Epidemiol. Infect. (2014), **142**, 2237–2247. © Cambridge University Press 2014
doi:10.1017/S0950268814001897

REVIEW ARTICLE
Impact of seafood regulations for *Vibrio parahaemolyticus* infection and verification by analyses of seafood contamination and infection

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Received 21 February 2014; Final revision 13 June 2014; Accepted 2 July 2014; first published online 31 July 2014

SUMMARY

Consumption of seafood contaminated with *Vibrio parahaemolyticus* causes foodborne infections, which are on the rise owing to increased consumption of raw seafood in Asia, Europe, North America, and other regions. *V. parahaemolyticus* infections have been common in Japan since the 1960s. Following an epidemic in 1997, the Japanese Ministry of Health, Labour, and Welfare instituted regulations for seafood in 1999, which appear to be reducing *V. parahaemolyticus* infections. In this review, we describe the scientific findings for these regulations. Analyses of the *V. parahaemolyticus* serotypes and isolate characteristics in samples from infected patients and contaminated seafood are discussed. In addition, based on the results of a survey, we show that new food safety regulations have led to improvements in food hygiene at many seafood retail shops, food service facilities, and restaurants. This example from Japan could be of immense help to control foodborne infections in other countries.

Key words: Infection, regulation, seafood, *Vibrio parahaemolyticus*.

Introduction

The first *Vibrio parahaemolyticus* outbreak occurred in Japan in 1950, with 272 total cases including 20 deaths [1], and was associated with boiled and semi-dried young sardine (*Engraulis japonica* Houttuyn) products (~50 kg) from a manufacturer in Japan. A new pathogen isolated from patients' samples and semi-dried young sardines were initially classified as *Pasteurella parahaemolyticus* but later as *V. parahaemolyticus*. Although *V. parahaemolyticus* has been a major foodborne pathogen for decades in Japan [2, 3], recently, the incidence of infections has been increasing in various countries of Asia [4–9], Europe [8, 9], North America [10–13], South America [14], and other countries [15].

The population of pathogenic *V. parahaemolyticus*, comprising thermostable direct haemolysin (TDH)- and/or TDH-related haemolysin (TRH)-positive strains, is generally smaller than that of non-pathogenic strains. In addition, it is difficult to distinguish pathogenic strains from non-pathogenic strains because their characteristics are similar. This contributes to the difficulties associated with surveying contamination and isolating pathogenic *V. parahaemolyticus* from seafood and environmental samples. However, since the mid-1990s, many studies have reported detection methods for pathogenic *V. parahaemolyticus* [16–19] and investigated contamination in various countries [20–24]. Novel approaches to control *V. parahaemolyticus* infections were

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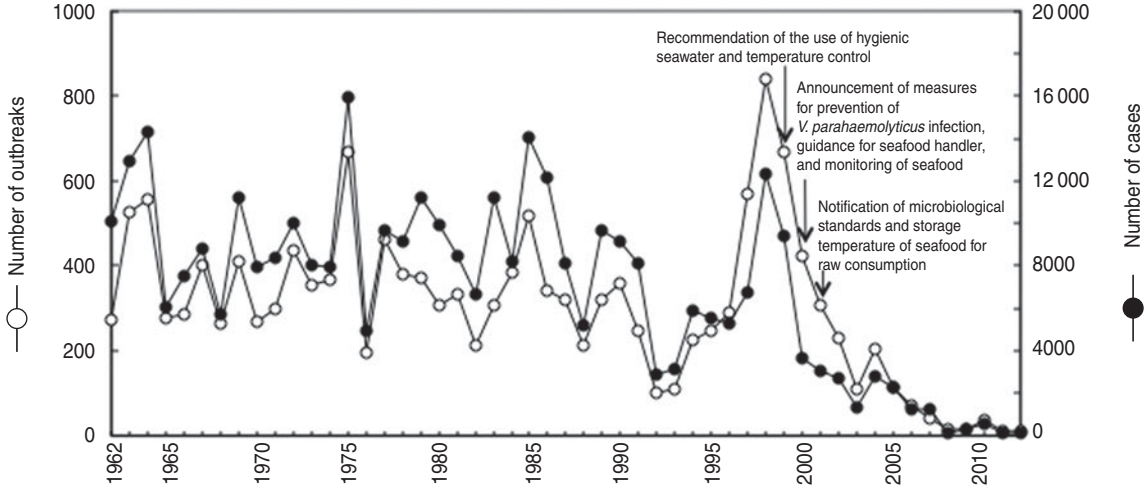

