Adverse health outcomes

T.K. Graczyk, K. Suresh and D. Lees

The popularity of molluscan shellfish in the diet is growing because shellfish contribute low-fat proteins, thought to enhance health (Rippey 1994; Munoz 1999; Wallace et al. 1999). However, concerns have been raised worldwide regarding health risks from molluscan shellfish contaminated with human pathogens of anthropogenic and agricultural origin (Feldhusen 1990; Todd et al. 1992; Potasman et al. 2002; Table 3.1). Most of the reports of outbreaks of infection have come from the United States, although there are some reports from Europe, Australia and Asia. Since the late 1800s when shellfish-related illnesses were first reported in the United States, there have been over 400 epidemics of foodborne diseases and over 14 000 gastroenteritis cases related to consumption of contaminated molluscan shellfish (Rippey 1994). In New York, USA alone from 1980 to 1994, over 85% of Norwalk-like virus (NLV) outbreaks, and all foodborne outbreaks of Vibrio spp. and Plesiomonas shigelloides, were associated with seafood consumption (Wallace et al. 1999). Molluscan shellfish accounted for 64% of all foodborne epidemics in which the
etologic agent was identified \((n = 204)\), and for 41% of outbreaks caused by an unknown etologic agent \((n = 12)\) (Wallace et al., 1999). In the mid-1990s, over 98% of the incidence reports and 99% of the case reports of \textit{Vibrio} spp. associated gastroenteritis and primary septicaemia were reported as due to consumption of raw oysters (Rippey 1994). Regarding non-\textit{Vibrio} spp. associated diarrhoeal diseases, oysters and hard shell clams were identified in more than 56% and 38% of foodborne outbreaks and in 54% and 44% of foodborne disease cases respectively (Rippey 1994).

Molluscan shellfish are well-recognized vectors of human enteropathogens and marine biotoxins. Oysters are more likely than other seafood items to contain infective microorganisms because they concentrate pathogens from surrounding waters and are very often eaten raw (Rippey 1994; Wallace et al. 1999). In the United States, 8% of approximately 33 million foodborne illnesses annually have been linked to the consumption of raw oysters (Altekruse et al. 1999). Clams, mussels, cockles and scallops are less of a public health concern because they are usually consumed cooked or steamed, which significantly alters the infectivity of potential pathogens (Rippey 1994).

Foodborne illnesses related to consumption of molluscan shellfish have been classified into three categories based on the origin of the etologic agent:

- human enteropathogens associated with raw sewage disposal, wastewater effluents, and overboard disposal of faeces;
- infectious agents or marine biotoxins indigenous to coastal waters, such as autochthonous bacteria, \textit{Vibrio} spp.; and
- post-harvest contamination (Rippey 1994).

### 3.1 BACTERIAL AND VIRAL GASTROENTERITIS RELATED TO WASTEWATER AND SEWAGE DISPOSAL

In the mid 1990s in the United States, over 75% of gastroenteritis outbreaks and over 79% of gastroenteritis cases associated with the consumption of shellfish contaminated by sewage or wastewater-derived pathogens were due to an unknown etologic agent (Rippey 1994). Gastroenteritis of unknown etiology occur more frequently in late spring and late fall, roughly coinciding with periods of the most intense shellfish feeding and associated pathogen bio-accumulation (Rippey 1994). It is believed that approximately 50% of foodborne outbreaks of unknown etiology related to the consumption of raw
Table 3.1 Reports of *Cryptosporidium* spp. in mollusc shellfish intended for human consumption (chronological order)

