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The role of seafood in foodborne diseases in the United States of America

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Summary

In the United States of America, seafood ranked third on the list of products which caused foodborne disease between 1983 and 1992. Outbreaks connected with fish vectors were caused by scombroid, ciguatoxin, bacteria and unknown agents; in shellfish, unknown agents, paralytic shellfish poisoning, *Vibrio* spp. and other bacteria, followed by hepatitis A virus, were responsible for the outbreaks.

At least ten genera of bacterial pathogens have been implicated in seafood-borne diseases. Over the past twenty-five years, bacterial pathogens associated with faecal contamination have represented only 4% of the shellfish-associated outbreaks, while naturally-occurring bacteria accounted for 20% of shellfish-related illnesses and 99% of the deaths. Most of these indigenous bacteria fall into the family Vibrionaceae which includes the genera *Vibrio*, *Aeromonas* and *Plesiomonas*. In general, *Vibrio* spp. are not associated with faecal contamination and therefore faecal indicators do not correlate with the presence of *Vibrio*. Viruses are the most significant cause of shellfish-associated disease: in New York State, for example, 33% and 62% of 196 outbreaks between 1981 and 1992 were caused by Norwalk virus and gastrointestinal viruses (small round structured viruses), respectively. In addition, several illnesses are a result of toxic algal blooms, the growth of naturally occurring bacteria and diatoms causing neurotoxic shellfish poisoning, paralytic shellfish poisoning, diarrhoetic shellfish poisoning, amnesic shellfish poisoning and ciguatera. Current estimates place the annual number of ciguatera cases at 20,000 world-wide.

Scombroid poisoning is the most significant cause of illness associated with seafood. Scombrotoxin is of bacterial origin and halophilic *Vibrio* spp. causing high histamine levels are implicated as the source. Scombroid poisoning is geographically diverse and many species have been implicated, namely: tuna, mahi-mahi, bluefish, sardines, mackerel, amberjack and abalone. Temperature abuse has been cited as a major cause of scombroid poisoning.

For routine work, the use of faecal indicators to predict the relative level of faecal contamination should not be disposed of. However, the main source of seafood illness is due to species which are not predicted by these organisms. In order to protect public health, routine surveillance using new pathogen-specific techniques such as polymerase chain reaction should be used. This, in combination with risk assessment methods and hazard analysis and critical control points, will begin to address the need for improvement in the safety of seafood.

Keywords

Bacteria – Enteric viruses – Outbreaks – Public health – Risk assessment – Seafood – Shellfish – Toxins – United States of America – Vibrio spp.

Introduction

Seafood contributes a significant proportion of the world food supply, and over 70 million tons are caught world-wide each year. Estimates report consumption averages of 13 kg per person per year for fish and shellfish (87). However, it is clear that seafood also remains an important source of foodborne disease.

Two books which give an extensive coverage of the topic of seafood safety were published in 1991 and 1994: these are 'Seafood safety' by the National Institute of Medicine (56) and 'Environmental indicators and shellfish safety' (47). From these books it is clear that contamination of seafood remains an important problem.

Data on foodborne disease outbreaks collected over a ten-year period (1983 to 1992) in the United States of America (USA) demonstrated that fish were the third most reported category according to vehicle of transmission: unknown vehicles ranked first and multiple vehicles ranked second (Fig. 1). Other countries have also reported the role of fish as a vehicle for foodborne disease: in Cuba, for example, fish and shellfish were associated with 12.8% of all foodborne disease outbreaks, a figure just slightly below pork and beef (each of which were associated with 15.4% of foodborne disease outbreaks) (112).

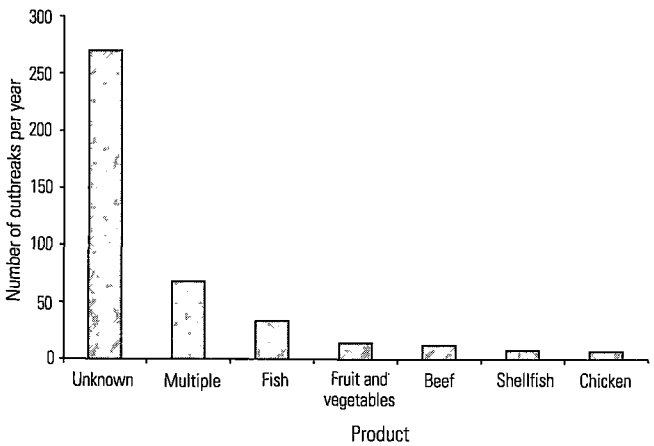


Fig. 1
Average outbreaks in the United States of America per year by vehicle of transmission, 1983-1992 (16, 24)

In the USA, the aetiological agents of seafood-borne disease associated with fish are, in order of occurrence, scombroid, ciguatoxin, bacteria and unknown agents. For shellfish-associated illness, causes are unknown agents, paralytic shellfish poisoning, *Vibrio* spp. and other bacteria, followed by hepatitis A virus (Figs 2 and 3).

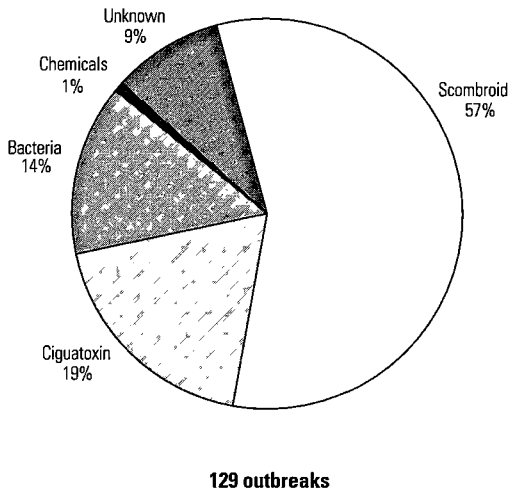


Fig. 2
Percentage of fish-associated disease occurring in the United States of America by aetiological agent, 1983-1992 (16, 24)

While the risk from consuming fish is largely associated with scombroid poisoning and post-harvest contamination, the risk posed by consumption of benthic invertebrates is linked to contamination of the source. Shellfish, particularly bivalve molluscs, are the most common seafood routes of human illness for viruses and *Vibrio* bacteria (56, 101, 109, 122). The disparity between the relative levels of bacterial contamination in fish and benthic invertebrates may best be explained by differences in the habitats and modes of feeding of the two groups. Fish (including bottom dwelling fish) tend not to be restricted to a small localised area and therefore are not restricted to a potentially contaminated area. Additionally, sediment and the sediment water interface (normal habitat for bivalve molluscs) are reservoirs for microbial pathogens. Bacterial concentrations here have been found at levels two to three times greater than those in the overlying water column

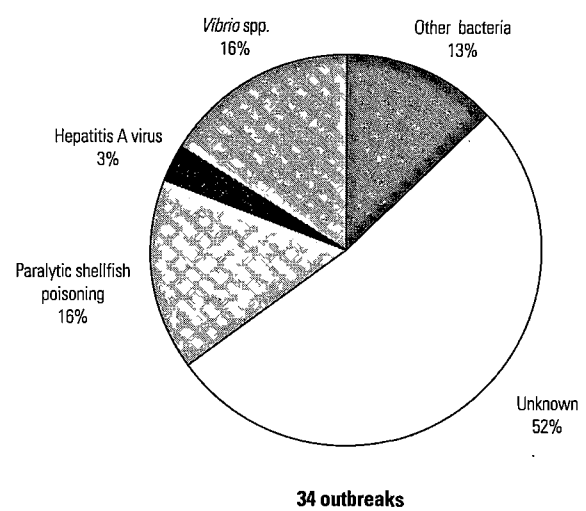


Fig. 3
Percentage of shellfish-associated disease occurring in the United States of America by aetiological agent, 1983-1992 (16, 24)

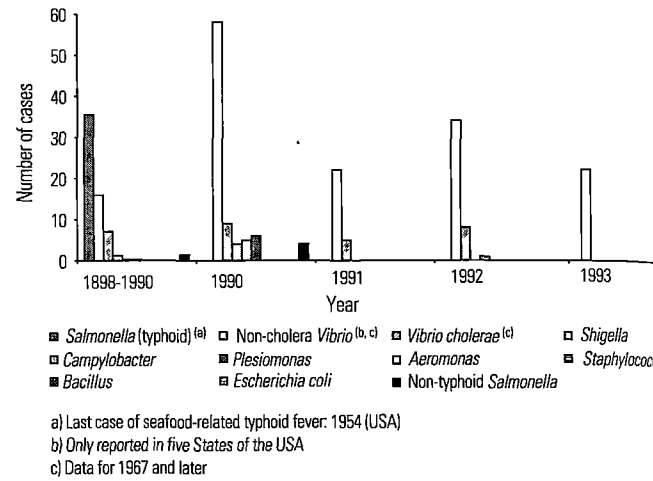
(77, 101). Virus levels may also be 100 times greater in the sediments than in the water column, where survival is greatly enhanced (116).

The close association between benthic invertebrates and sediment increases the likelihood of bacterial and viral contamination. Bivalve molluscs are implicated more than any other marine animal in seafood-borne illnesses. These invertebrates are sessile, thus cannot move out of potentially contaminated areas, and generally inhabit shallow areas close to shore and pollution sources (109). Furthermore, as filter feeders, these molluscs amass high levels of microbial pathogens within the internal tissues. Consequently, the consumption of raw bivalves is one the most common vectors of seafood-borne disease associated with contamination at the source (56, 101, 122).

The current system for prevention and control of seafood-asssociated foodborne disease has long been recognised as inadequate. Bacteriological standards or indicators have been used to define marine water quality, to evaluate potential contamination and to protect public health, and this has led to the use of coliform bacteria as indicators for water quality and sanitation of the food product. These standards have been shown to have some impact on the level of enteric bacterial outbreaks such as typhoid (115). However, there is now significant evidence that bacterial indicators do not adequately predict all microbial health risks. The group which remains undetected by such indicators includes bacterial pathogens such as *Vibrio vulnificus*. The relationships between indicator concentrations and risk of human illness caused by viral pathogens are particularly limited. No correlation between the indicator bacteria and the presence (or absence) of enteric viruses has been demonstrated for bivalve molluscan shellfish or the waters in which these shellfish are found (42, 117), and viral outbreaks in the USA associated with shellfish harvested from approved water continue to occur (35). A recent National Academy of Science report on 'Seafood Safety' concluded that the faecal coliform indicator is inadequate for determining the microbial quality and safety of marine waters and shellfish (56).

Bacterial pathogens in seafood

At least ten genera of bacterial pathogens have been implicated in seafood-borne diseases. Pathogenic bacteria associated with seafood can be categorised into two general groups: enteric bacteria which are present due to faecal contamination (either in the environment or due to poor handling and processing) and bacteria which are normal components of the marine or estuarine environment. Over the past 25 years, bacterial pathogens associated with faecal contamination have accounted for only 4% of the shellfish-associated outbreaks in the USA (101). Naturally-occurring bacteria accounted for only 20% of



a) Last case of seafood-related typhoid fever: 1954 (USA)
b) Only reported in five States of the USA
c) Data for 1967 and later

Fig. 4
Average number of annual cases associated with bacterial pathogens in the United States of America, 1898-1993 (101, 122)

shellfish-related illnesses but for 99% of the deaths (122) (Fig. 4). The remaining percentage of shellfish-related diseases is of unknown aetiology or is due to viral agents (Table I).

Pathogenic bacteria associated with faecal contamination

Important bacteria associated with faecal contamination of seafood include *Salmonella, Shigella, Campylobacter, Yersinia, Listeria, Clostridium, Staphylococcus* and *Escherichia coli* (36, 56). Few of these bacteria continue to pose a large-scale health threat through seafood consumption. The development of guidelines to minimise faecal contamination of shellfish and harvesting waters has greatly reduced the incidence of enteric bacteria in seafood (101). However, in some parts of the world these bacteria are still isolated from various seafoods, indicating the potential for transmission to humans (Table II).

Salmonella species

Until the mid-1950s, *Salmonella* Typhi was the most common bacterium associated with shellfish-vectored disease (56, 101). Due to more effective surveillance and improved water quality, the incidence of *S.* Typhi in shellfish harvested in the USA has declined. The last shellfish-related typhoid fever outbreak occurred in 1954 (USA) (56, 101). The risk of *S.* Typhi infection from shellfish is still quite low in the USA: however, infections continue to occur in other parts of the world (56). In the United Kingdom (UK), *S.* Typhi was detected in more than 1.6% of shellfish sampled from open harvesting waters (121).

