations with different random number seeds and the Latin hypercube method are shown. By averaging over five simulations, the result became reproducible as shown in Table VI. The probability of illness was estimated using three sets of dose-response parameters: the USDFDA parameters and our two new sets. To include uncertainties related to parameters α and β in the USFDA model, we used 21 sets of parameters and their likelihoods in the report[8] (cited as FDA1-21 in Table V); at each iteration of our simulation, we selected a combination of parameters with probability corresponding to the likelihood. The adjustment factor introduced for raw oysters by the USFDA RA was not used in our model because it is specific to the food. The uncertainties were not included for our new parameter sets. The results obtained using the USFDA parameter sets were used below unless stated otherwise because these have been widely used in previous RAs.[4,6,8]

The best-case scenario, which consists of (1) washing whole fish at landing, (2) storage and transportation in clean water, (3) no increase in

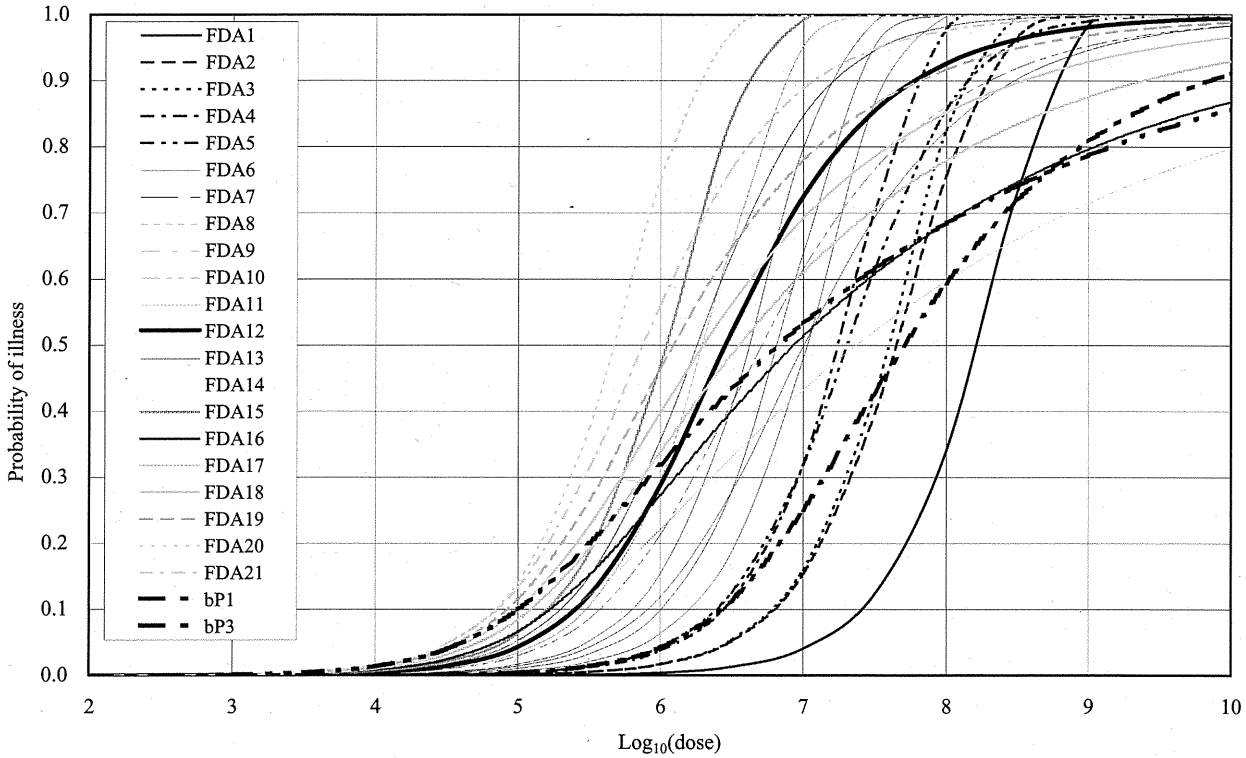

Fig. 4. Dose-response relationship of illness caused by *V. parahaemolyticus.* Probability of illness computed by the beta-Poisson model using parameter sets in Table V is shown.

temperature before preparation, and (4) washing fish visceral cavities during preparation, gave a mean number of nine pathogenic *V. parahaemolyticus* and a mean probability of illness of 5.6×10^{-6} per meal containing raw horse mackerel (Fig. 5).

The presence of contaminated water had a negligible effect, no wash at landing increased the probability of illness by 7%, exposure to higher temperature before preparation increased the risk by 50%, and no wash during preparation increased the risk 15-fold (by 1400%). The worst-case scenario, which consists of (1) no washing at landing, (2) storage and transportation in contaminated water, (3) exposure to high temperature before preparation, and (4) no washing during preparation, increased the mean number of pathogenic *V. parahaemolyticus* to 230 and the mean probability of illness to 1.4×10^{-4} per meal containing raw horse mackerel.

3.2. Effect of Input Variability

Sensitivity analysis demonstrated the following. The final outcome (the mean probability of illness) was bilinear with regard to the initial density of *V. parahaemolyticus* in the intestine and the fraction of pathogenic strains among total *V. parahaemolyticus*. This was due to the low dose in our model. At the low-dose limit, the probability of illness is proportional to dose, which is bilinear with regard to these input parameters because *V. parahaemolyticus* in fillets originate mainly in the intestine.

