表3　病原性関連遺伝子検出用PCRプライマーセット

Primers					
gene		forward	5'-3'	reverse	5'-3'
vvhA(Hill et al)	specific	vvhA-sf	CCGCGGTACAGGTTGGCGC	vvhA-sr	CGCCACCCACTTTCGGGCC
vuuA:	common	vuuA-cf	TTGAAAGTGCGGTAAAAGCAGG	vuuA-cr	CATCTGCATACTGTCAAAATCG
	specific	vuuA-sf	AGGTACCTTTCTTTAGGCGGCC	vuuA-sr	GATATGGTGAAAATCCTCCGCC
vvpE:	common	vvpE-cf	ATATTGCGGGTGAAGCGGCAGAG	vvpE-cr	GTGGTCGGTTTGCTGCCCGCTTT
	specific	vvpE-sf1	CCGTTCAAGCTCGTAGTCTTGC	vvpE-sr1	TAGCATTGGCATGCTTCACTTGG
		vvpE-sf2	CCTCGGGAAGCAGCGCCACCAAG	vvpE-sr2	CCACCGCCGTCGCATTGCGATCG
vpl:	common	vpl-cf	CCTATGTTCGCTGTTGGTATCG	vpl-cr	GCGTTAAAGATGTTGCCTGTATC
	specific	vpl-sf1	AGCTCTCTCCTGAAGCGATCAC	vpl-sr	CGCCCCTGCGTCAGTGAGTTTG
		vpl-sf2	CACATCACAAAACGTGATTAAG		
mshD :	common	mshD-cf	ACAACAGCAATTTTGATGGTGG	mshD-cr	TTTCCACTAAGGTAAAACCACG
	specific	mshD-sf	ACCAGTTTGCTCTTTCCCAATG	mshD-sr	GTATTGACCGGCGTGTATTGCC
vvpD :	common	vvpD-cf	CGTTACAATACTACCCTTGGCT	vvpD-cr	TCTCCGTAACCCATGCCTTCTTT
	specific	vvpD-sf	TTGGAAGTTCGGTTATGCACCG	vvpD-sr	TTGAGATACCAAGACATCAAGC
viuB:	common	viuB-cf	AAAGATTAGGTGAAAGAATGA	viuB-cr	CAATAAACACATAGCCTTTGGC
	specific	viuB-sf	CAGACAATATCGTGCGGCGGTG	viuB-sr	AGCAGACAGCGAGGTCGCTTTC
httpd	common	httpd-cf	GCATCGCTCGAAAGAAGCTTGG	httpd-cr	ATAGCGCTTTGAAGTTACCTTC
	specific	httpd-sf	TCAGCACTTAAGCACGCATTAA	httpd-sr	TGCGTAAATCACGCAATCGAGA
wza :	common	wza-cf	CATCTGAAGCAGGTAACTGGG	wza-cr	AGTGCTTCCGTCAGGCTCATGCC
	specific	wza-sf	CAGAACTTGATGTGCAGATCGC	wza-sr	TCGCCCGGCTCTAATAAACGG
hupA :	common	hupA-cf	TGTTGCTTTTGAAACCAAAGATCC	hupA-cr	TGGTGCTCTGAAACCTTGGCT
	specific	hupA-sf	TTCAAAAGCACCGTATGACGAC	hupA-sr	CTAAATAGTGCGCTTCTAATTCC
ela:	common	ela-cf	TTTACCAAAATGAATCTGGTGC	ela-cr	GGTTGATGATGCCACTGTTGAT
	specific	ela-sf	AATCGCGGCAATTTCGAAATGG	ela-sr	GCGGATGCCGTTTGAGTTTGGC
mntH :	common	mnt-cf	ACCTCTACAAAGTCGACATGGCG	mnt-cr	AGAATAATGCTATTCACTAAACC
	specific	mnt-sf	CTCTATTTCTACTCAAGTACCC	mnt-sr	TACCTAAGTCCCTCTAGCTAAC