Non-typhoidal *Salmonellae* have been associated with both fish and shellfish in recent years. *Salmonella* spp., including *S.* Paratyphi and *S.* Enteritidis, have been detected throughout the world in shrimp and bivalves (8, 31, 62, 65, 76, 121). Between 1984 and 1993, the United States Food and Drug Administration (FDA) received reports of eight shellfish-

Table I
Selected outbreaks of bacterial disease associated with the consumption of seafood since 1986

Bacteria	Year	Seafood	Cases	Location	Note	Reference
Shigella	1994	Shellfish	200	Mexico		94
Vibrio vulnificus	1994	Eel (suspect)	1	Denmark	First report of *V. vulnificus* in Denmark	14
Vibrio hollisae	1993	Oyster, crab	2	USA		1
Vibrio cholerae 0139	1993	Shrimp (suspect)	1	USA	Shrimp originated in India	22
Vibrio cholerae 01	1991	Crab	13	USA	Crab originated in Ecuador	20, 21
Vibrio cholerae non-01	1989	Fish or shellfish	24	USA	Isolated in the Gulf of Mexico	71
Vibrio parahaemolyticus	1989	Fish or shellfish	27	USA	Isolated in the Gulf of Mexico	71
Vibrio vulnificus	1989	Fish or shellfish	10	USA	Gulf of Mexico	71
Salmonella spp.	1986-1992	Fish, shellfish	74	Croatia	6.17 cases/year	99
Clostridium spp.	1986-1992	Fish, shellfish	18	Croatia	1.5 cases/year	99

related *Salmonella* infections (122). *Salmonella* infections due to seafood consumption are still low compared with salmonellosis associated with other foods.

Other sewage-related bacterial pathogens

Campylobacter jejuni was identified as an emerging infectious agent for diarrhoea in humans in 1977 (125). *Campylobacter* has a short survival time in marine waters and seafood-borne infections are not expected. However, the survival rate increases dramatically within shellfish, suggesting a protective relationship (5, 121).

In the USA, *Shigella* was implicated in 111 shellfish-related cases and four outbreaks from 1898 to 1990 (101). *Shigella* may be an important potential disease agent as it has a low infectious dose and long survival time in clams and oysters (56).

Yersinia enterocolitica infections result in appendicitis-like symptoms, including fever and abdominal pain, accompanied by diarrhoea or vomiting (39). Most *Yersinia* infections are not associated with a seafood vector (101). However, strains of *Y. enterocolitica* have been identified in fish and shellfish in both wild and aquaculture settings (56, 89). Additionally, *Yersinia* is a psychrotrophic bacterium which can multiply at low temperatures. This may increase the potential for cold-stored or frozen seafood to become a vector for human illness.

Listeria monocytogenes infections target specific groups. Spontaneous abortion and stillbirth have been caused in pregnant women by this bacterium. In infants and immuno-compromised individuals, infection leads to septicaemia and meningitis (125). *Listeria* spp. are also psychrotrophic and can grow under prolonged chilled conditions. Like *Yersinia, Listeria* has been isolated from a variety of fish and is potentially problematic for ready-to-eat products (13, 56, 89). Seafood-borne *Listeria* infections are believed to be under-reported in the USA (56).

Clostridium botulinum toxin type E is common in marine organisms. Botulism cases arising from seafood consumption

Table II
Sewage-related bacterial pathogens isolated from seafood, 1980 to 1997

Bacteria	Source of contamination	Seafood	Location	Reference
Salmonella Typhi	Environmental	Cockles, mussels, scallops, oysters	Ireland	121
Salmonella Enteritidis	Environmental	Bivalves	Ireland	121
Salmonella spp.	Environmental, market	Bivalves, shrimp	Ireland, Asia, Europe, South Africa, Indonesia, USA, Honduras, Bangladesh, Kuwait	31, 40, 62, 121
Campylobacter spp.	Environmental	Bivalves	South Africa, Australia	31, 62
Clostridium botulinum	Market	Whitefish	USA	15
Shigella spp.	Market	Shellfish	Mexico	94
Listeria spp.	Market	Trout, salmon, shrimp	Sweden, Asia, South-East Asia, Europe, North, Central and South America	40, 73

have been associated primarily with home-processed smoked or fermented fish products (54, 56).

Staphylococcus is commonly isolated from seafood in the USA. However, most outbreaks have been attributed to handling by infected persons (56). Seafood has not been an important vector in the transmission of *Escherichia coli* in the USA (56). To date, the emergent *E. coli* O157:H7 biotype has not been associated with seafood-related illness (101). However, cattle infected with this toxigenic strain may add to contaminated run-off waters reaching shellfish beds.

Naturally occurring bacterial pathogens: an emergent problem

Since the last outbreak of shellfish-related typhoid fever in the USA in the 1950s, the nature of seafood illnesses has changed. Prior to this, faecal contamination was the main source of bacterial pathogens in seafood. Over the last few decades, however, naturally-occurring bacteria have become the leading cause of shellfish-borne illness of known aetiology (122). Most of these indigenous bacteria belong to the family Vibrionaceae which includes the genera *Vibrio*, *Aeromonas* and *Plesiomonas*.

Members of the Vibrionaceae family are halophilic or halotolerant, in other words are characterised by their salt requirement. These are the dominant bacteria in warm marine and estuarine waters. All members of this group show an increase in abundance in warmer waters and an apparent reduction in numbers during cooler months. *Vibrio* spp. find reservoirs in the intestinal tract of fishes, within shellfish, in sediments and plankton (33, 37, 38, 83, 107). Most species give positive results when tested for chitinase activity and are often found colonising the exoskeletons of copepods and other zooplankton (55). In general, *Vibrio* spp. are not associated with faecal contamination and therefore faecal indicators do not correlate with the presence of *Vibrio* spp. (64).

Raw oyster consumption is the most common route for human infection with *Vibrio* spp. Ninety-five percent of cases in the USA are associated exclusively with the American oyster (*Crassostrea virginica*) (101). Gastroenteritis is the main disease associated with *Vibrio* spp.: however, systemic infections occur in high-risk groups and are responsible for a high mortality rate (72).

Plesiomonas and *Aeromonas* are common in estuarine waters and have been isolated in shellfish. Based on epidemiological investigations, *Plesiomonas shigelloides* has been a suspected cause of gastroenteritis for 40 years. Between 1978 and 1987 this bacterium was responsible for 0.5% of shellfish-related disease. While the pathogenicity of both *Aeromonas* and *Plesiomonas* has been questioned, Krovacek *et al.* found that marine and clinical isolates of *Aeromonas* had similar virulence characteristics (56, 66).

Vibrio species

Twelve pathogens are known within the *Vibrio* genus including *Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus*. These three are the dominant and emerging pathogenic species within the Vibrionaceae.

Vibrio cholerae

Vibrio cholerae is not strictly a naturally-occurring bacterium. In the last seven cholera pandemics, the main route of infection has been through the consumption of faecally-contaminated water (124). However, *V. cholerae* is halo-tolerant and survives well in warm waters of moderate to low salinity (27, 84, 96). In addition, *V. cholerae* has several reservoirs in the aquatic environment, which are of concern with regard to human infections (19, 124).

The primary vector for seafood-borne illness is consumption of raw oysters. In several cases, cooked crab has also been implicated (20, 21, 118). *V. cholerae* is responsible for the third-highest number of shellfish-related illnesses, after non-cholera *Vibrio* spp. and Norwalk viruses (122). Toxigenic O1 (epidemic biotype) infections are associated with profuse, watery diarrhoea. Non-toxigenic, non-O1 biotype (except O139) infections result in septicaemia and mild gastroenteritis (56).

Toxigenic, epidemic-type strains of *V. cholerae* have not been problematic in most developed countries due to effective sanitation and monitoring practices. However, given environmental reservoirs within fish, shellfish and even plankton, there is potential for these toxigenic strains to colonise novel regions. The practice of ships exchanging ballast waters close to shores illustrates this point. In 1991 and 1992, toxigenic *V. cholerae* O1 (El Tor) were isolated from bilge and ballast water of cargo ships docked in the northern Gulf of Mexico (74). Since then, the O1 El Tor (Latin American) biotype has been isolated from oysters in the northern Gulf (84).

Like other naturally-occurring bacteria, *V. cholerae* is not well correlated with faecal indicators such as *E. coli*. Despite having a faecal association with *E. coli*, *V. cholerae* survives longer and in higher numbers in surface waters (127).

The introduction of *V. cholerae* into novel regions, the presence of reservoirs and the emergence of new toxigenic strains create the potential for pandemics to reach regions which were previously free of the bacterium.

Non-cholera *Vibrio* species

Non-cholera *Vibrio* spp. account for more cases of shellfish-related disease than any other known agent. Between 1984 and 1993, 406 cases were attributed to this group (122).

This group shares the same reservoirs as *Vibrio cholerae* (fish, shellfish and plankton). The two groups also share a similar

seasonality. Infections and detection of *Vibrio* spp. in the environment are more likely to occur during the warm months (72, 101). Non-cholera species, though, are more halophilic, and the consumption of seafood, rather than contaminated water, is the main source of infection (72).

Vibrio parahaemolyticus

In the USA, *Vibrio parahaemolyticus* was implicated in 159 cases and 14 outbreaks of shellfish-related disease between 1967 and 1990 (101). In Japan, most seafood-borne disease is related exclusively to this species (39). Infections result in gastroenteritis primarily through consumption of raw or undercooked shellfish (101).

Vibrio parahaemolyticus has been isolated from a variety of fish and shellfish throughout the world. The pathogen has been isolated from temperate, subtropical and tropical coastal regions (7, 49, 60, 78). While *Vibrio* spp. thrive in warm waters, *V. parahaemolyticus* has also been isolated from several sources in the Pacific Northwest (USA). In Washington State, *V. parahaemolyticus* was detected in all sediments sampled, in oysters, in clams and in more than ten types of fish (7). Seventy-seven percent of sampled oysters from tropical regions (Brazil) revealed the presence of the pathogen (78).

Vibrio parahaemolyticus is generally found at ambient temperatures above 15°C. However, post-harvest temperature abuse can result in multiplication of bacteria by 1-4 orders of magnitude (30). During the summer months especially, shellfish should be placed on ice immediately: no growth has been observed at temperatures below 10°C. At the other end of this temperature spectrum is the incidence of *V. parahaemolyticus* in frozen fish products (85). This species shows psychrotrophic properties, and the bacterium has been recovered at frequencies of up to 25% in frozen peeled shrimp (123). The bacteria are apparently protected within the tissues of some shellfish.

As expected, given the indigenous nature of this species, faecal indicators do not predict the presence of this pathogen. Additionally, *V. parahaemolyticus* may be part of the natural flora of shellfish as it is not removed by controlled purification procedures (depuration) (59).

Vibrio vulnificus

Unlike other Vibrionaceae, *Vibrio vulnificus* infections rarely result in gastroenteritis. While wound infections are common due to recreational exposure, most infections of *V. vulnificus* result in septicaemia due to the consumption of raw oysters. Ninety-nine percent of all *V. vulnificus* infections are associated exclusively with the American oyster, *Crassostrea virginica* (122). *V. vulnificus* has also been detected in a variety of fish at higher concentrations than those found in oysters (33). However, rates of infection are low as fish are usually cooked thoroughly.

Vibrio vulnificus was responsible for 160 cases of shellfish-borne illness in the USA between 1967 and 1990, more than any other bacteria (101). The highest incidence of shellfish-related death (95%) is also due to this species (122). Additionally, *V. vulnificus* has a rapid onset and the highest mortality rate of any of the Vibrionaceae. In Florida (1981-1994), approximately 60% of people infected by eating raw oysters died (72). Similar fatality rates have been reported elsewhere (52, 53).

In general, healthy individuals are not affected by *Vibrio vulnificus*. Certain groups are at greater risk of infection and death as a result of the consumption of raw oysters. These include those with liver damage and the immuno-compromised (72, 91) (Fig. 5). In other individuals, iron in serum is bound to transferrin: as a result, there is insufficient free iron to assist growth of *V. vulnificus* in human blood. However, when the liver is damaged, there is often an overload of free (non-transferrin-bound) iron in serum. Given this excess iron, the bacteria can readily multiply (91).