Fig. 1. Annual incidence of *V. parahaemolyticus* infections between 1962 and 2012. This figure was prepared from data published in the annual *Handbook of Health and Welfare Statistics* from the Ministry of Health, Labour, and Welfare, Japan.

developed using data from such studies [25, 26]. In Japan, a council for *V. parahaemolyticus* control was established in 1998 to investigate effective regulatory actions to prevent *V. parahaemolyticus* infection, and the numbers of infections were successfully reduced. In this review, we describe the trends of *V. parahaemolyticus* infections and the regulations instituted by the Ministry of Health, Labour, and Welfare (MHLW) of Japan. Investigations on changes in *V. parahaemolyticus* contamination in seafood and the characteristics of *V. parahaemolyticus* isolates provide support for the positive impacts of such regulations.

Trends of *V. parahaemolyticus* infections in Japan

V. parahaemolyticus has been a dominant cause of foodborne infections in Japan since the 1960s, and infections have been continuously reported (Fig. 1). The most recent increase, since 1997, was coincident with a large wave of infections involving *V. parahaemolyticus* in Japan. The highest number (839) of outbreaks per year was recorded, and the number of cases each year reached 12318 at the peak of infection in 1998. From 1999 to 2001, the MHLW discussed and established step-by-step regulations for seafood safety from the production stage to the consumption stage (Fig. 1). Subsequently, the number of cases and outbreaks of *V. parahaemolyticus* infections decreased by 99- and 93-fold, respectively, from 1998 to 2012 (Fig. 1). Such a marked decrease has never previously been recorded for *V. parahaemolyticus* infections.

Based on analysis of 3955 *V. parahaemolyticus* outbreaks from 1989 to 1999 by the Committee of Milk, Meat and Seafood for the Food Sanitation Investigation Council, 2391 food-related outbreaks were identified, and 92% of the 2391 outbreaks were related to seafood [27]. In particular, 49% of the 2391 outbreaks were associated with sashimi (pieces of raw fish fillet) and sushi (vinegary rice balls with raw fish fillet); outbreaks were also caused by shellfish (16%), cooked seafood (12%) and boiled seafood such as crab (10%) and sea urchin (5%). Seven large-scale outbreaks of more than 500 cases have been reported, although they occurred far less frequently than small-scale outbreaks (1002 outbreaks of 1–10 cases). In 1996, a large outbreak including 703 cases occurred in association with boiled crab sold by a seafood shop. In 1997, a large outbreak including 527 cases occurred in association with boxed lunches prepared by a restaurant. In 1998, two large outbreaks were reported; one including 1167 cases associated with boxed lunch served by a restaurant, and the other including 742 cases associated with boxed lunch. In 1999, two large outbreaks were reported; one including 674 cases associated with sushi prepared by a food manufacturer, and the other including 509 cases associated with boiled crab cooked by a food manufacturer. In 2007, a large outbreak including 620 cases occurred in association with salted squid produced by a food manufacturer. Furthermore, facilities related to 2391 outbreaks were analysed, and 48%, 18%, 12%, 12%, 4% and 6% were restaurants,