表4　血清型参照株における病原性関連遺伝子PCR

Vibrio vulnificus			PCR											
O-serogroup	reference strain	由来	*vvh*	*vuuA*	*vvpE*	*vpl*	*wza*	*mshD*	*vvpD*	*ela*	*viuB*	*4-hppd*	*hupA*	*mntH*
O1（type strain）	ATCC27562	血液（CDC）	+	+	+	+	+	+	+	+	+	+	-	-
O1（H-血清作成用）	459-95	エビ（デンマーク）	+	-	+	+	+	-	+	+	-	-	-	+
O2	D3894	血液（CDC）	+	-	+	+	-	+	+	+	-	-	+	+
O3	E240	血液（CDC）	+	(+)	+	+	+	+	+	+	+	+	+	+
O3	E240-1(*vvh* -)		-	(+)	+	+	+	+	+	+	+	+	+	+
O4a	1115-80	血液（東大病院）	+	(+)	+	+	+	+	+	+	+	+	-	+
O5	E571	血液（CDC）	+	(+)	+	+	+	+	+	+	+	+	-	+
O6	91-81	血液（京都市）	+	+	+	+	+	+	+	+	+	-	+	+
O7	1338-80	アサリ（神戸市）	+	+	+	+	+	+	+	+	+	-	-	+
O8	191-87	カキ（韓国）	+	-	+	+	-	+	+	+	+	-	+	+
O9	189-87	カキ（韓国）	+	+	+	+	+	+	+	+	+	-	+	+
O10	586-88	エビ（インド）	-	-	-	-	-	-	-	-	-	-	-	-
O11	705-88	血液（韓国）	+	-	+	+	+	+	+	+	+	+	+	+
O12	371-89	血液（タイ）	+	+	+	+	+	+	+	+	+	+	+	+
O13	W-vv5	膿（韓国）	+	-	+	+	+	+	+	+	+	+	+	+
O14	C84-7-4-vv1	血液（韓国）	+	+	+	+	+	+	+	+	+	-	-	+
O15	889-89	ウナギ（ノルウェー）	+	+	+	+	+	+	+	+	+	-	-	-
O16	611-95	海水（ドイツ）	+	+	+	+	-	+	+	+	+	-	-	-

（+）はバンドは検出されたものの、サイズが異なっていたもの

表5　患者由来株、環境由来株の病原性関連遺伝子PCR

Vibrio vulnificus

患者由来株			PCR											
O-serogroup	No. of isolates	(%)	*vvh*	*vuuA*	*vvpE*	*vpl*	*wza*	*mshD*	*vvpD*	*ela*	*viuB*	*4-hppd*	*hupA*	*mntH*
O1	6	26.1%	6/6	1/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	2/6	0/6	5/6
O4	12	52.2%	12/12	2/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	5/12	7/12	6/12
O5	1	4.3%	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1
O6	1	4.3%	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1
O7	3	13.0%	3/3	0/3	3/3	3/3	3/3	2/3	3/3	3/3	3/3	3/3	3/3	(3)/3
Total	23	100.0%	23/23	3/23	23/23	23/23	23/23	22/23	23/23	23/23	23/23	11/23	11/23	16/23
(positive%)			(100%)	(13%)	(100%)	(100%)	(100%)	(96%)	(100%)	(100%)	(100%)	(48%)	(48%)	(70%)

環境由来株			PCR											
O-serogroup	No. of isolates	(%)	*vvh*	*vuuA*	*vvpE*	*vpl*	*wza*	*mshD*	*vvpD*	*ela*	*viuB*	*4-hppd*	*hupA*	*mntH*
O1	6	28.6%	6/6	6/6	3/6	6/6	6/6	6/6	6/6	6/6	6/6	0/6	0/6	6/6
O3	1	4.8%	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1
O4	6	28.6%	6/6	(4)/6	6/6	6/6	5/6	6/6	6/6	6/6	6/6	5/6	2/6	6/6
O6	2	9.5%	2/2	1/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/2	2/2	2/2
O8	2	9.5%	2/2	2/2	2/2	2/2	0/2	2/2	2/2	2/2	2/2	0/2	2/2	2/2
O14	1	4.8%	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1
O16	2	9.5%	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/2	2/2	2/2
R	1	4.8%	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	0/1	1/1
Total	21	100.0%	21/21	18/21	18/21	21/21	18/21	21/21	21/21	21/21	21/21	6/21	9/21	21/21
(positive%)			(100%)	(86%)	(86%)	(100%)	(86%)	(100%)	(100%)	(100%)	(100%)	(29%)	(43%)	(100%)