<table>
<thead>
<tr>
<th>Shellfish species and reference</th>
<th>Geographic location</th>
<th>Identification level; detection techniques</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crassostrea virginica</em> (Fayer et al. 1998)</td>
<td>Choptank, Severn, Miles, Wye, Potomac, and Wicomico Rivers; Chesapeake Bay, USA.</td>
<td><em>Cryptosporidium parvum</em>; IFA, mouse bioassay.</td>
<td>Infectious oocysts in hemolymph and gills, most infected oysters at a site near a large cattle farm.</td>
</tr>
<tr>
<td><em>C. virginica</em> (Fayer et al. 1999)</td>
<td>Fishing Bay, Tangier Sound, and Wicomico, Nanticoke, Potomac, and Patuxent Rivers; Chesapeake Bay, USA.</td>
<td><em>C. parvum</em> Genotype 1 and 2; IFA, mouse bioassay, PCR, PCR-RFLP.</td>
<td>Oocysts detected in oysters and water, infectious oocysts in hemolymph and gills.</td>
</tr>
<tr>
<td><em>Dosinia exoleta</em>, <em>Venerupis pullastra</em>, <em>Venus rhomboideus</em>, <em>Venus verrucosa</em>, <em>Mytilus galloprovincialis</em>, <em>Ostrea edulis</em>, <em>Ruditapes philippinarum</em> (Freire-Santos et al. 2000).</td>
<td>Galicia, Spain; Italy; England.</td>
<td><em>Cryptosporidium</em> spp.; malachite green, safranine, methylene blue, carbol-fuchsin, auramine-rhodamine, IFA.</td>
<td>Depuration ineffective for <em>Cryptosporidium</em> spp. positive relationships between faecal coliforms and <em>Cryptosporidium</em> spp. in shellfish.</td>
</tr>
<tr>
<td><em>Mytilus edulis</em> (Lowery et al. 2001)</td>
<td>Shores of Belfast Lough, Ireland.</td>
<td><em>C. parvum</em> Genotype 1; IMS-IFA, PCR, PCR-RFLP.</td>
<td>Anthropogenic source(s) of contamination.</td>
</tr>
<tr>
<td><em>C. virginica</em> (Fayer et al. 2002)</td>
<td>Chesapeake Bay, USA.</td>
<td><em>Cryptosporidium</em> sp.; IFA</td>
<td>Oyster contamination coincided with rainfalls and increased stream-flow.</td>
</tr>
<tr>
<td><em>C. virginica</em> (Fayer et al. 2003)</td>
<td>Atlantic coast from Maine to Florida, USA.</td>
<td><em>C. parvum</em>, <em>C. hominis</em>; <em>C. meleagridis</em>, IFA, PCR, genotyping, mouse bioassay.</td>
<td>65% commercial harvesting sites contaminated with <em>Cryptosporidium</em> spp.</td>
</tr>
<tr>
<td><em>C. virginica</em> (Gomez-Couso et al. 2004)</td>
<td>United Kingdom.</td>
<td><em>C. parvum</em>, <em>C. hominis</em>; multiplexed nested PCR.</td>
<td><em>C. parvum</em> and <em>C. hominis</em> detected in 11% of sampled bivalves.</td>
</tr>
</tbody>
</table>

IFA, immunofluorescent antibodies; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restricted fragment length polymorphism.

Adverse health outcomes
oysters, are due to viral agents (Wilson and Moore 1996). In over 93% of molluscan shellfish-associated outbreaks, the shellfish were probably contaminated at the sites from which they were harvested as opposed to post-harvest contamination (Wallace et al. 1999).

3.1.1 Viral gastroenteritis

The predominant viral agents implicated in molluscan shellfish outbreaks include the diverse group of NLVs (noroviruses; caliciviruses) and hepatitis A (HAV) (Rippey 1994; Munoz 1999; Wallace et al. 1999). Outbreaks of HAV have been reported consistently from around the world since the early 1960s (see for example, Rippey 1994; Wallace et al. 1999; Furuta et al. 2003). However, in the past two decades NLVs have been the predominant cause of viral gastroenteritis (see for example Huppatz et al. 2008). The principal presentation of NLV infection is an acute onset of vomiting or diarrhoea, or both. The illness is generally mild and self-limiting, with symptoms lasting 12 to 48 hours. In seven separate outbreaks of NLV gastroenteritis, the median incubation period was 31 hours (range: 2 to 69 hours) and the median duration of illness 48 hours (range: 10 hours to 7 days) (Anonymous 1993; 1996). The attack rate was 91% among people who consumed more than five dozen oysters, and 46% among those who consumed less than one dozen (Anonymous 1993). A study in the United States showed that the attack rate in multi-state outbreaks due to consumption of raw or steamed oysters contaminated with NLV ranged from 43% to 100% (Anonymous 1994). The attack rates of NLV gastroenteritis due to overboard disposal of faeces into the oyster bed, with subsequent harvesting and distribution of contaminated oysters, varied from 58% to 83% (Kohn et al. 1995; McDonnell et al. 1997).