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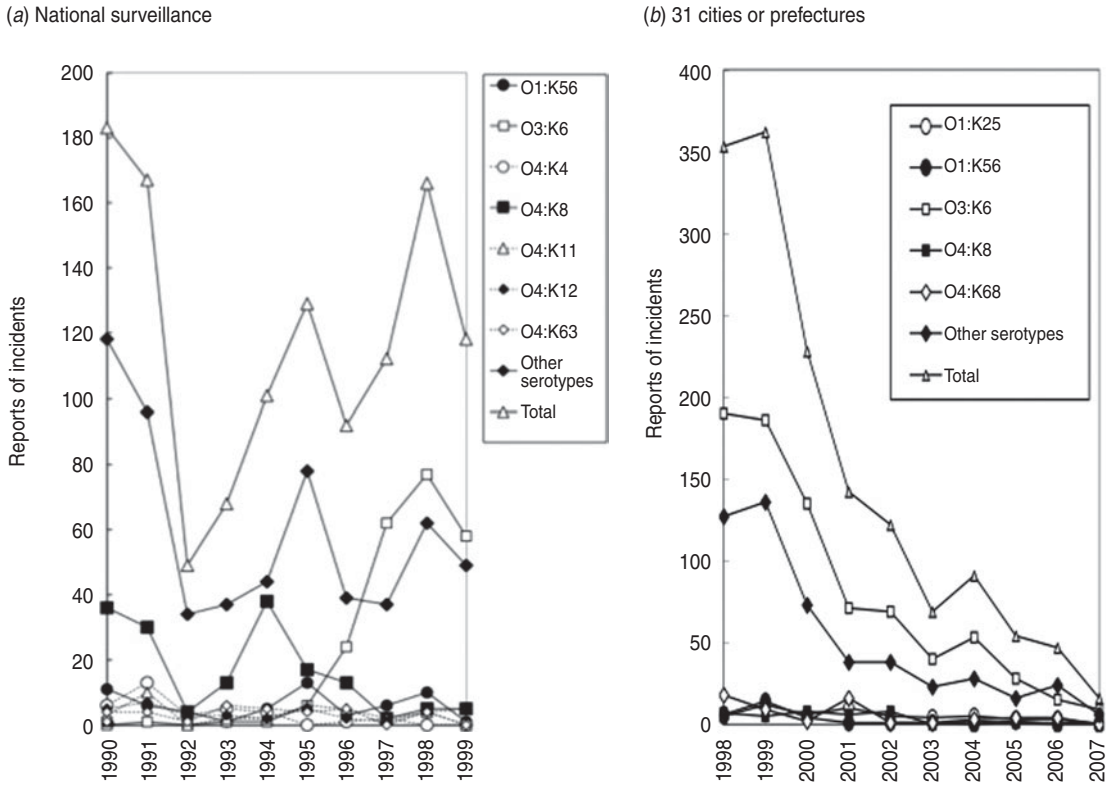

Fig. 2. Serotypes of *V. parahaemolyticus* isolates from humans between 1990 and 2008. (*a*) Nationwide surveillance by the National Institute of Infectious Disease in Japan between 1990 and 1999, and (*b*) surveillance by 31 local governments in Japan between 1998 and 2007.

hotels, caterers, shops making boxed lunches, home, seafood facilities and other facilities, respectively.

Based on a surveillance report of infections that occurred over 22 years in Shizuoka prefecture, Japan since 1963, the major serotypes initially involved in infections were O2:K3, O3:K7, O4:K8, O4:K12, O5:K15, and O4:K63 although other serotypes accounted for more than half of the subsequent infections [28]. Nationwide surveillance by the National Institute of Infectious Disease (NIID) of Japan identified the serotypes of isolates from patients between 1990 and 1999. Until 1995, O1:K56, O4:K4, O4:K8, O4:K11, O4:K12, and O4:K63 were the predominant serotypes, and other serotypes accounted for more than half of the infections (Fig. 2*a*) [29]. The proportion of cases with serotype O3:K6 rapidly increased beginning in 1996, and this serotype became the most predominant serotype between 1997 and 1999. Serotype O3:K6 accounted for more than half of the total cases between 1997 and 1999, which was the first time that a particular serotype accounted for more than half of the total incidents. From 1998 to 2007, 31 local governments collected serotype data as part of a research project focusing on the *V. parahaemolyticus* serotypes involved in outbreaks [29]. Serotype O3:K6 was determined to be responsible for about 50% of the total number of outbreaks reported each year (Fig. 2*b*) [29]. Serotypes O1:K25, O1:K56, O4:K8, and O4:K68 were also relatively dominant among more than 60 serotypes during this time. However, various serotypes were associated with each outbreak, and there was a decrease in the number of outbreaks independent of serotype. Thus, the increase in *V. parahaemolyticus* infections from 1996 to 1998 reflected mainly the increase in serotype O3:K6 infections, but the following decrease in *V. parahaemolyticus* infections reflected the decrease not only in serotype O3:K6 but also in other serotypes infections. The regulations by MHLW of Japan for seafood safety in 1999–2001 might achieve unprecedented results in the reduction of *V. parahaemolyticus* infections. In the next section, the regulations and the scientific foundation are introduced.