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Vibrio vulnificus septicaemia in Japan; An estimated number of infections and physicians' knowledge of the syndrome.

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Key words: *Vibrio vulnificus*, septicemia, incidence and physicians' knowledge

Heading: *V.vulnificus* septicemia in Japan

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Abstract

Questionnaire surveys were implemented to study the incidence and physician's knowledge of *Vibrio vulnificus* infections in Japan. Registered emergency physicians were selected by stratified random sampling for a questionnaire survey. A total of 235 out of 386 physicians (61%) responded to the questionnaire and 12 *V. vulnificus* septicaemia cases were reported from 10 respondents. The annual estimated number of *V. vulnificus* septicaemia was calculated as 425 (95% CI: 238 – 752). The study also revealed that only 15.7% (95% CI =11.3 - 21.0) of responding physicians had a basic knowledge of *V. vulnificus* infection. Education for both physicians and people at high-risk group of developing the infection (e.q. immunocompromised, chronic liver disease) will be necessary for the prevention, early diagnosis and appropriate treatment of the disease.

Vibrio vulnificus grows in warm estuaries and seawater. It can cause septicaemia, which is life-threatening, especially for the immunocompromised or people with chronic liver disease. The infection has been a leading cause of seafood-related deaths [1]. The food implicated in most reported cases has been raw oysters. Illness occurs after ingestion of the seafood or exposure to estuarine water; oysters harvested in warmer months are major vehicles of the disease in the USA [2]. The clinical course is very acute and severe and one third of the patients with *V.vulnificus* infection go into endotoxic shock in a few days with up to a 50% case fatality rate [3, 4].Despite the limited data available on the epidemiology of the infection, approximately 70-90 cases of *V.vulnificus* infections are annually reported in USA [5] and 40-50 septicaemia cases are reported in South Korea [6].

Japan is the largest importer and biggest consumer of seafood in the world [7]. Many types of seafood, especially oysters, shrimp and the other crustaceans are often eaten raw or undercooked. Most Japanese people may therefore be at high risk for food-borne microbial infections from seafood. There were more than 400 outbreaks reported each year due to *Vibrio parahaemolyticus* involving 3,000-9,000 people and most of them were associated with consumption of raw or undercooked seafood [8]. Furthermore, more than two million people are estimated to suffer from chronic liver diseases, an important risk factor for *V.vulnificus* infections [9]. Sampling tests showed about 30% of local oysters (species not suitable for human consumption) were

contaminated with *V.vulnificus* [10].

As a result of risk behaviour and host factors, 7 cases of *V. vulnificus* infection with 3 fatalities were identified in Kumamoto prefecture during the summer season (June 29- July 18). Most cases were associated with consumption of uncooked Japanese mantis shrimp (*Oratosquilla oratoria*) [11].

However, the overall public health impact of *V. vulnificus* infections has not been evaluated in Japan. The infection is not widely recognized; the infection has not been included in the national notifiable diseases list. From 1978 through 1999, only 93 *V. vulnificus* infections were reported in Japanese scientific journals [12]. To assess the public health impact of *V. vulnificus* infections, we estimated its annual number of septicaemias and examined the knowledge of physicians of the disease by means of a questionnaire survey.