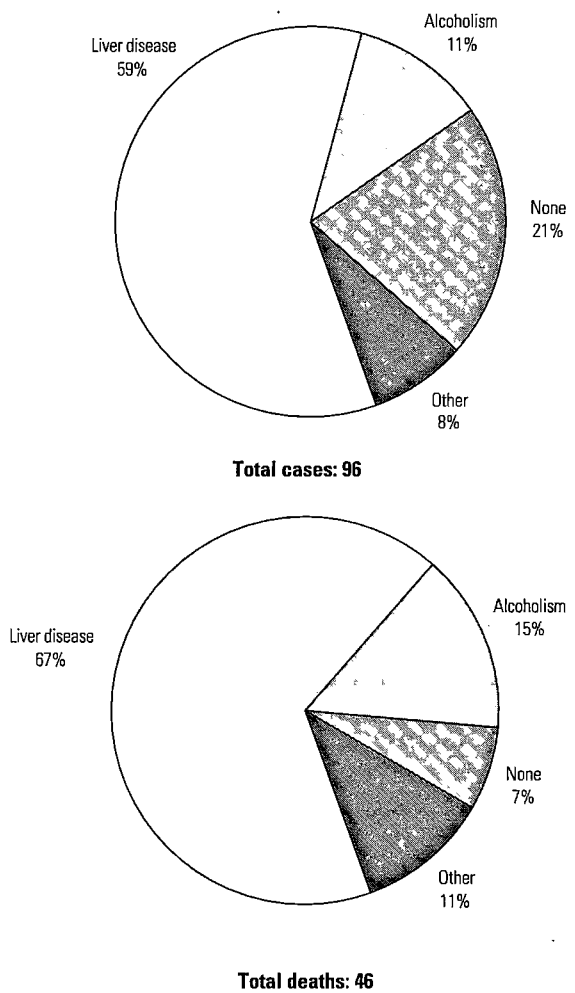


Fig. 5
***Vibrio vulnificus* infections and deaths associated with high-risk conditions in Florida, 1981-1994 (72)**

While all of the *Vibrio* spp. show seasonality in the environment, in *V. vulnificus* the seasonality is more pronounced (63). Infection with pathogenic *Vibrio* spp. due to the consumption of oysters from Apalachicola Bay, Florida, occurred in every month of the year except April. Only *V. vulnificus* showed a marked seasonality, with a cluster of infections between July and October (63). Of cases reported in Florida between 1981 and 1994, 92% of *V. vulnificus* infections and 91% of deaths occurred between April and August (72).

The seasonality of *Vibrio vulnificus* infections can be explained in two ways. First, growth of this bacterium is strongly related to temperature. The optimal conditions are 13°C-22°C and salinity of 10 (61). Below 10°C, *V. vulnificus* ceases to multiply and begins to enter a 'viable but non-culturable' state (30, 92). This 'over-wintering' stage explains the apparent reduction of *V. vulnificus* in cooler months. Secondly, *V. vulnificus* cells may appear as one of two morphotypes: opaque and virulent; or translucent and less virulent. The opacity of the virulent morphotype is due to an acidic polysaccharide coating (102). This coat is an antiphagocytic surface antigen which allows the cells to resist bacteriocidal action (both in humans and in oysters) (50, 91). Additionally, free iron in serum is not required for growth of this morphotype. The cells seem able to use transferrin-bound iron (91). These more virulent cells are more prevalent in warmer months (122).

Vibrio vulnificus occurs throughout the world, primarily in warm waters of moderate salinity. In Brazil, 12% of the oysters found in retail markets gave positive results for *V. vulnificus* (78). In Chesapeake Bay, *V. vulnificus* accounted for 0.6% to 17.4% of the total bacterial population in warm months. Throughout May and June, all plankton samples gave positive results for the opaque morphotype (126). Even as far north as Canada (Prince Edward Island), New Hampshire and Maine, *V. vulnificus* has been isolated from water, fish and shellfish (49, 93). *V. vulnificus* has been isolated less frequently in Europe. The first case of *V. vulnificus* infection in Sweden was reported in 1994 (80). In the Netherlands, *V. vulnificus* was isolated in three of 11 water samples taken during summer. Positive results were only found when the water temperature reached 20°C (114). The coastal regions of the Gulf of Mexico are the primary source of shellfish implicated in *V. vulnificus* infections (53). For those cases in which the source of the shellfish could be traced, 23% were from the Gulf Coast (122). Only five States of the USA (Alabama, Florida, Louisiana, Texas and Mississippi) currently report *V. vulnificus* infections to the Centers for Disease Control: these States border the Gulf of Mexico (26).

Vibrio vulnificus is a naturally-occurring bacterium and a commensal organism within oysters (49). Therefore, the presence of *V. vulnificus* in waters or shellfish is not predictable through the presence of indicator organisms (63). Additionally, *V. vulnificus* is selectively retained in oyster tissues during controlled purification procedures (depuration) (44, 59). Most of the time there was no significant change between *V. vulnificus* levels in pre- and post-depurated oysters: however, in one case the concentration actually increased by three orders of magnitude (59). This suggests that *V. vulnificus* may colonise and multiply within tissues of oysters.

Vibrio vulnificus may multiply quickly after harvesting if proper temperature conditions are not maintained. If oysters are immediately placed at 10°C or less, no multiplication of the bacteria occurs in the shellfish. Yet, if the oysters are stored for one day at 22°C to 30°C, the level of *V. vulnificus* increases by one to two orders of magnitude (30). Substantial multiplication may take place during post-harvest transport. Currently, no specific storage regulations for transport exist in the USA, and summer temperatures are warm enough to promote appreciable multiplication (30). Freezing and vacuum-packaging does decrease the levels of *V. vulnificus* in oysters with time. However, even after 30 days at –20°C, two log units of the bacteria were still detectable (95).

Vibrio vulnificus presents a special problem of public health significance. While infection may be rapidly fatal for a few high-risk groups, the majority of consumers will experience no adverse effects. Given the ubiquitous nature of this bacterium in warm estuarine waters, the potential for outbreaks always exists. Over the last 16 years, the incidence of *V. vulnificus* infections has increased substantially (Fig. 6). Consumer education and better detection are the most realistic options to protect public health.

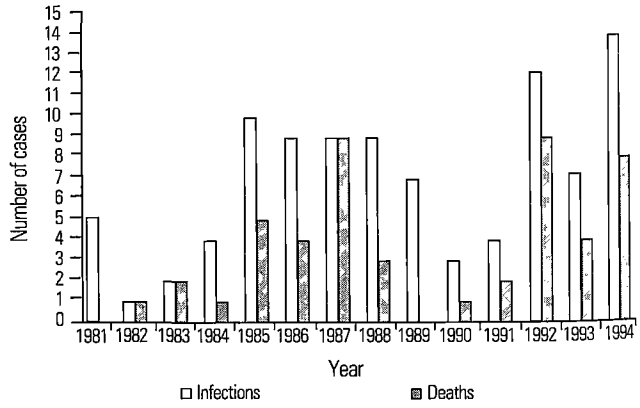


Fig. 6
Annual number of *Vibrio vulnificus* infections and deaths in Florida, 1981-1994 (72)

Human pathogenic viruses

Enteric viruses are obligate human pathogens. These agents are small (only nanometers in size) and have a simple structure of a protein coat surrounding a core of genetic material (ribonucleic acid [RNA] or deoxyribonucleic acid

[DNA]). These viruses produce an immense range of symptoms in humans, for example, diarrhoea, aseptic meningitis, paralysis, conjunctivitis, myocarditis and hepatitis. Viral replication occurs initially within the intestinal tract and leads to excretion of large numbers of virus particles in the faeces, after which survival in the environment can be prolonged due to the structure of viruses. There are over 120 enteric viruses which may be found in human sewage. The viruses include the enteroviruses (poliovirus, coxsackie A and B viruses, echoviruses), hepatitis A virus, non-A, non-B hepatitis virus (hepatitis E virus), adenoviruses, rotavirus and small round viruses (calicivirus, astrovirus, Snow Mountain agent, Norwalk virus and small rounded structured viruses) (Table III). In raw sewage, levels as high as 492,000 viral units per litre have been detected and in secondary effluent following disinfection, levels can be reduced to between 2 and 7,150 viral units per litre (81, 104). There is a significant body of information linking hepatitis and viral gastroenteritis to the consumption of contaminated shellfish in the USA and throughout the world (3, 32, 41, 57, 100). Over 100 outbreaks have been documented in the USA, increasing from less than 10 in the years 1966-1970 to more than 50 in the years 1981-1985 after more routine use was made of the electron microscope for detection (100). This trend reflects the identification of new viruses and better methods for detection. Table IV summarises some noteworthy outbreaks. There are many enteric viruses which have not been linked epidemiologically with contaminated shellfish or which have only recently been identified: some of these have been detected in shellfish. There has been speculation that more than 50% of the outbreaks of unknown aetiology are due to viruses.

Perhaps the most comprehensive data on surveillance of virus-associated disease comes from New York State (119). Twelve years of data collection have shown that of 196 outbreaks, 4 were caused by Snow Mountain agent, 65 by Norwalk virus, 6 by hepatitis A virus (HAV) and 121 by gastrointestinal viruses (small round structured viruses, non-identified). Shellfish was the vehicle in 40% of all virus outbreaks: more than any other food category (Fig. 7). Similarly, data collected during 22 years of surveillance in England and Wales demonstrate that of 98 outbreaks, 11 were caused by HAV, 57 were due to other gastrointestinal viruses and 26 were of unknown cause (Fig. 8) (3).

Hepatitis viruses

Both hepatitis A and non-A, and non-B hepatitis have been linked epidemiologically to shellfish-associated illness (32, 57). In studies conducted in the USA and the UK, an estimated 19% to 25% of hepatitis cases may be due to contaminated shellfish (17, 32, 57). Studies in Germany found that 20% of cases of infectious hepatitis were associated with shellfish (110). Therefore, shellfish may be an important source of sporadic cases and may be responsible for the endemic levels of hepatitis.

Enteroviruses

The enteroviruses in polluted waters have been studied since the 1940s, and methods for the recovery and detection in water, sediment and shellfish, and the survival ability have been evaluated extensively (57, 105). These viruses have been commonly isolated from marine waters and shellfish (82). In surveys performed in the USA, between 28% and 63% of the samples of shellfish gave positive results for enteroviruses in areas closed to harvesting (0.2 to 224 plaque forming units [PFU]/100 g). In areas open to harvesting, viruses were recovered from 9% to 40% of the samples and levels ranged from 0.3 to 200 PFU/100 g and 2.9 to 46 PFU/100 l of water (106). Waters are often closed to harvesting due to influences of rainfall, tidal flushing, sewage treatment plant discharges and septic tanks. Bioconcentration and accumulation in shellfish may increase the concentration of viruses by 10 to 100 fold compared to the water column or sediment (57).