3.1.2 Bacterial gastroenteritis

In general, the bacterial agents of shellfish-vectored illnesses represent a small portion of all outbreaks and cases, 4.0% and 3.8%, respectively (Rippey 1994). Identified bacteria listed include the causative agent of typhoid fever (Salmonella spp., such as S. typhi), Shigella spp., Campylobacter spp., Plesiomonas shigelloides, Aeromonas hydrophila, and Escherichia coli (Rippey 1994; Weber et al. 1994; Munoz 1999; Potasman et al. 2002). Historically, typhoid fever was a significant public health problem among consumers of raw oysters (Rippey 1999). After the deaths of several citizens in New York, USA in the early 1900s following the consumption of contaminated oysters,
outbreaks due to *S. typhi* were successfully eliminated by:

- implementation of more effective sewage treatment;
- reconstruction of storm and sewerage systems; and
- institution of national surveillance systems for infectious disease outbreaks (Rippey 1999).

The last case of oyster-related typhoid fever reported in the United States was in 1954 (Rippey 1999).

### 3.2 SHELLFISH-VECTORED ILLNESSES RELATED TO AUTOCHTHONOUS BACTERIA

Several *Vibrio* spp. have been identified as the causative agents of molluscan shellfish-related diseases with the severity of the disease depending on the contracted species of *Vibrio* (Blake 1983; Rippey 1994; Weber *et al.* 1994; Shapiro *et al.* 1998). These include: *V. parahaemolyticus, V. vulnificus, V. cholerae O1* and non-O1 serotypes, *V. fluvialis, V. hollisae, V. mimicus, V. damsella, V. metschnikovii, V. furnissi,* and *V. alginolyticus* (Blake 1983; Rippey 1994; Weber *et al.* 1994; Shapiro *et al.* 1998). *Listeria* spp. have also been detected in frozen and fresh shellfish and in coastal waters (Todd *et al.* 1992; Richards 2003).

#### 3.2.1 *Vibrio* spp. infections

In general, gastroenteritis associated with *Vibrio* spp. is much more severe than diarrhetic diseases of viral etiology (Rippey 1994). *V. vulnificus* can cause infections resulting in fulminant primary septicaemia (often with necrotizing cutaneous lesions) associated with a high mortality rate that can reach up to 61% (Hlady *et al.* 1993; Rippey 1994; Weber *et al.* 1994; Wallace *et al.* 1999; Table 3.2). Beside diarrhoeal disease, *V. vulnificus, V. fluvialis, V. hollisae, V. mimicus,* and *V. parahaemolyticus* can cause extra-intestinal infections (cholecystis) and wound and ear infections (Blake, 1983; Shapiro *et al.* 1998, Table 3.2). The population at risk for *V. vulnificus* septicaemia include people with pre-existing conditions such as:

- liver diseases due to cirrhosis, hepatitis, or alcohol overuse;
- renal disease; certain medical conditions (e.g., diabetes, hemochromatosis, leukaemia and anaemia); and
The case–fatality ranges from 50% to 63% among this group (Hlady et al. 1993; Rippey 1994; Weber et al. 1994; Wallace et al. 1999); this population is at 80 times greater risk of illness and over 200 times greater risk of death (Hlady et al. 1993). Morbidity and mortality due to V. cholerae O1 and non-O1 serotypes have been sporadically reported among shellfish consumers in the United States (Rippey 1994; Weber et al. 1994). In one outbreak, raw oysters were associated with eight cases of V. cholerae O1 (Weber et al. 1994). All implicated oyster lots had been harvested from the Gulf Coast waters and shipped interstate (Weber et al. 1994). V. cholerae non-O1 infections have been associated with mortality, although this serotype does not cause such severe gastroenteritis as O1 serotype (Rippey 1994; Weber et al. 1994). Interestingly, the use of antacids predisposes a person to foodborne Vibrio spp. infections by neutralizing the protective gastric acid barrier (Munoz 1999).