Regulations for seafood safety

MHLW of Japan established step-by-step regulations for seafood safety from the production stage to the

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consumption stage in 1999–2001, and the four main points of these regulations were as follows:

Regulation 1: Hygienic seawater. Seafood handlers were advised to use disinfected or artificial seawater, or potable water to wash and process shellfish and finfish.
Regulation 2: Temperature control. The temperature for seafood during distribution and storage was recommended to be $\leqslant 4$ °C, but was ultimately set at $\leqslant 10$ °C.
Regulation 3: V. parahaemolyticus levels in seafood. The microbiological standards of *V. parahaemolyticus* levels in seafood were set at $\leqslant 100$ most probable number (MPN)/g for raw consumption, and non-detectable (ND) levels/25 g for ready-to-eat boiled seafood.
Regulation 4: Rapid consumption. Consumers were advised to consume seafood within 2 h after removal from the refrigerator. Restaurants were advised to serve seafood to consumers immediately after taking the seafood out of the refrigerator.

Scientific findings in surveys of *V. parahaemolyticus* contamination and experiments *in vitro* were used to formulate these regulations.

Regulation 1: Hygienic seawater

In 1999, 256 seafood markets throughout Japan responded to a questionnaire conducted by the MHLW on the seawater used in their facilities. The results showed that 90 (35%) of 256 seafood markets had used natural seawater obtained from the seacoast for washing, soaking, and preserving fish, and 72 (80%) of the 90 markets had used such natural seawater without sanitation treatment [30, 31]. Therefore the seafood handled in 28% of the seafood markets was considered to be possibly contaminated with *V. parahaemolyticus* derived from natural seawater.

Coastal seawater used in seafood markets was surveyed for the presence of the *tdh* gene and the total *V. parahaemolyticus* level in 1999 [32, 33]. The results showed that the frequency of *tdh*-positive *V. parahaemolyticus* increased depending on the total *V. parahaemolyticus* level. Over 70% of 29 seawater samples were positive for *tdh* when the total *V. parahaemolyticus* level was higher than 10^4 MPN/l. However, 28% of 157 seawater samples were positive for *tdh* even at low levels of total *V. parahaemolyticus*, from ND levels to 10^2 MPN/l. These findings suggested that coastal seawater was the most likely source of contamination of seafood with *tdh*-positive *V. parahaemolyticus*, of which the frequency may increase with total *V. parahaemolyticus* levels. Many outbreaks of *V. parahaemolyticus* were associated with boiled seafood such as crab. Because coastal seawater is often used to cool boiled seafood in processing plants, the boiled food might be contaminated with *V. parahaemolyticus*.

Regulation 2: Temperature control

Because temperatures <5 °C are known to reduce *V. parahaemolyticus* population [34], temperatures $\leqslant 4$ °C were initially recommended for seafood storage by the MHLW. However, this temperature was difficult for the seafood industry to accept because of the extra costs needed to install equipment for cooling at $\leqslant 4$ °C instead of $\leqslant 10$ °C. Therefore, to study the growth of *V. parahaemolyticus* including serotype O3:K6 strains at 10 °C, ten strains of TDH-producing *V. parahaemolyticus*, five strains of serotype O3:K6, and one strain each of serotypes O1:K25, O1:K56, O4:K8, O4:K68, and O5:K15 were cultured in buffered peptone water (BPW) in triplicate with 3% NaCl at 10 °C and 12 °C, and the number of bacteria in the BPW was determined at 8 h, 24 h, 48 h, and 72 h. The growth results were compared between strains cultured at 10 °C and 12 °C. *V. parahaemolyticus* rapidly grew at 12 °C after 8 h, and increased to $10^{6{\cdot}5}$ c.f.u./ml at 72 h, while growth at 10 °C was slower and increased by 10- and 100-fold at 48 h and 72 h, respectively. There were significant differences between the growth of 10 strains of *V. parahaemolyticus* at 10 °C and 12 °C ($P<0{\cdot}01$). Seafood for raw consumption is generally consumed while the seafood is fresh, usually within 48 h, and therefore keeping fresh seafood at $\leqslant 10$ °C was considered acceptable for raw consumption.