Table III
Characteristics of enteric viruses associated with faecal-oral transmission

Viruses	Size (nm)	Nucleic acid	Diseases
Enteroviruses: poliovirus, coxsackievirus A and B, echovirus	20-30	RNA	Paralysis, aseptic meningitis, herpangia, respiratory illness, pleurodynia, pericarditis, myocarditis, congenial heart anomalies, nephritis, diarrhoea, fever, rash
Hepatitis A virus (HAV), epidemic non-A, non-B (hepatitis E virus)	27	RNA	Infectious hepatitis
Adenovirus	68-85	DNA	Acute conjunctivitis, diarrhoea, respiratory illness, eye infection
Rotavirus		RNA (double stranded)	Infantile gastroenteritis
Norwalk virus, calicivirus, astrovirus, Snow Mountain agent, small round structured virus (SRSV), (known collectively as small round viruses, SRVs)	27	RNA	Gastroenteritis

RNA: ribonucleic acid DNA: deoxyribonucleic acid

Table IV
Noteworthy virus outbreaks associated with shellfish

Virus	Year	Shellfish	Cases	Location	Note	Reference
HAV	1955	Clams		Sweden	First documented outbreak	103
HAV	1961	Oysters	84	Mississippi/Alabama, USA	Sewage pollution, with 40% developing non-specific gastroenteritis 15-24 hours post exposure	76
HAV	1973	Oysters	263	Louisiana, USA	Harvested from approved oyster-growing areas	97
HAV	1978	Mussels (boiled and steamed)	41	England	Boiled and steamed after going to a cleansing station	9
HAV	1984	Mussels/clams	75	Livorno, Italy	Identified as annual incidence rate doubled	79
HAV	1996	Many shellfish	271	Puglia, Italy	Risk factor associated with shellfish stored in water	75
HAV	1988	Clams	300,000	Shanghai, China	Largest outbreak documented	48
Norwalk virus	1978	Oysters	2,000	Sydney, Australia	First Norwalk outbreak	86
Snow Mountain agent	1977	Clams	83	Massachusetts, USA	First outbreak, clams were baked	113
Small round viruses: astrovirus, calicivirus, parvovirus, SRSV	1985 to 1986	Oysters, cockles, mussels	NR	United Kingdom	Six outbreaks demonstrating the importance of shellfish-associated viral gastroenteritis and newly recognised viruses	3
SRSV	1993	Oysters	180	Louisiana, USA	Raw or steamed oysters affected, 14 States received the product	23

HAV: hepatitis A virus
SRSV: small round structured virus
NR: not reported

Enteroviruses may remain infectious for nine days in marine waters and nineteen days in sediment, and up to one month in shellfish (98).

In addition to shellfish, shrimp and other benthic invertebrates may be an unrecognised risk associated with seafood-borne disease. Botero *et al.* (10) reported that 40% of all shrimp harvested from contaminated waters in Venezuela yielded enteroviruses.

These viruses cause a wide range of diseases in different individuals (from aseptic meningitis which may be very mild, to chronic diseases such as myocarditis) and may not be easily related to a common source outbreak. Outbreaks associated with shellfish have not been readily documented. Based on the data obtained by monitoring and survival studies, exposure to enteroviruses clearly does occur through the ingestion of contaminated shellfish. The public health impact is probably not fully appreciated.

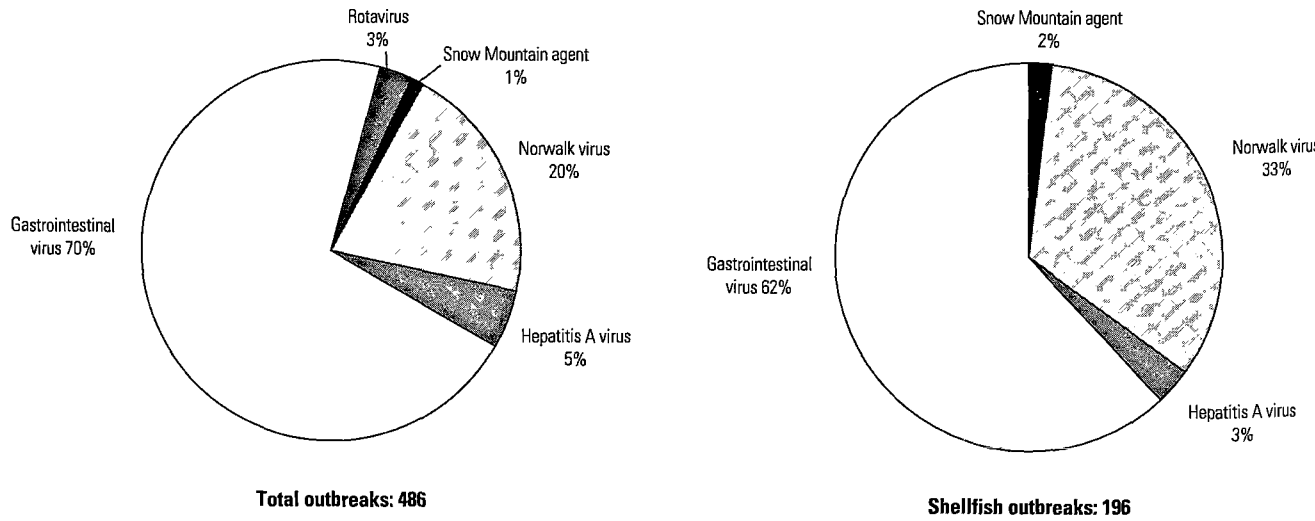


Fig. 7
Viruses associated with shellfish-associated disease outbreaks in New York State, 1981-1992 (119)

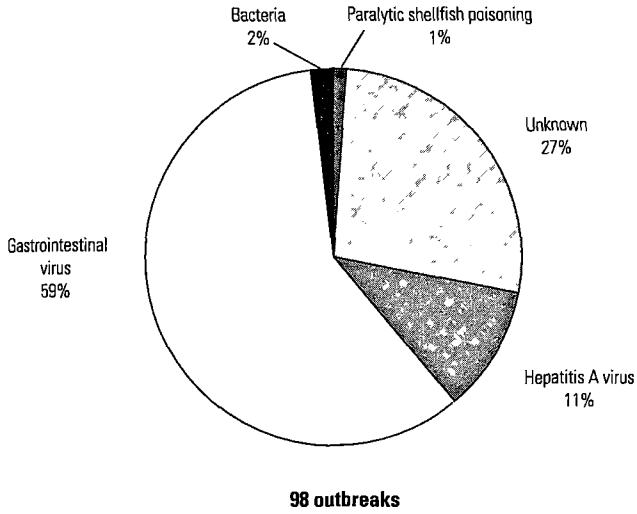


Fig. 8
Aetiological agents associated with disease outbreaks caused by shellfish in England and Wales, 1965-1986 (3)

Small round viruses

Small round viruses (SRVs) were first identified with the use of the electron microscope: the group includes Norwalk virus, Snow Mountain agent, astroviruses, caliciviruses and other small round structured viruses (SRSVs). There are extensive data showing these viruses to be a major cause of shellfish-associated disease: SRVs are possibly the most significant group of viruses causing adult gastroenteritis (3, 32, 57). Symptoms include vomiting, diarrhoea, fever and in some cases respiratory illness (16). The viruses are heat-stable and are more resistant to chlorine than the bacteria which are used to disinfect waste water.

Rotavirus and adenovirus

These viruses have not been documented as causing shellfish-associated disease. This may be due to low survival in the marine environment or poor diagnostics. Rotaviruses and adenoviruses types 40 and 41 are often associated with infant diarrhoea but may not be considered in adults. In addition, adults may excrete only low quantities of the virus which could be missed under the electron microscope.

Toxins in shellfish and fish

Several illnesses are associated with the consumption of shellfish and fish as a result of toxic algal blooms, naturally-occurring bacteria and diatoms (Table V) (56). Diseases include neurotoxic shellfish poisoning, paralytic shellfish poisoning, diarrhoetic shellfish poisoning, amnesic shellfish poisoning and ciguatera. Puffer fish poisoning and scombroid poisoning have been associated with toxins derived from naturally-occurring marine bacteria (56, 130). Many of these intoxications result in minor symptoms and the number of cases world-wide is probably under-reported. Scientists believe that the frequency of harmful algal blooms is increasing world-wide. This increase may reflect coastal

Table V
Natural toxins associated with seafood (56)

	Paralytic shellfish poisoning (PSP)	Puffer fish poisoning (PFP)	Ciguatera	Diarrhoetic shellfish poisoning (DSP)	Neurotoxic shellfish poisoning (NSP)	Scombroid fish poisoning	Amnesic shellfish poisoning (ASP)
Seafood involved	Mussels, clams, some fish; poison in digestive gland, siphon	Puffer or globefish (tetrodon), poison in liver, gonads and roe	Most common in barracuda, kahala, snapper and grouper	Mussels, clams, scallops; toxin in digestive gland	Bivalves and most plankton feeders	Mahi-mahi, tuna, bluefish, mackerel, skipjack	Mussels and clams
Source of poison or toxin	Toxic dinoflagellates: *Alexandrium catenella* (Pacific); *A. tamarensis* excavate (Atlantic)	May be produced by fish, some evidence from dinoflagellates	Not definitely known, possible *Gambierdiscus toxicus*	Dinoflagellates: *Dinophysis fortii, D. acuminata*	Dinoflagellate: *Gymnodinium breve*	Bacterial action on fish with high levels of histidine	Diatom: *Nitzschia pungens*
Location	NW and NE North America, south Chile, North Sea area, Japan	Areas of Pacific around China and Japan, rare in USA	Tropical areas around world, in USA mainly around Florida	Heaviest around Japan and Europe, no case in USA	Mostly west coast of Florida, Caribbean	World-wide	Eastern Canada; north-east USA
Type of poison or toxin*	Neurotoxin, purine base, very water soluble	Neurotoxin, slightly water soluble	Lipid-soluble, polyether multicomponent	Substrate okadaic acid, lipid-soluble multicomponent	Neurotoxin, lipid-soluble polyether	Histamine and histamine-like substances	Neurotoxin and cytotoxin, domoic acid
Extent of problem in the USA and world-wide	Local areas world-wide, few cases now	Important seafood in Japan; 100 cases, 50 deaths per year; rare in USA	Largest seafood problem, 50,000 cases per year, < 0.1% mortality	High morbidity rate, potentially world-wide problem, none in USA	Massive fish kills, environmental problems	Japan 100-1,000 cases, some in USA	Canada (1987) 103 cases and 3 deaths; no known cases in USA

* All toxins appear to be heat-stable

nutrification and dispersal of toxic species over shipping routes and with ocean currents (108).

Neurotoxic shellfish poisoning

Neurotoxic shellfish poisoning (NSP) causes symptoms of numbness, gastrointestinal effects, dizziness and muscular aches in humans. NSP is caused by a concentration of the red tide dinoflagellate *Gymnodinium breve*, and its neurotoxin (brevetoxin) in shellfish. *G. breve* occurs primarily in the Gulf of Mexico, and most cases of disease are associated with Gulf shellfish. However, these planktonic algae have been documented as far north as Cape Hatteras off North Carolina in the Atlantic Ocean (108). The Gulf Stream current system effectively transported *G. breve* to this new location (56).

Between 1973 and 1988, 53 cases of NSP were reported in the USA. However, symptoms are often mild and may go unreported. The closure of shellfish harvesting beds during red tide events effectively curtails the disease in humans but this action has economic impacts (56).

Diarrhoetic shellfish poisoning

Diarrhoetic shellfish poisoning (DSP) causes gastrointestinal distress and abdominal cramping (56). DSP is the result of okadaic acid production by dinoflagellates concentrated in mussel, oyster and scallop tissues (39). Algae most often implicated in disease outbreaks are *Dinophysis* spp., including *D. fortii* and *D. acuminata*. *Prorocentrum* spp. is another potential DSP dinoflagellate. DSP has been a problem primarily in Japan, and occasionally in Europe. No cases of DSP have been reported in the USA, although *Dinophysis* has been recovered from USA waters (56).

Amnesic shellfish poisoning

Amnesic shellfish poisoning (ASP) results in gastroenteritis and neurological symptoms, particularly memory loss (111). Symptoms often occur in the elderly and resemble Alzheimer's disease (39). ASP was the first known intoxication due to a diatom. In 1987, *Nitzschia pungens* was identified as a producer of domoic acid, which was responsible for the first outbreak of ASP (Canada, 107 cases) (56). Domoic acid-producing *Pseudonitzschia* spp. have been isolated world-wide (111).

Paralytic shellfish poisoning

Paralytic shellfish poisoning (PSP) is a serious illness which leads to neurological symptoms, paralysis and sometimes death. Toxigenic dinoflagellates, producing 20 derivatives of saxitoxin, accumulate within shellfish (especially mussels, cockles, clams and scallops) (39). Saxitoxins are considered the most potent of the algal toxins (56). *Alexandrium* (formerly *Gonyaulax*) *tamarensis* and *A. catenella* have both been implicated in PSP in the USA (56). The cyanobacterium *Anabaena circinalis*, which has been isolated from freshwater mussels, can produce two types of saxitoxin; C-toxins and gonyautoxin (90). This represents another potential vehicle for illness. Generally, PSP is heat-stable, and outbreaks have occurred from consumption of cooked shellfish (18). Yet in lobster, more water-soluble toxins showed some decrease with cooking (67). In the USA, 282 cases of PSP were reported between 1978 and 1986 (56). Outbreaks have been documented primarily in cool waters, for example in Alaska, California, Maine, Massachusetts, Oregon and Washington (18, 56). The closure of shellfishing areas during blooms has helped to reduce exposure to PSP toxins.