We developed a self-administered survey that included questions about working place (i.e. Prefecture), clinical specialties, years since medical school graduation, number of patients diagnosed with *V. vulnificus* infections during the year 2000, and basic knowledge of *V. vulnificus* infections (i.e. mode of transmission and clinical symptoms). Physicians in emergency rooms and intensive care units were specifically targeted to find emergency cases. The Japanese Association for Acute Medicine (JAAM) is the largest medical association for emergency medicine in Japan with a membership of 8,324 physicians, 3.3% of all physicians in Japan. In addition to the

emergency room, JAAM members serve in other specialties such as internal medicine and surgery. After implementing a pilot study, 386 member physicians from 323 facilities were selected by prefecture-stratified random sampling using the year 2000 JAAM membership directory. Questionnaires were mailed to the physicians' working places in February 2001, and responders were requested to reply anonymously by the end of March 2001. A follow up letter was sent to all targeted physicians. For statistical analyses, the chi-square test was used using EPINFO 6.04b software.

Of 386 JAAM members selected, 235 (61%) responded to the questionnaire. Twelve *V. vulnificus* septicaemia cases were reported from 10 respondents. Two cases were reported from each of three prefectures (Tokyo, Fukuoka, and Saga); the other six cases were from Hokkaido, Aomori, Ehime, Kochi, Nagasaki, and Kumamoto (Figure 1). Except for the Hokkaido case, the other 11 cases were from locations where *V. vulnificus* infections had been previously reported. The total annual number of cases was estimated by the following equation:

$$\mathbf{N = \text{Reported No. of cases from the survey} \times \frac{\text{Total number of JAAM members}}{\text{Number of responding physicians}}}$$

Using the above survey data, the annual number of *V. vulnificus* septicaemia cases in Japan was estimated as 425 (95% CI: 238 – 752)

Among the 235 respondents, 198 (85.3%) stated that they had no knowledge of *V.*

vulnificus infections. The percentage of respondents who had appropriate knowledge of the infection was significantly higher in the western region (23.9% 95%CI [15.6, 33.9]) than the eastern region (10.1% 95%CI [5.7, 16.4]) (P<0.05), and it was higher in emergency physicians (28.6%) than surgeons (3.2%)(P<0.05). On the other hand, knowledge was not associated with number of years since medical school graduation (P>0.44).

This was the first epidemiological survey to estimate the disease burden of *V. vulnificus* infections in Japan. In this study, we targeted only registered JAAM members. There was a possibility of underestimating the number of patients. Patients may have failed to see doctors and they might have been treated in departments other than those of emergency physicians. Furthermore, emergency room physicians might have treated affected patients without confirming the causative pathogen.

When we compared our estimated number with the other countries (South Korea and USA), our estimate was the highest of the three. Although the USA has 2.5 times more people than Japan, the frequency of eating raw or undercooked seafood is relatively low in the US: oysters are main seafood consumed raw. South Korea has approximately one third the population of Japan but is located in a colder climate[jmk1]. The frequency and degree of contamination of seafood with *V vulnificus* may be higher in Japan, because the seawater temperature influences the proliferation of the bacteria. Considering that Korean data is also based on a passive reporting system, it seems

reasonable that Japan has the highest number of *V. vulnificus* septicaemia.

This study also demonstrated that the awareness of *V. vulnificus* infections among Japanese physicians was low. Emergency room physicians and those who work in the western region of the country had more knowledge of the problem, but the length of a physician's career did not appear to improve knowledge. These results indicate that those who are aware of *V. vulnificus* infections probably gained their knowledge through clinical experience or other sources rather than medical school education. Education about *V. vulnificus* infections should be addressed, not only to emergency room physicians, but also to general physicians who can urge high risk patients.(e.g. those suffering from chronic liver diseases or the immunocompromised) to avoid eating raw seafood This study is the first step to estimate the public health impact of *V. vulnificus* infections in Japan. Inter-disciplinary interventions involving physicians, microbiologists, public health professionals, and high-risk populations and their families are needed.

Acknowledgements

We greatly appreciate Dr. John Kobayashi and Dr. Michael Kramer for advice on the study. This study was planned and developed with support of the Japanese Ministry of Health, Labour, and Welfare scientific research grant on emerging and re-emerging infectious diseases.