Regulation 3: V. parahaemolyticus *levels in seafood*

It is known that the population of TDH-producing *V. parahaemolyticus* in environments including seawater and seafood is much smaller than that of non-pathogenic *V. parahaemolyticus*, and therefore it is difficult to distinguish TDH-producing *V. parahaemolyticus* strains from a large number of non-pathogenic *V. parahaemolyticus* strains. Therefore, total *V. parahaemolyticus* was considered as an indicator to conduct an inspection test for contamination with TDH-producing *V. parahaemolyticus*. The level of total *V. parahaemolyticus* in seafood was surveyed in 2000. Most seafood samples (99·3% of 3641

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Table 1. *Levels of total* V. parahaemolyticus *in seafood associated with outbreaks*

	Level of total *V. parahaemolyticus* (c.f.u. or MPN/g)				
	⩽1	>1 to ⩽10	>10 to ⩽100	>100 to ⩽1000	>1000
Number of outbreaks	1	1	1	6	2
Leftover food	Tuna	Boiled crab	Sea urchin	Short-neck clam, Oyster, Bloody clam, Pen shell, Sashimi, Cooked egg	Omelette, Clam

c.f.u., Colony-forming unit; MPN, most probable number.

samples), including seafood for raw consumption and boiled crab/boiled octopus, contained ⩽50 *V. parahaemolyticus* MPN/g food, and 0·6% samples contained >100 MPN/g [35]. It is indicated that the ⩽50 MPN/g regulation level would limit the sale and consumption of most seafood distributed in Japan.

To set up a microbiological standard for *V. parahaemolyticus* in seafood for raw consumption, the levels of total *V. parahaemolyticus* in seafood were sought based on data of *tdh*-positive and total *V. parahaemolyticus* contamination in seawater and seafood, and based on the assumptions that an infectious dose of TDH-producing *V. parahaemolyticus* is 100 cells/serving and that the amount of consumption of raw seafood is equivalent to 100 g/serving. In a study on the level of *V. parahaemolyticus* in seawater, the ratios of *tdh*-positive *V. parahaemolyticus* to total *V. parahaemolyticus* in six *tdh*-positive seawater samples were 1/14, 1/23, 1/150, 1/500, 1/700 and 1/1000 [36]. The data on seawater were extrapolated to seafood contamination. If seafood contained total *V. parahaemolyticus* at a level of 101 cells/g, the levels of *tdh*-positive *V. parahaemolyticus* in seafood were calculated as 7·2, 4·4, 0·7, 0·2, 0·1 and 0·1 MPN/g (721, 439, 67, 20, 10 and 10 MPN/100 g). This indicates that 2/6 seafood samples cause *V. parahaemolyticus* infections because they contained over 100 *tdh*-positive *V. parahaemolyticus* cells/100 g. Therefore, a microbiological standard set at the level of 100 cells/g was expected to prevent *V. parahaemolyticus* infections caused by seafood contaminated with total *V. parahaemolyticus* at the level of 100 cells/g, although a microbiological standard set at 1000 cells/g would not prevent the infections at all. In another study using seven *tdh*-positive seawater samples, the ratios of *tdh*-positive *V. parahaemolyticus* to total *V. parahaemolyticus* were 1/23, 1/63, 1/600, 1/830, 1/1000, 1/5010 and 1/21400 [31]. From calculations, it is indicated that 2/7 seafood samples cause *V. parahaemolyticus* infections if seafood contained total *V. parahaemolyticus* at a level of 100 cells/g. Therefore, this level of microbiological standard was also expected to reduce *V. parahaemolyticus* infections. The results of both studies suggest that a large number of *V. parahaemolyticus* infections would be prevented by setting a microbiological standard of total *V. parahaemolyticus* at a level of 100 cells/g, and also suggest that the impact of a level of ⩽1000 total *V. parahaemolyticus* cells/g as a microbiological standard would be far smaller than setting a level of 100 cells/g.

Furthermore, total *V. parahaemolyticus* levels in seafood associated with 11 outbreaks from 1998 were analysed. The contamination levels in 8/11 outbreaks were >100 *V. parahaemolyticus* MPN/g food (Table 1) [37], which suggested that the regulatory level of ⩽100 *V. parahaemolyticus* MPN/g is effective for food control. A level of ⩽10 MPN/g may be more effective, but huge amounts of seafood free from *tdh*-positive strains would have to be removed from raw consumption.

Thus, the microbiological standard of *V. parahaemolyticus* levels for raw consumption was set at ⩽100 MPN/g seafood. In addition, the microbiological standard of *V. parahaemolyticus* levels for ready-to-eat boiled seafood were set at a ND level/25 g seafood because the population of *V. parahaemolyticus* in seafood markedly reduces to ND levels by boiling.