### 3.2.2 Seasonal pattern and distribution of Vibrio spp. infections

Case reports of V. vulnificus due to consumption of raw oysters show a seasonal pattern with the highest frequencies from midsummer through to late autumn (Blake 1983; Rippey 1994; Shapiro et al. 1998). This bacterium has been identified in oysters at the highest densities when the water temperature exceeds 15°C (Rippey 1994; Anonymous 1996; Shapiro et al. 1998; Wallace et al. 1999). V. vulnificus can occur in oysters legally harvested from open oyster beds and properly handled prior to their consumption in a raw form (Shapiro et al. 1998).
3.3 POST-HARVEST CONTAMINATION OF SHELLFISH BY BACTERIA

The bacteria involved in this type of contamination, *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens*, are derived from equipment, utensils and premises used for processing of molluscan shellfish, and from food handlers (Todd *et al.* 1992; Rippey 1994).

3.4 HUMAN WATERBORNE PARASITES AND MOLLUSCAN SHELLFISH

*Cryptosporidium parvum*, *Giardia lamblia*, *Cyclospora cayetanensis* and *Toxoplasma gondii* are human protozoan enteropathogens in which transmission is associated with water (Wolfe 1992; Ortega *et al.* 1993; Graczyk *et al.* 1997; Lindsay *et al.* 2001). *C. parvum*, *G. lamblia*, and *C. cayetanensis* infections cause gastroenteritis, which is predominantly manifested by diarrhoea (Wolfe 1992; Ortega *et al.* 1993; Graczyk *et al.* 1997d). *T. gondii* causes serious congenital complications in foetuses born to mothers infected for the first time during pregnancy. Medically, the most important is *Cryptosporidium* spp. as it significantly contributes to the mortality of people with impaired immune systems (Graczyk *et al.* 1997). Although *G. lamblia* (syn. *G. intestinalis*, *G. duodenalis*) and *C. cayetanensis* cause serious prolonged diarrheal illness in adults and children worldwide, the infections usually respond well to pharmacologic treatment (Wolfe 1992; Ortega *et al.* 1993). *C. parvum*, *G. lamblia* and *T. gondii* are anthropozoonotic pathogens (Wolfe 1992; Graczyk *et al.* 1997; Lindsay *et al.* 2001). All of these parasites produce a long-lasting and environmentally resistant infectious stage – *Cryptosporidium* spp. *Cyclospora* spp. and *Toxoplasma* spp. produce oocysts and *Giardia* spp. cysts, which can be transmitted via water. *Cryptosporidium* spp. oocysts pollute coastal waters via point and diffuse sources of contamination, such as wastewater discharges, leaky septic tanks, urban runoff, recreational activities, and agricultural runoff predominantly from livestock operations, namely cattle farms (Graczyk *et al.* 2000a; 2000b). Clinical infections are mainly confined to calves, which can shed up to $10^6$ oocysts per gram of their faeces, and exceed $10^9$ oocysts in daily output (Anderson 1981). As many as $10^6$ oocysts per ml can be found in human diarrhetic faeces (Rose 1997). The infectious dose of *C. parvum* for immunosuppressed people has not been established, but it is believed that the disease can be caused by a single oocyst (Rose 1997). Mortality rates due to *C. parvum* among these individuals vary from 52% to 68% (Rose 1997).
In addition to Cryptosporidium spp., Giardia spp., Toxoplasma spp. and Cyclospora spp., human-infectious microsporidia such as Encephalitozoon intestinalis, E. hellem and Enterocytozoon bieneusi are emerging anthropozoonotic pathogens that inflict considerable morbidity on healthy people and can be associated with mortality in immunosuppressed populations (Bryan and Schwartz 1999). The transmissive stages of all these parasites, oocysts, cysts and spores respectively, are resistant to environmental stressors and are therefore relatively widespread in the environment (Wolfe 1992; Rose et al. 1997; Kucerova-Pospisilova et al. 1999). Cryptosporidium spp. and Giardia spp. are very frequently transmitted via water (Wolfe 1992; Rose et al. 1997). Considerable evidence gathered to date also indicates the involvement of water in the epidemiology of microsporidia (Sparfel et al. 1997; Dowd et al. 1998; Cotte et al. 1999; Fournier et al. 2000).