Ciguatera

Ciguatera is a clinical syndrome which causes several gastrointestinal and neurological disorders. Ciguatera usually results from the consumption of tropical reef fish or higher carnivores from tropical or subtropical regions (56). Several benthic dinoflagellates are associated with a range of ciguatoxins. The epiphytic *Gambierdiscus toxicus* may be the main source of the toxins. Other dinoflagellates implicated in ciguatera include *Amphidinium carteri, A. klebsi, Coolia monotis, Ostreopsis ovata, O. siamensis, Prorocentrum concavum, P. lima* and *P. rhathymum* (128). The principal toxins produced are ciguatoxin, scaritoxin, maiotoxin and palytoxin (43, 56, 129). Ciguatera-related toxins are bioaccumulated. Herbivorous fishes which feed on toxic benthic algae are consumed by higher carnivores. The level of toxins in fish tissues increases further up the food chain. An example of this is a 500-case outbreak of ciguatera in Madagascar due to the consumption of shark meat. Twelve percent of those affected died. As a top carnivore, the shark probably carried very high levels of ciguatoxins (46).

Current estimates place the world-wide annual number of ciguatera cases at 20,000 (129). Between 1978 and 1987, 791 cases were reported in the USA (56). It is believed that disturbances to reefs, for example during hurricanes and human recreational use, will open a niche for more toxic algae. This may subsequently cause an increase in ciguatera poisonings.

Puffer fish poisoning

Puffer fish poisoning (PFP) results in similar symptoms to those of PSP. Illness results from the consumption of fish from the Tetraodontidae family, including puffer fish. Unlike the toxins discussed thus far, the source is not considered to be diatoms or dinoflagellates. The microbial flora of the fishes are responsible for production of tetrodotoxin (39). Bacteria implicated thus far include *Vibrio* spp., *Listonella pelagia, Alteromonas* spp. and *Shewanella* spp. (130). Cases are most prevalent in Japan, where an estimated 200 people per year are affected by PFP. The mortality rate approaches 50% (39). Outbreaks are rare in the USA: three cases were reported in 1996 but the source was puffer fish imported from Japan (25).

Scombroid poisoning

Symptoms of scombroid poisoning are gastrointestinal and neurological in nature. The scombrotoxin produced is

believed to be of bacterial origin: the most commonly implicated group are halophilic *Vibrio* spp. (56). The nature of the poisoning is caused by the ingestion of fish with high histamine levels (39). Scombroid poisoning occurs in geographically diverse regions, and several types of fish have been implicated. These include tuna, mahi-mahi, bluefish, sardines, mackerel, amberjack and abalone (39).

Temperature abuse has been cited as a major cause of scombroid poisoning (39, 56). Multiplication of fish-associated *Vibrio* spp. and histamine production are maximised at or near 30°C (56). Cooking has no impact on the toxic effect (39).

Like ciguatera, this illness is more prevalent than the toxic shellfish-associated illnesses. Between 1978 and 1987, 757 cases of scombroid poisoning were reported in the USA (56). Under-reporting of this illness is a world-wide problem (39).

Monitoring data and risk assessment for use with hazard analysis and critical control points

The seafood hazard analysis and critical control points (HACCP) concept is focused on the identification of sources and points of contamination, levels, transmission, fate and transport of micro-organisms including regrowth and inactivation potential, and finally on the possibility of exposure of the consumer to the contaminant. Once these pathways have been identified, the most effective control strategies can be implemented and the best means for monitoring the control points can be established. For shellfish- and viral-associated diseases, the data suggest that harvesting from unapproved sources is associated with more than 30% of the outbreaks. The current tagging system appears to be inadequate. Even reliance on depuration or relaying (the moving of shellfish from contaminated harvesting waters to pristine waters for a period of time, usually one to two weeks) may not provide the protection needed.

The purpose of HACCP is to identify and monitor points where there is the greatest risk of contamination. Methods are needed for this and risk assessment methods are needed to determine the outcome associated with failure of the control points and the benefits achieved in regard to protection of public health.

Methods

Better characterisation of the quality of harvesting waters and shellfish is clearly needed. The bacteriological indicator system is severely limited, thus the development and use of new methods for direct detection of the viruses, naturally-occurring *Vibrio* spp. and the seafood toxin-associated micro-organisms in waters, sediments and shellfish will become necessary. Although methods for the biotoxins are available, the reliance on animal tests will deter most laboratories from widespread monitoring.

Bacteria

Some basic problems exist for preventing bacterial infections resulting from consumption of contaminated seafood. The faecal indicator system for shellfish-harvesting waters has been very effective in protecting consumers against general types of faecal contamination. However, several pathogenic bacteria are not predicted by this system. This is expected for naturally-occurring bacteria, but appears also to be true for some faecal-related bacteria (*Salmonella* and *Campylobacter*) (31, 62, 76, 121). Additionally, several pathogenic bacteria are known to enter a viable but non-culturable (VBNC) state which is provoked by certain environmental stimuli. These cells may still have an important role as pathogens, yet they cannot be detected by routine culture procedures. Therefore, during certain times of the year false negative results for the presence of particular pathogens may be common. A shift in monitoring practices and methods of detection is necessary to overcome these problems. New detection techniques, specific to pathogenic bacteria, show promise and are preferable to antiquated indicator methods.

Immuno-detection methods have been successfully used for the detection of *Salmonella*, *V. vulnificus* and *V. cholerae*. Enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies was used to detect *Salmonella* Paratyphi in prawns. The total assay time was 20 hours, which is much shorter than with standard cultivation (65). *V. vulnificus* and *V. cholerae* can be directly detected in oyster tissues by immunoelectron microscopy (2).

The most promising of the new detection methods are based on molecular techniques. DNA hybridisation and the polymerase chain reaction (PCR) have been used to isolate pathogens from a variety of sources. A major advantage of these techniques are specificity for particular pathogens, sensitivity (detection of one cell is not uncommon) and speed (most assays are complete within a few hours). As few as 1-10 *Salmonella* cells per gram of oyster meat have been detected by PCR in seeded samples (8). Similar recoveries have also been shown for *Vibrio* spp. in water samples (4, 12, 29). *V. vulnificus*, *V. cholerae* and *V. parahaemolyticus* have also been detected by PCR in shellfish tissues (34). The limit of detection is greater for these samples (100-1,800 cells per gram) (28, 51). This difference is due to the inhibitory properties of oyster tissue on the PCR.

Besides rapid detection and excellent sensitivity, a major advantage in using molecular detection methods is that these are not limited to culturable bacteria. The levels of total bacteria, both culturable and VBNC, can be obtained. Coleman and Oliver (29) optimised the PCR to detect VBNC *V. vulnificus* in water samples. The limit of detection was 10 cells. If viable but non culturable cells are prevalent, the number of cycles in the PCR must be nearly doubled. Approximately 500 times more DNA from VBNC cells is necessary to view bands by gel electrophoresis (11, 29).

Viruses

Williams and Fout (120) have reviewed methods for the recovery and detection of viruses from shellfish. Both extraction and concentration, as well as adsorption-elution-concentration procedures, have been developed to recover viruses. The efficiency of the methods for recovery may range from 2% to 47%. While cell culture systems have been widely used for the detection of viable viruses, these detect and quantify only a fraction of the hundreds of viruses present in wastewater or contaminated coastal water and shellfish. As previously mentioned for the bacteria, new procedures, such as PCR amplification of target viral genomes, provide more rapid, specific and sensitive approaches for detection of viruses, especially the fastidious viruses (i.e., HAV and Norwalk virus) (58, 68, 69, 70). Routine assessment of waters, sediments and shellfish with PCR will have to address in the future both the inhibition and potential detection of inactivated viruses. However, as a tool for undertaking vulnerability, PCR can provide a broader assessment of viruses present and the potential for exposure. During the investigation of an outbreak of HAV associated with the consumption of raw oysters, PCR was used to detect the presence of HAV in oysters and scallops from both unapproved and approved waters. PCR has now been used for SRSV as well as Norwalk virus in shellfish (6, 35, 69, 70).

Toxins

For detection of natural toxins, a mouse assay system has been used, although an immunoassay method has been adopted in certain situations. A useful method for the future would be to screen for the micro-organisms rather than the toxin itself. For scombroid poisoning, 87% of cases were associated with inadequate refrigeration, therefore regrowth models associated with various temperature-abuse scenarios are needed for the various enteric bacteria associated with the production of histamine.

Risk assessment

Risk assessment models can be used to examine infectious disease risks from foods and water contaminated with microbial pathogens. The risk of infection is a function of the micro-organism, dose and the host-microbe interaction. This defines a probability of infectivity from a given unit dose; a dose-response function which can be modelled (45). Each pathogen or strain has an intrinsic ability to cause infection or disease. The host population would also influence the model, and a different model might be developed for each population with varying sensitivities to the pathogen. Host factors include general and specific immunity, genetic factors, age, sex and other underlying diseases or conditions which might influence susceptibility.

Exposure depends on the initial concentration of the pathogen in the food or water, processes which would decrease the numbers (i.e. wastewater treatment) and environmental conditions which would influence microbial survival or potentially the regrowth. The final level of the pathogen in the food or water and the amount or volume consumed determine the risk of exposure.

This type of assessment has been used for shellfish harvested from approved waters in the USA (88, 106).

Using echovirus-12 and rotavirus probability models, the individual risk has been determined for consumption of raw shellfish. Individuals consuming a single serving of raw shellfish from approved waters in the USA may have a 1/100 probability of becoming infected with a moderately infectious enteric virus. However, the risk of infection becomes a 1/2 probability (with a lower 95% confidence limit of a 2/5 risk) if exposed to a highly infectious virus, such as the rotavirus, at an average virus exposure of 6 PFU/60 g (Fig. 9).

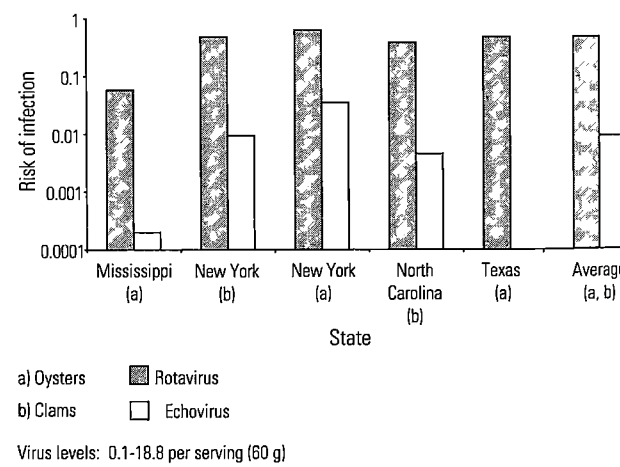


Fig. 9
Risk of viral infection from consumption of raw shellfish using dose-response modelling and monitoring data (106)

The risk of infection is the initial step in microbial dose-response assessment. Estimates of morbidity and mortality can then be made from the infectivity estimates based on asymptomatic to symptomatic ratios and case/fatality ratios for various pathogens. Secondary and tertiary transmission by a person-to-person route should also

be taken into account. Finally, the overall risk must be assessed in comparison to other relative risks.

More data are required on the occurrence and exposure for the various microbial hazards associated with seafood. These data, along with dose-response models, can be used for risk assessment and can be fed into the HACCP approach as control data, so that the most cost-effective and reliable strategy for reducing risks and improving seafood safety for any particular hazard or geographical location can be more adequately defined and prioritised. Significant data gaps can be identified and research can be supported to address the greatest uncertainties.

For routine work, the use of faecal indicators to predict a relative level of faecal contamination should not be disposed of. However, the main source of seafood illness is species which are not predicted by these organisms. In order to ensure public health, routine surveillance using pathogen-specific techniques should be implemented. This, in combination with risk assessment and HACCP, will begin to address the improvements needed for enhancing the safety of seafood.

Conclusion

Scombroid illness appears to be the greatest cause of seafood-associated disease linked to post-harvest contamination and improper storage of fish, and is caused by a variety of enteric bacteria.