Changes in hygienic practices in seafood handling facilities

A total of 300 seafood retail shops, food service facilities, and restaurants in Shizuoka prefecture, Japan,

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Table 2. *Questionnaire on seafood regulations by prefecture governments in Japan, that was sent to seafood retail shops, food service facilities and restaurants*

Question		No. of facilities* (%)
(a)	Was government control of seafood hygiene strengthened after 2001?	
	Yes	158 (70·5)
	No	66 (29·5)
	Total	224 (100)
(b)	Did you reform or rebuild your kitchen after 2001?	
	Yes	60 (26·0)
	No	171 (74·0)
	Total	231 (100)
(c)	Did you change the ways you store or serve seafood after 2001?	
	(i) Serving seafood on ice	5 (3·8)
	(ii) Avoiding serving seafood	29 (21·8)
	(iii) Store seafood in the refrigerator just before serving	65 (48·9)
	(i) & (ii); (i) & (iii); or (ii) & (iii)	6 (4·5)
	(iv) No change	28 (21·0)
	Total	133 (100)
(d)	Did you change your kitchen equipment after 2001?	
	(i) Use of a plastic cutting board instead of a wooden cutting board	11 (7·0)
	(ii) Use of cutting boards exclusive to foods for raw consumption that are different from cutting boards for other foods	22 (13·0)
	(iii) Use of a different kitchen knife for each category of food	22 (13·0)
	(i); (ii) & (iii)	43 (26·0)
	(i) & (ii); (i) & (iii); or (ii) & (iii)	36 (21·0)
	(iv) No change	34 (20·0)
	Total	168 (100)
(e)	Did you change the way you handled and cooked seafood after 2001?	
	(i) Purchase of ready-to-eat raw seafood for serve it	26 (23·0)
	(ii) More often wash of cutting boards and knives using fresh water	37 (32·0)
	(iii) Sterilization of cutting boards and knives with hot water	15 (13·0)
	(i) & (ii); (i), (ii) & (iii); or (ii) & (iii)	9 (8·0)
	(iv) No change	27 (24·0)
	Total	114 (100)

* A total of 244 facilities responded.

were invited to complete a questionnaire, of which 244 shops responded to a survey on changes in food hygiene practices implemented after 2001 for securing seafood safety [38]. In total, 224/244 (91·8%) facilities responded to questions on alterations in the control by food safety agencies (Table 2, question a). Two-thirds of the facilities responded that the control of food safety agencies was strengthened after 2001. In addition, 231/244 (94·7%) facilities responded to questions on reforming or renovating kitchens (Table 2, question b). One-quarter of these had reformed or renovated their kitchens after 2001 but three-quarters of these answered that they performed no change. There may be financial constraints for improvements pertaining to seafood hygiene. Furthermore, 133/244 (55·2%) facilities responded to questions on seafood preservation methods (Table 2, question c). Half of the respondents stored seafood in a refrigerator just before serving. This indicates that importance of rapid consumption was widely known. A total of 168/244 (68·9%) facilities responded to questions related to cooking equipment (Table 2, question d). Four-fifths of these respondents had changed their cooking equipment, in particular replacing wooden cutting boards with plastic cutting boards, separating cutting boards for raw seafood from those used for other seafood, or using separate cooking knives for each kind of food item. A total of 114/244 (46·7%) facilities responded to questions on methods used to handle and cook seafood (Table 2, question e). Half of these respondents had changed their seafood purchasing and kitchen sanitation practices; they

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Table 3. *The frequencies of* tdh*-positive seafood samples in food with total* V. parahaemolyticus *at levels of <100 MPN/g and >100 MPN/g in 2001 and 2007–2009*

Year	Seafood	Origin	Level of total *V. parahaemolyticus*	
			<100 MPN/g	⩾100 MPN/g
2001	Horse mackerel	Domestic	0/15 (0%)*	0/2 (0%)
	Hen clam	Domestic	1/88 (1·1%)	5/20 (25·0%)
	Short-neck clam	Domestic	2/10 (20·0%)	5/24 (20·8%)
	Total seafood†	Domestic	3/118 (2·5%)	14/55 (25·5%)
2007–2009	Horse mackerel	Domestic	4/181 (2·2%)	2/25 (8·0%)
	Hen clam	Domestic	7/64 (10·9%)	2/37 (5·4%)
	Short-neck clam	Domestic and imported	11/198 (5·6%)	17/92 (18·5%)
	Bloody clam	Domestic and imported	15/108 (13·9%)	4/23 (17·4%)
	Total seafood‡	Domestic and imported	39/676 (5·8)	26/166 (15·7%)