Molluscan shellfish are suspension- or sediment-feeding organisms, which filter unicellular algae, bacteria, other microorganisms and detritus in approximately the 1–30 µm particle size range (McMahon 1991; Kennedy et al. 1996). The diameter of Cryptosporidium spp., Cyclospora spp. and Toxoplasma spp. oocysts does not exceed 6, 8 and 10 µm, respectively, while Giardia spp. cysts are oval and no longer than 15 µm (Wolfe 1992; Ortega et al. 1993; Graczyk et al. 1997; Lindsay et al. 2001). Microsporidian spores range from 1.5 to 4 µm (Graczyk et al. 2004). Thus, cystic stages of these parasites fall within the range of particles filtered by bivalve molluscs.

 Historically, C. parvum oocysts of waterborne origin were first identified in the tissue of blue mussels in Ireland (Chalmers et al. 1997), initiating worldwide investigation of this pathogen in molluscan shellfish (Graczyk 2003a; 2003b). Since then, multiple studies have demonstrated that these filter-feeding organisms can harbour environmentally-derived protozoan parasites as a result of concentrating the recovered particles (Graczyk 2003a; 2003b).

An interesting epidemiological discovery is the identification, for the first time, of human-infectious microsporidia spores, E. intestinalis and E. bieneusi, in a molluscan shellfish, the zebra mussel (Dreissena polymorpha) (Graczyk et al. 2004). Microsporidia infect a variety of vertebrate and invertebrate hosts, and approximately 14 species have been reported to infect people (Kotler and Orenstein 1999). Of these E. intestinalis and E. bieneusi have been recorded as zoonotic, involved in the infection of domestic animals and livestock (Deplazes et al. 1996; Bornay-Llinares et al. 1998; Breitenmoser et al. 1999; Rinder et al. 2000; Buckholt et al. 2002; Graczyk et al. 2004). In humans they cause serious gastroenteritis, as well as urinary and sometimes ocular infections (Graczyk et al. 2004). Although the actual transmission route of this specific spore species is not known, it is quite possible that infectious spores of human or animal origin
passed to the aquatic environments via faeces or urine (Bryan and Schwartz 1999). Spores of microsporidia have been detected in a variety of surface waters (Avery and Undeen 1987), which becomes a source of human infections (Cotte et al. 1999). In addition, spores of *E. intestinalis* and *E. bieneusi* have been detected previously in surface waters (Sparfel et al. 1997; Dowd et al. 1998).

### 3.4.1 The public health threat from molluscan shellfish contaminated with *Cryptosporidium* spp.

Prior to 1992, the association between contamination derived from animal faecal wastes and the occurrence of shellfish-vectored illnesses was inconclusive (Stelma and McCabe 1992). In 1994, enterohemorrhagic *Escherichia coli* O157 became a major concern (Rippey 1994). Beginning in 1998, multiple studies worldwide indicated that molluscan shellfish intended for human consumption can be contaminated with *Cryptosporidium* spp. (Table 3.1). So far there has been no reported outbreak (or case) of foodborne cryptosporidiosis linked to consumption of raw oysters (Potasman et al. 2002); however,

- a large proportion of foodborne infections linked to oyster consumption are in the category of an unknown etiologic agent (Anonymous 1996);
- epidemiology of enteric infections (cryptosporidiosis) indicates an association with consumption of raw shellfish; and
- it is believed that the true incidence of shellfish-vectored gastroenteritis is considerably underestimated (Potasman et al. 2002).
- there is no mandatory requirement for reporting of gastroenteritis of an unspecified nature and physicians and health departments may not forward case reports to authorities (Rippey 1994; Wallace et al. 1999).