Viruses such as the small round structured viruses (SRSV) are an emerging concern associated with the consumption of shellfish. Better protection of harvesting waters is imperative as many outbreaks have been associated with cooked or depurated products.

Sensitive populations, such as the immuno-compromised, diabetics and those with impaired liver functions, are at an increased risk of severe outcomes from infection with *Vibrio* spp. These groups are also at increased risk of death due to such organisms.

Indicator bacteria are inadequate to protect the consumer against enteric viruses carried in seafood.

New methods allow for determination of contamination and exposure to be used in conjunction with risk assessment and identification of critical control points.

Vibrio spp. account for the highest number of infections of known bacterial aetiology from shellfish consumption. As naturally-occuring bacteria, *Vibrio* spp. cannot be eliminated from the source, therefore the most practical measures to minimise human infection include strict guidelines against temperature abuse, consumer education and better detection.

The occurrence of microbial pathogens and natural toxins in fish and shellfish harvested in one geographical area can have an impact on many geographically distinct populations as a result of widespread distribution and transport of seafoods, and the problem will not be rectified without the implementation of increased sanitation practices.

■

Le rôle des poissons et des fruits de mer dans les toxi-infections alimentaires aux États-Unis d'Amérique

E.K. Lipp & J.B. Rose

Résumé

Aux États-Unis d'Amérique, de 1983 à 1992, les poissons et fruits de mer occupaient le troisième rang parmi les catégories d'aliments responsables de toxi-infections alimentaires. S'agissant de la consommation de poissons, les vecteurs ou agents le plus souvent incriminés étaient des poissons scombridés, des ciguatoxines, des bactéries et d'autres agents indéterminés ; concernant les mollusques et crustacés, les toxi-infections étaient dues à des agents indéterminés, aux agents de l'intoxication paralysante des mollusques et crustacés, à *Vibrio* spp. et à d'autres bactéries, suivis par le virus de l'hépatite A. On dénombre au moins dix genres de bactéries responsables de toxi-infections alimentaires transmises par les fruits de mer. Au cours des 25 dernières années,

les agents bactériens associés à une contamination fécale n'ont représenté que 4 % des maladies liées à la consommation de mollusques et crustacés, tandis que les bactéries endogènes représentaient 20 % des toxi-infections alimentaires et étaient responsables de 99 % des décès. La plupart des ces bactéries indigènes appartiennent à la famille des Vibrionaceae qui comprend les genres *Vibrio*, *Aeromonas* et *Plesiomonas*. En général, *Vibrio* spp. n'est pas associé à des contaminations fécales ; aussi n'y a-t-il pas de corrélation entre les indicateurs de cette contamination et la présence de *Vibrio*. Les virus sont la principale cause de toxi-infections associées à la consommation de mollusques et de crustacés : par exemple, entre 1981 et 1992, sur 196 foyers signalés dans l'État de New York, 33 % étaient dus au virus de Norwalk et 62 à des virus gastro-intestinaux (petits virus à structure arrondie). De plus, plusieurs maladies résultent d'une efflorescence toxique des algues, du développement de bactéries endogènes et de diatomées responsables d'intoxication neurotoxique (*neurotoxic shellfish poisoning*), d'intoxication paralysante (*paralytic shellfish poisoning*), d'intoxication diarrhéique (*diarrhoetic shellfish poisoning*), d'intoxication avec effet d'amnésie (*amnesic shellfish poisoning*), ainsi que de ciguatera. D'après les estimations actuelles, on dénombre 20 000 cas de ciguatera chaque année dans le monde.

L'intoxication par l'histamine (intoxication par les scombridés) est la première cause de toxi-infection alimentaire associée à la consommation de poissons et de fruits de mer. La scombrotoxine est d'origine bactérienne avec, à la source, des vibrions halophiles induisant des niveaux élevés d'histamine. L'intoxication par l'histamine varie selon les régions géographiques, et plusieurs espèces sont impliquées, notamment le thon, le mahi-mahi, le tassergal, la sardine, le maquereau, la sériole et l'ormeau. Une température excessive est reconnue comme le principal facteur favorisant l'intoxication par l'histamine.

Dans la pratique, il ne faut pas négliger l'étude des indicateurs fécaux pour évaluer le niveau relatif de contamination. Toutefois, les espèces à l'origine des principales intoxications par les fruits de mer ne peuvent être détectées par le simple recours à ces indicateurs fécaux. Pour protéger la santé publique, une surveillance régulière, basée sur de nouvelles techniques spécifiques à un agent pathogène, telles que l'amplification en chaîne par polymérase, devrait être mise en œuvre. Ces techniques, ajoutées à l'évaluation des risques et à la méthode de l'analyse des risques, points critiques pour leur maîtrise, permettront de garantir l'innocuité des poissons et des fruits de mer pour l'homme.

Mots-clés
Bactéries – Entérovirus – États-Unis d'Amérique – Évaluation des risques – Foyers – Fruits de mer – Mollusques et crustacés – Poissons – Santé publique – Toxines – Vibrions.

■

El papel de los alimentos de origen marino en las toxi-infecciones alimentarias en Estados Unidos de América

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Resumen
En Estados Unidos de América, entre 1983 y 1992, los alimentos de origen marino fueron la tercera categoría de alimentos responsables de toxi-infecciones alimentarias. En lo que al pescado se refiere, los vectores y agentes más citados fueron los escómbridos, las ciguatoxinas, las bacterias y otros agentes

desconocidos; en cuanto al marisco, agentes desconocidos, el envenenamiento paralítico por marisco, *Vibrio* spp. y otras bacterias, seguidos por el virus de la hepatitis A, fueron las causas de las toxi-infecciones registradas.

Hay por lo menos diez géneros de patógenos bacterianos involucrados en toxi-infecciones alimentarias asociadas al consumo de mariscos. En el curso de los últimos 25 años, los patógenos bacterianos asociados con la contaminación fecal estuvieron implicados en sólo el 4% de los brotes relacionados con marisco, mientras que la flora bacteriana endógena daba cuenta del 20% de las afecciones y del 99% de las muertes. La mayoría de esas bacterias indígenas pertenecen a la familia Vibrionaceae, que comprende a los géneros *Vibrio, Aeromonas* y *Plesiomonas. Vibrio* spp. no suele venir asociado a la contaminación fecal, por lo que los indicadores de ese tipo de contaminación no presentan correlación con la presencia de *Vibrio.* Los virus constituyen la causa más frecuente de toxi-infecciones asociadas al marisco: por ejemplo, en el Estado de Nueva York, entre 1981 y 1992, de un total de 196 brotes, un 33% y un 62% fueron causados respectivamente por el virus de Norwalk y por virus gastrointestinales (pequeños virus de estructura globular). Por otra parte, diversas enfermedades son consecuencia de florescencias tóxicas de algas, del crecimiento de flora bacteriana endógena y de diatomeas que causan envenenamiento neurotóxico (*neurotoxic shellfish poisoning*), envenenamiento paralítico (*paralytic shellfish poisoning*), envenenamiento diarreico (*diarrhoetic shellfish poisoning*), envenenamiento amnésico (*amnesic shellfish poisoning*) y ciguatera. Las estimaciones actuales cifran en 20.000 el número de casos anuales de ciguatera en el mundo.

La toxicidad histamínica (envenenamiento de escómbridos) constituye la causa más importante de toxi-infecciones ligadas al consumo de productos marinos. La escombrotoxina, de origen bacteriano, es causada por *Vibrio* spp. halóficos que provocan une elevación de los niveles de histamina. Las características del envenenamiento de escómbridos varían geográficamente, y son numerosas las especies involucradas, a saber: el atún, el mahi-mahi, la anjova, la sardina, la caballa, el pez limón y la oreja de mar. Una de las principales causas de envenenamiento de escómbridos que suele citarse es el exceso de temperatura.

En la práctica, no se debe descartar el estudio de indicadores fecales para predecir el nivel relativo de contaminación; sin embargo, gran parte de las especies responsables de toxi-infecciones alimentarias debidas al consumo de mariscos no pueden ser detectadas mediante estos indicadores fecales. Velar eficazmente por la salud pública exigiría aplicar una vigilancia sistemática basada en nuevas técnicas de detección específica de patógenos, como la reacción en cadena de la polimerasa. El uso de tales técnicas, combinado con el de métodos de evaluación de riesgos y de análisis de riesgos y control de puntos críticos, constituiría un primer paso hacia la necesaria mejora del nivel de seguridad que ofrecen los alimentos de origen marino.

Palabras clave

Alimentos de origen marino – Bacterias – Brotes – Estados Unidos de América – Evaluación de riesgos – Marisco – Moluscos y crustáceos – Salud pública – Toxinas – Vibrio – Virus entéricos.

References

1. Abbott S. & Janda J.M. (1994). – Severe gastroenteritis associated with *Vibrio hollisae* infection: report of two cases and review. *Clin. infect. Dis.*, **18**, 310-312.

2. Aldrich H.C., McDowell L.M., Tamplin M.L., Frase C., Murphree R. & Jackson J.K. (1995). – Detection of *Vibrio vulnificus* and *Vibrio cholerae* O1 in oyster tissue using immunoelectron microscopy. *J. Shellfish Res.*, **14**, 493-499.

3. Appleton H. (1987). – Small round viruses: classification and role in foodborne infections. *In* Novel diarrhoea viruses. John Wiley & Sons, Chichester, 108-137.

4. Arias C.R., Garay E. & Aznar R. (1995). – Nested PCR method for rapid and sensitive detection of *Vibrio vulnificus* in fish, sediments and water. *Appl. environ. Microbiol.*, **61**, 3476-3478.

5. Arumugaswamy R.K. & Proudford R.W. (1987). – The occurrence of *Campylobacter jejuni* and *Campylobacter coli* in Sydney rock oyster (*Crassostrea commercialis*). *Int. J. Food Microbiol.*, **4**, 101-104.

6. Atmar R.L., Neill F.H., Woodley C.M., Manger R., Fout G.S., Burkhardy W., Leja L., McGovern E.R., Le Guyader F., Metcalf T.G. & Estes M.K. (1996). – Collaborative evaluation of a method for the detection of Norwalk virus in shellfish tissues by PCR. *Appl. Microbiol.*, **62**, 254-258.

7. Baross J. & Liston J. (1970). – Occurrence of *Vibrio parahaemolyticus* and related hemolytic Vibrios in marine environments of Washington State. *Appl. Microbiol.*, **25**, 179-186.

8. Bej A.K., Mahbubani M.H., Boyce M.J. & Atlas R.M. (1994). – Detection of *Salmonella* spp. in oysters by PCR. *Appl. environ. Microbiol.*, **60**, 368-373.

9. Bostock A.D., Mepham P. & Phillips S. (1979). – Hepatitis A infection associated with the consumption of mussels. *J. Infect.*, **1**, 171-177.

10. Botero L., Montiel M. & Porto L. (1996). – Enteroviruses in shrimp harvested from contaminated marine waters. *Int. J. environ. Hlth Res.*, **6**, 103-108.

11. Brauns L.A., Hudson M.C. & Oliver J.D. (1991). – Use of the polymerase chain reaction in detection of culturable and nonculturable *Vibrio vulnificus* cells. *Appl. environ. Microbiol.*, **57**, 2651-2655.

12. Brauns L.A. & Oliver J.D. (1994). – Polymerase chain reaction of whole cell lysates for the detection of *Vibrio vulnificus*. *Food Biotechnol.*, **8**, 1-6.

13. Bremer P.J. & Osborne C.M. (1995). – Efficacy of marinades against *Listeria monocytogenes* cells in suspension or associated with Green Shell Mussels (*Perna canaliculus*). *Appl. environ. Microbiol.*, **61**, 1514-1519.

14. Brock T., Christensen N., Riewerts Eriksen N.H., Winter S., Rygaard H. & Jorgensen F. (1994). – The first fatal case of *Vibrio vulnificus* infection in Denmark. *Acta pathol. microbiol. scand.*, **102**, 874-876.

15. Centers for Disease Control (1987). – International outbreak of type E botulism associated with ungutted, salted whitefish. *Morbidity and Mortality Weekly Report*, **36** (49), 812-813.

16. Centers for Disease Control (1990). – Waterborne disease outbreaks, 1986-1988 and Foodborne disease outbreaks, 5-year summary, 1983-1987. *Morbidity and Mortality Weekly Report*, **39** (Surveillance Summary 1).