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Legend of figures

Fig.1 A map of Japan shows the number and distribution of *V.vulnificus* septicemias cases reported in each prefecture.

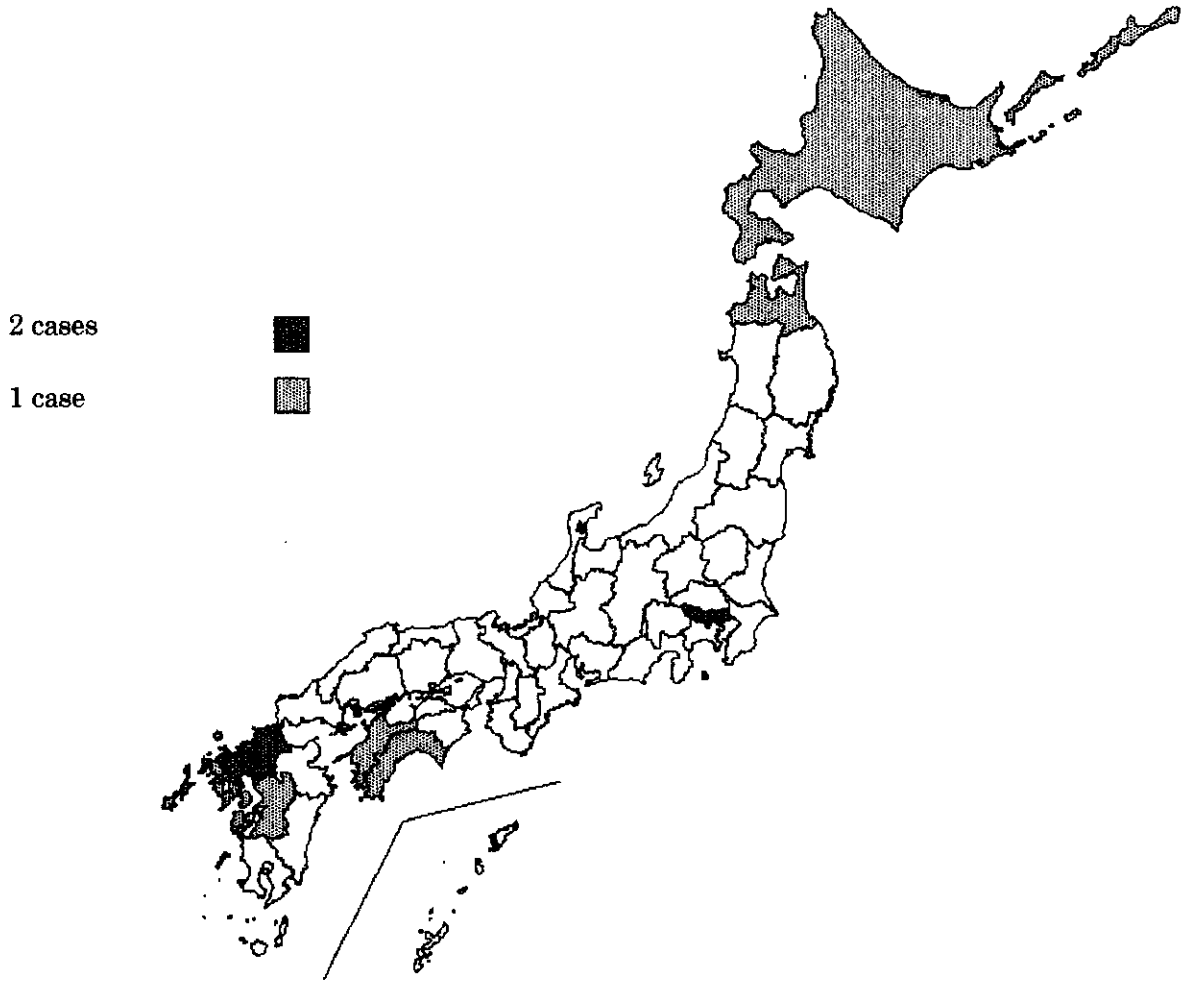

Figure 1