MPN, Most probable number.
* The number of *tdh*-positive samples/the number of samples tested.
† Total samples, including horse mackerel, hen clam, short-necked clam, rock oysters, scallops, and other molluscan shellfish.
‡ Total samples, including horse mackerel, hen clam, short-necked clam, bloody clam, rock oysters, scallops, and other molluscan shellfish.

purchased only cleaned fish and often washed and hot water-sterilized their cutting boards and knives. The results of questions on cooking equipment, and methods used to handle and cook seafood suggest that preventing cross-contamination by hygienic cooking was implemented. The overall results of the survey suggested that food safety regulations led to improvements in food hygiene at seafood retail shops, food service facilities, and restaurants.

Changes in *V. parahaemolyticus* contamination in seafood after regulations were proposed

Frequencies of total and tdh*-positive* V. parahaemolyticus

Two studies comparing *V. parahaemolyticus* contamination in seafood between *V. parahaemolyticus* epidemic and non-epidemic periods were conducted with similar seafood targets using the same methods. A total of 329 samples in 2001 [39] and 842 samples from 2007 to 2009 [29] were qualitatively and quantitatively tested for *tdh*. Although seafood in Japan was highly contaminated with total *V. parahaemolyticus* in both periods (95·4% in 2001 and 85·2% from 2007 to 2009), contamination with *tdh*-positive *V. parahaemolyticus* was observed in 33 samples (10·0%) in 2001 and 65 samples (7·7%) from 2007 to 2009.

There were no significant differences between the frequencies of *tdh*-positive seafood samples in 2001 and those from 2007 to 2009. However, the number of cases and outbreaks decreased by 18- and 17-fold from 2001 to 2007–2009, respectively.

In a report on *V. parahaemolyticus* contamination in the western region of Japan between 2002 and 2004, about 80% of the seafood tested was found to be contaminated with *V. parahaemolyticus*, and 10·6% and 14·9% of the seafood was positive for *tdh* and *trh*, respectively [40]. TDH-producing *V. parahaemolyticus* and TRH2-producing bacteria were isolated from 3·7% and 6·2% of seafood samples, respectively. In another report on contamination in the central region of Japan, 11·7% of seafood samples tested was positive for *tdh* [41]. However, both reports indicated that molluscs, such as short-neck clams, were highly *tdh*-positive, showing 27–35% positivity rates. In 2010, 5·1%, 18·6%, and 5·6% of seafood from various regions in Japan were positive for *tdh, trh*, and both *tdh* and *trh*, respectively (S. Saito *et al*., unpublished data). Although the surveys of these reports were conducted with samples from different regions, the frequency of *trh* was consistently higher than that of *tdh*.

Relationship between total and tdh*-positive* V. parahaemolyticus *levels*

The relationship between total and *tdh*-positive *V. parahaemolyticus* levels in seafood purchased in markets in 2001 [39] and 2007–2009 [29] was investigated in two studies.

The density of *tdh*-positive *V. parahaemolyticus* ranged from <0·3 to 9·3 MPN/g in the results in

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Table 4. *Analysis of the characteristics of TDH-producing* V. parahaemolyticus *serotype O3:K6 from seafood and patients*

PFGE pattern		Isolates			
		Seafood		Patients	
*Not*I	*Sfi*I	Type (origin, year)	No. of isolates	Year of isolation	No. of isolates
1	b			2006	2
				2007	1
2	b			2008	1
3		Short-neck clam (domestic, 2001)	1		
4	b	Rock oyster (domestic, 2001)	2	1998	2
				1999	1
		Bloody clam (Korea, 2009)	1	2005	1
				2009	1
	d			2001	1
	g			2007	1
	c			2007	1
	b	Rock oyster (domestic, 2001)	2	2007	1
5		Short-neck clam (domestic, 2008)	2		
	f	Hen clam (domestic, 2001)	2	2006	1
	i			1998	1
6	a	Hen clam (domestic, 2001)	1		
	h			1997	1
7	f	Rock oyster (domestic, 2001)	1		
8				1999	1
9	a			2008	1
10	e	Bloody clam (China, 2009)	1		
11				2001	1
12		Short-neck clam (domestic, 2008)	1		
13	g	Short-neck clam (domestic, 2008)	1	2007	1
14	a			2005	1
15		Bloody clam (China, 2009)	5		
16				2007	1
17				2009	1
18				2007	1