17. Centers for Disease Control (1991). – Summary of notifiable diseases, United States. *Morbidity and Mortality Weekly Report*, **39**, 11-26.

18. Centers for Disease Control (1991). – Paralytic shellfish poisoning – Massachusetts and Alaska, 1990. *Morbidity and Mortality Weekly Report*, **40** (10), 157-160.

19. Centers for Disease Control (1991). – Update: cholera outbreak – Peru, Ecuador, and Colombia. *Morbidity and Mortality Weekly Report*, **40** (13), 225-227.

20. Centers for Disease Control (1991). – Cholera – New Jersey and Florida. *Morbidity and Mortality Weekly Report*, **40** (17), 287-289.

21. Centers for Disease Control (1991). – Cholera – New York, 1991. *Morbidity and Mortality Weekly Report*, **40** (30), 516-518.

22. Centers for Disease Control (1993). – Imported cholera associated with a newly described toxigenic *Vibrio cholerae* O139 strain – California, 1993. *Morbidity and Mortality Weekly Report*, **42** (26), 501-503.

23. Centers for Disease Control (1993). – Multistate outbreak of viral gastroenteritis related to consumption of oysters – Louisiana, Maryland, Mississippi, and North Carolina, 1993. *Morbidity and Mortality Weekly Report*, **42** (49), 945-948.

24. Centers for Disease Control (1996). – Surveillance for foodborne disease outbreaks – United States, 1988-1992. *Morbidity and Mortality Weekly Report*, **45** (Surveillance Summary 5).

25. Centers for Disease Control (1996). – Tetrodotoxin poisoning associated with eating puffer fish transported from Japan – California 1996. *Morbidity and Mortality Weekly Report*, **45** (19), 389-391.

26. Centers for Disease Control (1996). – *Vibrio vulnificus* infections associated with eating raw oysters – Los Angeles, 1996. *Morbidity and Mortality Weekly Report*, **45** (29), 621-623.

27. Chowdhury M.A.R., Miyoshi S.-I., Yamanaka H. & Shinoda S. (1992). – Ecology and distribution of toxigenic *Vibrio cholerae* in aquatic environments of a temperate region. *Microbios*, **72**, 203-213.

28. Coleman S.S., Melanson D.M., Biosca E.G. & Oliver J.D. (1996). – Detection of *Vibrio vulnificus* biotypes 1 and 2 in eels and oysters by PCR amplification. *Appl. environ. Microbiol.*, **62**, 1378-1382.

29. Coleman S.S. & Oliver J.D. (1996). – Optimization of conditions for the polymerase chain reaction amplification of DNA from culturable and non-culturable cells of *Vibrio vulnificus*. *FEMS Microbiol. Lett.*, **19**, 127-132.

30. Cook D.W. & Ruple A.D. (1989). – Indicator bacteria and Vibrionaceae multiplication in post-harvest shellstock oysters. *J. Food Protec.*, **52**, 343-349.

31. D'Aoust J.Y., Gelinas R. & Maishment C. (1980). – Presence of indicator organisms and recovery of *Salmonella* in fish and shellfish. *J. Food Protec.*, **43**, 679-682.

32. Deleon R. & Gerba C.P. (1990). – Viral disease and transmission by seafood. *In* Food contamination from environmental sources (J.O. Nriagu & M.S. Simmons, eds). John Wiley & Sons, Inc., New York, 785 pp.

33. Depaola A., Capers G.M. & Alexander D. (1994). – Densities of *Vibrio vulnificus* in the intestines of fish from the US Gulf coast. *Appl. environ. Microbiol.*, **60**, 984-988.

34. Depaola A. & Hwang G.-C. (1995). – Effect of dilution, incubation time, and temperature of enrichment on cultural and PCR detection of *Vibrio cholerae* obtained from the oyster *Crassostrea virginica*. *Mol. cell. Probes*, **9**, 75-81.

35. Desenclos J.C.A., Klontz K.C., Wilder M.H., Nainan O.V., Margolis H.S. & Gunn R.A. (1991). – A multi-state outbreak of hepatitis A caused by the consumption of raw oysters. *Am. J. publ. Hlth*, **81**, 1268-1272.

36. Doyle M.P. (ed.) (1989). – Foodborne bacterial pathogens. Marcel Dekker Inc., New York, 796 pp.

37. Epstein P.R. (1993). – Algal blooms in the spread and persistence of cholera. *BioSystems*, **31**, 209-221.

38. Epstein P.R. (1995). – Emerging diseases and ecosystem instability: new threats to public health. *Am. J. publ. Hlth*, **85**, 168-172.

39. Food and Drug Administration (1992). – Foodborne pathogenic microorganisms and natural toxins. Center for Food Safety and Applied Nutrition, Rockville, Maryland, 161 pp.

40. Gecan J.S., Bandler R. & Staruszkiewicz W.F. (1994). – Fresh and frozen shrimp: a profile of filth, microbiological contamination, and decomposition. *J. Food Protec.*, **57**, 154-158.

41. Gerba C.P. (1988). – Viral disease transmission by seafoods. *Food Technol.*, **41**, 99-103.

42. Gerba C.P., Goyal R.L., Cech I. & Bogdan G.F. (1979). – Failure of indicator bacteria to reflect the occurrence of enteroviruses in marine waters. *Am. J. publ. Hlth*, **69**, 116-119.

43. Gleibs S., Mebs D. & Werding B. (1995). – Studies on the origin and distribution of palytoxin in a Caribbean coral reef. *Toxicon*, **33**, 1531-1537.

44. Groubert T.N. & Oliver J.D. (1994). – Interaction of *Vibrio vulnificus* and the eastern oyster, *Crassostrea virginica*. *J. Food Protec.*, **57**, 224-228.

45. Haas C. (1983). – Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. *Am. J. Epidemiol.*, **118**, 573-582.

46. Habermehl G.G., Krebs H.C., Rasoanaivo P. & Ramialihariosa A. (1994). – Severe ciguatera poisoning in Madagascar: a case report. *Toxicon*, **32**, 1539-1542.

47. Hackney C.R. & Pierson M.D. (1994). – Environmental indicators and shellfish safety. Chapman & Hall, New York, 523 pp.

48. Halliday M.L., Kang L.-Y., Zhou T.-K., Hu M.-D., Pan Q.-C., Fu T.-Y., Huang Y.-S. & Hu S.-L. (1991). – An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *J. infect. Dis.*, **164**, 852-859.

49. Hariharan H., Giles J.S., Heaney S.B., Arsenault G., McNair N. & Rainnie D.J. (1995). – Bacteriological studies on mussels and oysters from six river systems in Prince Edward Island, Canada. *J. Shellfish Res.*, **14**, 527-532.

50. Harris-Young L., Tamplin M.L., Mason J.W., Aldrich H.C. & Jackson J.K. (1995). – Viability of *Vibrio vulnificus* in association with hemocytes of the American oyster (*Crassostrea virginica*). *Appl. environ. Microbiol.*, **61**, 52-57.

51. Hill W.E., Keasler S.P., Trucksess M.W., Feng P., Kaysner C.A. & Lampel K.A. (1991). – Polymerase chain reaction identification of *Vibrio vulnificus* in artificially contaminated oysters. *Appl. environ. Microbiol.*, **57**, 707-711.

52. Hlady W.G., Mullen R.C. & Hopkins R.S. (1993). – *Vibrio vulnificus* from raw oysters: leading cause of reported deaths from foodborne illness in Florida. *J. Florida med. Assoc.*, **80**, 536-538.

53. Hlady W.G. & Klontz K.C. (1996). – The epidemiology of *Vibrio* infections in Florida, 1981-1993. *J. infect. Dis.*, **173**, 1176-1183.

54. Hobbs G. (1976). – *Clostridium botulinum* and its importance in fishery products. *In* Advances in food research (C.O. Chichester, E.M. Mrak & G.F. Stewart, eds). Academic Press, New York, 135-169.

55. Huq A., Xu B., Chowdhury M.A.R., Islam M.S., Montilla R. & Colwell R.R. (1996). – A simple filteration method to remove plankton-associated *Vibrio cholerae* in raw water supplies in developing countries. *Appl. environ. Microbiol.*, **62**, 2508-2512.

56. Institute of Medicine (1991). – Seafood safety. National Academy Press, Washington, DC, 486 pp.

57. Jaykus L.A., Hemard M.T. & Sobsey M.D. (1993). – Human enteric pathogenic viruses. *In* Environmental indicators of shellfish safety (M.D. Pierson & C.R. Hackney, eds). Van Nostrand Reinhold, New York, 92-153.

58. Jaykus L.A., De Leon R. & Sobsey M.D. (1996). – A virion concentration method for detection of human enteric viruses in oysters by PCR and oligoprobe hybridization. *Appl. environ. Microbiol.*, **62**, 2074-2080.

59. Jones S.H., Howell T.L. & O'Neill K.R. (1991). – Differential elimination of indicator bacteria and pathogenic *Vibrio* spp. from Easter oysters (*Crassostrea virginica* Gmelin, 1791) in a commercial controlled purification facility in Maine. *J. Shellfish Res.*, **10**, 105-112.

60. Joseph S.W., Colwell R.R. & Kaper J.B. (1980). – *Vibrio parahaemolyticus* and related halophilic vibrios. *CRC Crit. Rev. Microbiol.*, **10**, 77-125.

61. Kaspar C.W. & Tamplin M.L. (1993). – Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. *Appl. environ. Microbiol.*, **59**, 2425-2429.

62. Kfir R., Burger J.S. & Idema G.K. (1993). – Detection of Salmonella in shellfish grown in polluted seawater. *Water Sci. Technol.*, **27**, 41-44.

63. Klontz K.C., Williams L., Baldy L.M. & Campos M. (1993). – Raw oyster-associated *Vibrio* infections: linking epidemiologic data with laboratory testing of oysters obtained from a retail outlet. *J. Food Protec.*, **56**, 977-979.

64. Koh E.G.L., Huyn J.-H. & Larock P.A. (1994). – Pertinence of indicator organisms and sampling variables to *Vibrio* concentrations. *Appl. environ. Microbiol.*, **60**, 3897-3900.

65. Korbsrisate S., Saraombath S., Janyapoon K., Ekpo P. & Pongsunk S. (1994). – Immunological detection of *Salmonella* Paratyphi A in raw prawns. *Appl. environ. Microbiol.*, **60**, 4612-4613.

66. Krovacek K., Pasquale V., Baloda S.B., Soprano V., Conte M. & Dumontet S. (1994). – Comparison of virulence factors in *Aeromonas hydrophila* strains isolated from the marine environment and human diarrheal cases in southern Italy. *Appl. environ. Microbiol.*, **60**, 1379-1382.

67. Lawrence J.F., Maher M. & Watson-Wright W. (1994). – Effect of cooking on the concentration of toxins associated with paralytic shellfish poison in lobster hepatopancreas. *Toxicon*, **32**, 57-64.

68. Lees D.N., Henshilwood K. & Dore W.J. (1994). – Development of a method for detection of enteroviruses in shellfish by PCR with poliovirus as a model. *Appl. environ. Microbiol.*, **60**, 2999-3005.

69. Lees D.N., Henshilwood K. & Brown D.W.G. (1995). – Detection of small round structured viruses in shellfish by reverse transcription PCR. *Appl. environ. Microbiol.*, **61**, 4418-4424.

70. Le Guyader F., Neill F.H., Estes M.K., Monroe S.S., Ando T. & Atmar R.L. (1996). – Detection and analysis of small round-structured virus strain in oysters implicated in an outbreak of acute gastroenteritis. *Appl. environ. Microbiol.*, **62**, 4268-4272.

71. Levine W.C. & Griffin P.M. (1993). – Vibrio infections on the Gulf Coast: results of first year of regional surveillance. Written in association with The Gulf Coast Vibrio Working Group. *J. infect. Dis.*, **167**, 479-483.

72. Lipp E.K. & Hammond R. (1996). – Ecology and virulence of halophilic Vibrios in Florida. *Florida J. environ. Hlth*, **153**, 6-10.

73. Loncarevic S., Tham W. & Danielsson-Tham M.-L. (1996). – Prevalence of *Listeria monocytogenes* and other *Listeria* spp. in smoked and 'Gravad' fish. *Acta vet. scand.*, **37**, 13-18.