3.3. Storage Temperature and Duration

Fig. 6 shows the results of sensitivity analysis of storage temperature and duration in our model from fishing to preparation, in the optional high-temperature period during transportation, and after preparation. Note that this figure shows only the change in risk due to the change in variables within the range of their assumed distributions.[14] The first period from fishing to preparation was long, but the

[14] Exposure to high temperature for a long period beyond the assumed distributions at any point in the journey from sea to table greatly increases risk.

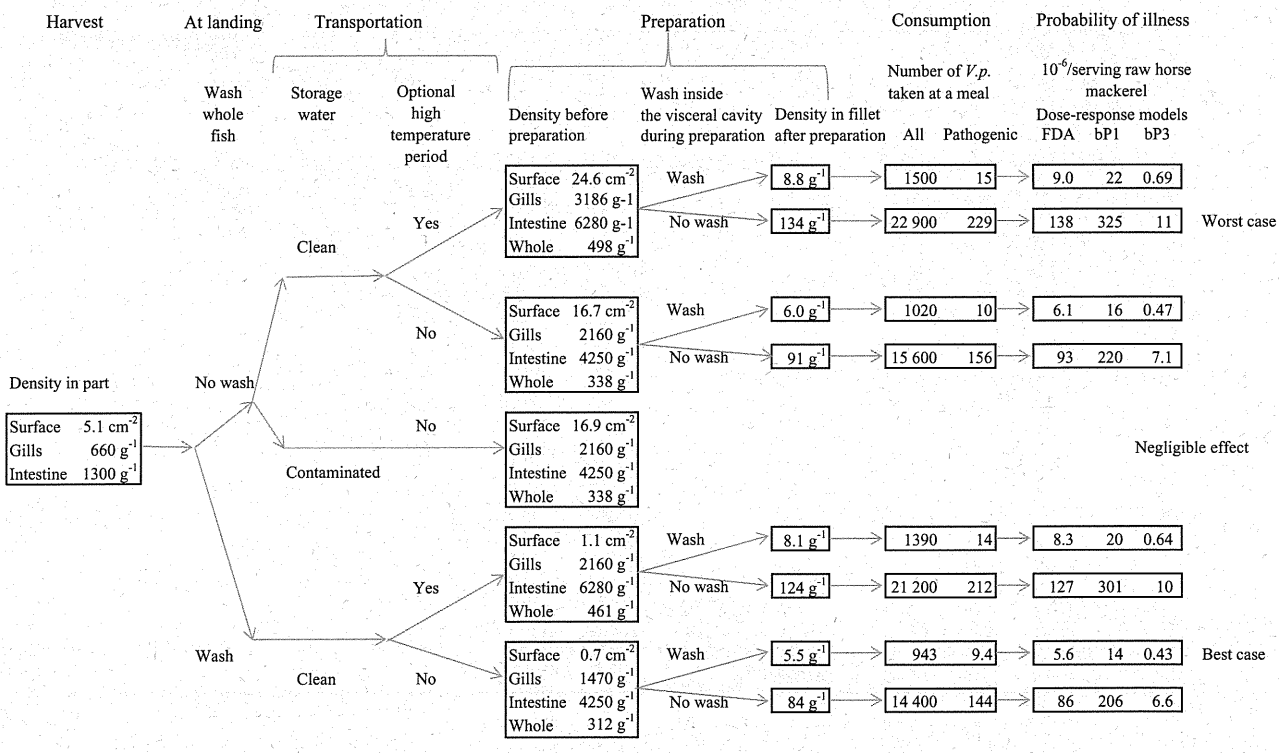

Fig. 5. Results of Monte Carlo simulations by @RISK4.5. Effects of washing whole fish at landing, washing inside the visceral cavity during preparation, and an optional high-temperature period are shown. Storage in contaminated water has an insignificant effect, and hence only the result before preparation and without washing whole fish is shown. See Table V for notation of dose-response models FDA, bP1, and bP3.

Table VI. Convergence of Monte Carlo Simulation

Number of Iterations	Mean Number of Pathogenic *V.p.*			Probability of Illness		
	Average	Min	Max	Average	Min	Max
1,000	10.23	8.60	12.32	6.13	4.94	7.55
5,000	10.10	9.52	11.08	6.19	5.68	6.59
10,000	10.03	9.57	10.66	6.09	5.81	6.59
30,000	10.30	9.87	10.72	6.21	5.94	6.60
100,000	10.20	9.94	10.51	6.14	6.04	6.29
300,000	10.20	10.10	10.35	6.17	6.04	6.40

Notes: Average, minimum, and maximum of 10 simulations are tabulated for the baseline scenario (no washing whole fish at landing, storage in clean water, no high-temperature period before preparation, and washing inside the visceral cavitiy during preparation). Mean number of pathogenic *V.p.* is the number of the organisms ingested at a meal with raw horse mackerel. Probability of illness is the probability in 10^6 servings of raw horse mackerel estimated by using the dose-response parameters in the USFDA RA without the adjustment factor for oysters.

temperature was usually low enough to minimize growth.

3.4. Comparison of Dose-Response Relations

The results described above were obtained using the USFDA dose-response model. The new dose-response models determined from the original human feeding trials gave a mean probability of illness of 250% of the USFDA value when based on the illness criterion of diarrhea and detection of fecal *V. parahaemolyticus* (bP1), and of 8% of the USFDA value when based on the illness criterion of three or more episodes of diarrhea (bP3).

At the low-dose limit, the number of illnesses is proportional to the total number of pathogenic organisms ingested by the population.[25] The proportional constant is the probability of illness from ingestion of a single pathogenic *V. parahaemolyticus*, which is given as P_{ill}/dose in Table V. The above comparison agrees with the estimation based on P_{ill}/dose.