2001 and from <0·3 to 2·1 MPN/g in the results from 2007 to 2009. The density of *tdh*-positive *V. parahaemolyticus* did not exactly correlate with the number of total *V. parahaemolyticus*. However, the incidence of *tdh*-positive *V. parahaemolyticus* was higher in samples contaminated with relatively high levels of total *V. parahaemolyticus*. The frequencies of *tdh*-positive *V. parahaemolyticus* in samples contaminated with total *V. parahaemolyticus* at a level >100 MPN/g (25·2% in 2001, 15·7% from 2007 to 2009) were about 10 and three times higher than those of *tdh*-positive samples below this level (2·5% in 2001, 5·8% from 2007 to 2009) (Table 3). The data suggest that regulating the levels of total *V. parahaemolyticus* to 100 MPN/g would reduce the consumption of seafood contaminated with *tdh*-positive *V. parahaemolyticus*. The rate of samples for which the total *V. parahaemolyticus* level exceeded 100 MPN/g was 31·8% (55/173) and 19·7% (166/842) in 2001 and 2007–2009, respectively. The reduction of rate was smaller than the decrease of *V. parahaemolyticus* foodborne infections. Seafood exceeding the suggested levels of *V. parahaemolyticus* is still distributed, but regulations such as temperature control might help to inhibit *tdh*-positive *V. parahaemolyticus* in the steps prior to consumption.

Characteristics of *V. parahaemolyticus* isolates

The characteristics of *tdh*-positive, *trh*-negative and pandemic O3:K6 strains isolated from seafood and patients from 2007 to 2009 were analysed and

https://doi.org/10.1017/S0950268814001897 Published online by Cambridge University Press

compared to those of O3:K6 strains isolated from 1997 to 2006 [29]. The strains were classified using the *Not*I and *Sfi*I restriction profiles in pulsed-field gel electrophoresis analysis (Table 4). Four combinations of *Not*I and *Sfi*I restriction profiles (*Not*I: 4, *Sfi*I: b; *Not*I: 5, *Sfi*I: b; *Not*I: 5, *Sfi*I: f; *Not*I: 13, *Sfi*I: g) were identical in strains isolated from seafood and patients. These findings demonstrate that the pandemic O3:K6 strains continued to inhabit areas in or around Japan up to 2009.

Furthermore, a study conducted in 2010 (S. Saito *et al*., unpublished data) showed that the serotype O3:K6 was still present in Japan because TDH-producing O3:K6 was isolated from 7/8 TDH-producing *V. parahaemolyticus* samples. All of these samples were from molluscs, such as the short-neck clam, and were from various regions throughout Japan. Therefore, it appears that TDH-producing O3:K6-contaminated seafood is currently being consumed in Japan.

Conclusions

V. parahaemolyticus has continued to cause many foodborne infections since the 1960s in Japan, and a large pandemic of O3:K6 infections in 1998 and a rapid decrease of *V. parahaemolyticus* by all serotypes including O3:K6 since 1999 was observed. We demonstrated that the rapid and considerable decrease in *V. parahaemolyticus* infections was not due to changes in the levels of *V. parahaemolyticus* contamination in seafood in association with possible natural changes in the prevalence of pathogenic *V. parahaemolyticus*. Instead, this marked decline can be attributed to a series of governmental regulations implemented from 1999 to 2001 that may have facilitated improvements in hygiene conditions throughout the supply chain, spanning the distribution to consumption stages of seafood. These regulations have probably contributed to reductions in *V. parahaemolyticus* infections caused by not only serotype O3:K6 but also other serotypes in Japan. Therefore, this review suggests that broadly implementing similar food regulations would help control foodborne infections with *Vibrio* spp., especially *V. parahaemolyticus*, throughout the world.

Acknowledgements

We thank the governments of the prefectures and cities, and research centres in Japan for their collaborations.

Declaration of Interest

None.

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