74. McCarthy S.A. & Khambaty F.M. (1994). – International dissemination of epidemic *Vibrio cholerae* by cargo ship ballast and other nonpotable waters. *Appl. environ. Microbiol.*, **60**, 2597-2601.

75. Malfait P., Lopalco P.L., Salmaso S., Germinario C., Salamina G., Quarto M. & Barbuti S. (1996). – An outbreak of hepatitis A in Puglia, Italy, 1996. *EuroSurveillance*, **1** (5), 33-35.

76. Martinez-Manzanares E., Morinigo M.A., Castro D., Balebona M.C., Sanchez J.M. & Borrego J.J. (1992). – Influence of the faecal pollution of marine sediments on the microbial content of shellfish. *Mar. Pollut. Bull.*, **24**, 342-349.

77. Mason J.O. & McClean W.R. (1962). – Infectious hepatitis traced to the consumption of raw oysters. *Am. J. Hyg.*, **75** (1), 1-22.

78. Matte G.R., Matte M.H., Rivera I.G. & Martins M.T. (1994). – Distribution of potentially pathogenic vibrios in oysters from a tropical region. *J. Food Protec.*, **57**, 870-873.

79. Mele A., Rastelli M.G., Gill O.N., Dibisceglie D., Rosmini F., Pardelli G., Valtriani C. & Patriarchi P. (1989). – Recurrent epidemic hepatitis A associated with consumption of raw shellfish, probably controlled through public health measures. *Am. J. Epidemiol.*, **130**, 540-546.

80. Melhus A., Holmdahl T. & Tjernberg I. (1995). – First documented case of bacteremia with *Vibrio vulnificus* in Sweden. *Scand. J. infect. Dis.*, **27**, 81-82.

81. Melnick J.L. & Gerba C.P. (1980). – The ecology of enteroviruses in natural waters. *CRC Crit. Rev. environ. Control*, **10** (1), 65-93.

82. Metcalf T.G. & Stiles W.C. (1964). – The accumulation of enteric viruses by the oyster *Crassostrea virginica*. *J. infect. Dis.*, **115**, 68-76.

83. Montilla R., Palomar J., Santmarti M., Fuste C. & Viñas M. (1994). – Isolation and characterization of halophilic *Vibrio* from bivalves bred in nurseries at the Ebre Delta. *J. Invert. Pathol.*, **63** (2), 178-181.

84. Motes M., Depaola A., Zywno-Van Ginkel S. & McPhearsson M. (1994). – Occurrence of toxigenic *Vibrio cholerae* O1 in oysters in Mobile Bay, Alabama: an ecological investigation. *J. Food Protec.*, **57**, 975-980.

85. Muntada-Garriga J.M., Rodriguez-Jerez J.J., Lopez-Sabater E.I. & Mora-Ventura M.T. (1995). – Effect of chill and freezing temperature on survival of *Vibrio parahaemolyticus* inoculated in homogenates of oyster meat. *Appl. Microbiol.*, **20**, 225-227.

86. Murphy A.M., Grohmann G.S., Christopher R.J., Lopez W.A., Davey G.R. & Millsom R.H. (1979). – An Australian-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. *Med. J. Australia*, **2**, 329-333.

87. National Marine Fisheries Service (1997). – Fisheries of the United States, 1996, current fisheries statistics, No. 9500. US Goverment Printing Office, Washington, DC, 126 pp.

88. National Research Council (1993). – Managing wastewater in coastal urban areas. National Academy of Sciences, National Academy Press, Washington, DC, 477 pp.

89. Nedoluha P.C. & Westhoff D. (1995). – Microbiological analysis of striped bass (*Morone saxatilis*) grown in flow-through tanks. *J. Food Protec.*, **58**, 1363-1368.

90. Negri A.P. & Jones G.J. (1995). – Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussel *Alathyria condola*. *Toxicon*, **33**, 667-678.

91. Oliver J.D. (1989). – *Vibrio vulnificus*. *In* Foodborne bacterial pathogens (M.P. Doyle, ed.). Marcel Dekker Inc., New York, 569-600.

92. Oliver J.D., Hite F., McDougald D., Andon N.L. & Simpson L.M. (1995). – Entry into, and resuscitation from, the viable but nonculturable state by *Vibrio vulnificus* in an estuarine environment. *Appl. environ. Microbiol.*, **61**, 2624-2630.

93. O'Neill K.R., Jones S.H. & Grimes D.J. (1990). – Incidence of *Vibrio vulnificus* in northern New England waters and shellfish. *FEMS Microbiol. Lett.*, **72**, 163-168.

94. Pan American Health Organization (1995). – Regional information system for epidemiological surveillance of food borne disease (SIRVE-ETA) period 1993-1994. Pan American Sanitary Bureau, Regional Office of the World Health Organization, Washington, DC, 10.

95. Parker R.W., Maurer E.M., Childers A.B. & Lewis D.H. (1994). – Effect of frozen storage and vacuum-packaging on survival of *Vibrio vulnificus* in Gulf Coast oysters (*Crassostrea virginica*). *J. Food Protec.*, **57**, 604-606.

96. Perez-Rosas N. & Hazen T.C. (1989). – *In situ* survival of *Vibrio cholerae* and *Escherichia coli* in a tropical rain forest watershed. *Appl. environ. Microbiol.*, **Feb**, 495-499.

97. Portnoy B.L., Mackowiak P.A., Caraway C.T., Walker J.A., McKinley T.W. & Klein C.A. (1975). – Oyster-associated hepatitis: failure of shellfish certification programs to prevent outbreaks. *J. Am. med. Assoc.*, **233**, 1065-1068.

98. Rao V.C., Seidel K.M., Goyal S.M., Metcalf T.G. & Melnick J.L. (1984). – Isolation of enteroviruses from water, suspended solids, and sediments from Galveston Bay: survival of poliovirus and rotavirus adsorbed to sediments. *Appl. environ. Microbiol.*, **48**, 404-409.

99. Razem D. & Katusin-Razem B. (1994). – The incidence of cases of foodborne diseases in Croatia. *J. Food Protec.*, **57**, 746-753.

100. Richards G.P. (1985). – Outbreaks of shellfish associated enteric virus illness in the United States: requisite for development of viral guidelines. *J. Food Protec.*, **48**, 815-823.

101. Rippey S.R. (1994). – Infectious diseases associated with molluscan shellfish consumption. *Clin. Microbiol. Rev.*, **7**, 419-425.

102. Rodrick G., Collins M. & Heil D. (1994). – The causes, prevention and control of *Vibrio* infections from molluscan shellfish consumption. *Florida J. environ. Hlth*, **145**, 18-22.

103. Roos R. (1956). – Hepatitis epidemic conveyed by oysters. *Sven. Lakartidningen*, **53**, 989-1003.

104. Rose J.B. (1986). – Microbial aspects of wastewater reuse for irrigation. *CRC Crit. Rev. environ. Control*, **16**, 231-256.

105. Rose J.B. & Gerba C.P. (1991). – Assessing potential health risks from viruses and parasites in reclaimed water in Arizona and Florida. *Water Sci. Technol.*, **23**, 2091-2098.

106. Rose J.B. & Sobsey M.D. (1993). – Quantitative risk assessment for viral contamination of shellfish and coastal waters. *J. Food Protec.*, **56**, 1042-1050.

107. Shukla B.N., Singh D.V. & Sanyal S.C. (1995). – Attachment of non-culturable toxigenic *Vibrio cholerae* O1 and non-O1 and *Aeromonas* spp. to the aquatic arthropod *Gerris spinolae* and plants in the River Ganga, Varanasi. *FEMS Immunol. med. Microbiol.*, **12**, 113-120.

108. Smayda T.J. (1989). – Primary production and the global epidemic of phytoplankton blooms in the sea: a linkage? *In* Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms (E.M. Cosper, V.M. Bricelj & E.J. Carpenter, eds). Springer-Verlag, Berlin, 449-484.

109. Spalding B.J. (1995). – Better tests needed to meet goal of safer seafood supply. *Am. Soc. Microbiol.*, **61**, 639-641.

110. Stille W., Kunkel B. & Nerger K. (1972). – Austern hepatitis. *Deutsche Med. Wschr.*, **97**, 145-147.

111. Todd E.C.D. (1993). – Domoic acid and amnesic shellfish poisoning – a review. *J. Food Protec.*, **56**, 69-83.

112. Todd E.C.D. (1996). – Worldwide surveillance of foodborne disease: the need to improve. *J. Food Protec.*, **59**, 82-92.

113. Truman B.I., Madore H.P., Memegus M.A., Nitzkin J.L. & Dolin R. (1987). – Snow Mountain agent gastroenteritis from clams. *Am. J. Epidemiol.*, **126**, 516-525.

114. Veenstra J., Rietra P.J.G.M., Coster J.M., Slaats E. & Dirks-Go S. (1994). – Seasonal variations in the occurrence of *Vibrio vulnificus* along the Dutch coast. *Epidemiol. Infect.*, **112**, 285-290.

115. Verber J.L. (1984). – Shellfish borne outbreaks. United States Public Health Service, Food and Drug Administration, Davisville, 6-14.

116. Volterra L., Tosti E., Vero A. & Izzo G. (1985). – Microbiological pollution of marine sediments in the southern stretch of the Gulf of Naples. *Water Air Soil Poll.*, **26**, 175-184.

117. Wait D.A., Hackney C.R., Carrick R.J., Lovelace G. & Sobsey M.D. (1983). – Enteric bacterial and viral pathogens and indicator bacteria in hard shell clams. *J. Food Protec.*, **46**, 493-496.

118. Weber T., Mintz E.D., Canizares R., Semiglia A., Gomez I., Sempertegui R., Davila A., Greene K.D., Puhr N.D., Cameron D.N., Tenover F.C., Barrett T.J., Bean N.H., Ivey C., Tauxe R.V. & Blake P.A. (1994). – Epidemic cholera in Ecuador: multidrug-resistance and transmission by water and seafood. *Epidemiol. Infect.*, **112**, 1-11.

119. Weingold S.E., Guzewich J.J. & Fudala J.K. (1994). – Use of foodborne disease data for HACCP risk assessment. *J. Food Protec.*, **57**, 820-830.

120. Williams F.P. & Fout G.S. (1992). – Contamination of shellfish by stool-shed viruses: methods of detection. *Environ. Sci. Technol.*, **26**, 689-696.

121. Wilson I.G. & Moore J.E. (1996). – Presence of *Salmonella* spp. and *Campylobacter* spp. in shellfish. *Epidemiol. Infect.*, **116**, 147-153.

122. Wittman R.J. & Flick G.J. (1995). – Microbial contamination of shellfish: prevalence, risk to human health, and control strategies. *Ann. Rev. publ. Hlth*, **16**, 123-140.

123. Wong H.-C., Chen L.-L. & Yu C.-M. (1995). – Occurrence of vibrios in frozen seafoods and survival of psychrotrophic *Vibrio cholerae* in broth and shrimp homogenate at low temperatures. *J. Food Protec.*, **58**, 263-267.

124. World Health Organisation (1996). – Emerging and other communicable diseases (EMC) cholera fact sheet. Fact sheet N107, March, Geneva, 2 pp.

125. World Health Organisation (1996). – Emerging foodborne diseases. Fact sheet N124, Geneva, 2 pp.

126. Wright A.C., Hill R.T., Johnson J.A., Roghman M.-C., Colwell R.R. & Morris G. Jr (1996). – Distribution of *Vibrio vulnificus* in the Chesapeake Bay. *Appl. environ. Microbiol.*, **62**, 717-724.

127. Xu H.-S., Roberts N., Singleton F.L., Attwell R.W., Grimes D.J. & Colwell R.R. (1982). – Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. *Microb. Ecol.*, **8**, 313-323.

128. Yasumoto T., Seino N., Murakami Y. & Murata M. (1987). – Toxins produced by benthic dinoflagellates. *Biol. Bull.*, **172**, 128-131.

129. Yasumoto T. & Satake M. (1996). – Chemistry, etiology and determination methods of ciguatera toxins. *J. Toxicol. Toxin Reviews*, **15**, 91-107.

130. Yasumoto T. & Yotsu-Yamashita M. (1996). – Chemical and etiological studies on tetrodotoxin and its analogs. *J. Toxicol. Toxin Reviews*, **15**, 81-90.