### 3.4.2 Methods used for identification of human protozoan parasites in molluscan shellfish

Methods for identification of human protozoan parasites in the tissue of molluscan shellfish include:

- immunofluorescent antibodies (IFA) alone or in combination with immunomagnetic separation (IMS);
- polymerase chain reaction (PCR) alone or combined with Restricted Fragment Length Polymorphism (RFLP) for genotyping;
- multiplexed nested PCR;
- fluorescent *in situ* hybridization (FISH).
Infectivity of the parasites recovered from the shellfish is usually assessed by mouse bioassays (Graczyk 2003a; 2003b).

Because *Cryptosporidium* spp., *Giardia* spp. and microsporidia can infect a variety of non-human hosts (Wolfe 1992; Graczyk et al. 1997; Kotler and Orenstein 1999), identification of human–infectious species is a challenge. Another challenge is the determination of the viability of these environmentally recovered pathogens, as they may be non-viable and thus have no epidemiological significance. Although molecular methods are very sensitive and specific they do not assess infectivity of the pathogens recovered from shellfish. Both challenges are met by the FISH technique. FISH employs fluorescently labeled oligonucleotide probes targeted to species-specific sequences of 18S rRNA, and therefore identification of pathogens is species-specific (Graczyk et al. 2004). Also, as rRNA has a short half-life and is only present in numerous copies in viable organisms, FISH allows for differentiation between viable and non-viable pathogens (Jenkins et al. 2003; Graczyk et al. 2004). FISH has been combined with direct IFA against the wall antigens of *Cryptosporidium* spp. and *Giardia* spp. and this approach has been successful for detection of *C. parvum* and *G. lamblia* in shellfish samples (Graczyk et al. 2004). For identification of viable pathogens such as *C. parvum*, *G. lamblia* or human-infective microsporidia, FISH is advantageous over other techniques including PCR because it allows simultaneous species-specific identification, visualization and viability assessment of single pathogen cells. Such resolution is either unavailable, or extremely impractical with any other technique. For example, using recently developed highly sensitive RT-PCR, the lowest number of *C. parvum* oocysts which can be assessed for viability is $10^3$ (Jenkins et al. 2000).

### 3.5 BIAS IN REPORTING OF MOLLUSCAN SHELLFISH-VECTORED ILLNESSES

The data reported in medical literature most likely represents only a small portion of actual gastroenteritis cases, as the true incidence of shellfish-vectored illnesses is believed to be considerably underestimated (Hauschild and Bryan 1980; Mead et al. 1999). There are several reasons for such under-reporting, including:

- a lack of mandatory requirements for reporting of gastroenteritis of an unspecified nature because gastroenteritis is not a reportable illness (Rippey 1994; Wallace et al. 1999);
- many cases of gastroenteritis are mild and self-limiting and hence do not require treatment by a physician (Rippey 1994; Wallace et al. 1999);
not all outbreaks are recognized or reported and sporadic cases of foodborne illnesses are not detected by the existing foodborne disease surveillance system (Wallace et al. 1999);

it is difficult to epidemiologically ascribe a diarrhetic disease outbreak to a specific food item, particularly when small numbers of people are showing symptoms (Rippey 1994; Archer and Kvenberg 1995; Wallace et al. 1999);

for some infectious agents, symptoms may not become apparent immediately, but instead appear long after the implicated food items have been consumed or discarded (Rippey 1994; Wallace et al. 1999); and

the accuracy of the tagging system is not perfect (Rippey 1994).

3.6 METHODS OF SHELLFISH SANITIZATION

Molluscan shellfish destined for human consumption can be subjected to processing such as cooking/heating (pasteurization), relaying, depuration, irradiation, ozonation and high hydrostatic pressure in order to remove or inactivate potential microbiological contaminants. These methods have been applied predominantly to purge or inactivate bacterial and viral agents (Richards 2003), and the published information on their efficiency for protozoan parasites is limited. Gomez-Couso et al. (2003a) demonstrated that depuration was ineffective in removing C. parvum oocysts from mussels, oysters, clams and cockles harvested from contaminated waters. Gomez-Couso et al. (2003b) also demonstrated that molluscan shellfish contaminated with C. parvum oocysts can spread this contamination within the commercial depuration plants to other aquatic organisms processed in such facilities.

3.7 WHY ARE ILLNESSES CAUSED BY SHELLFISH CONSUMPTION NOT ANTICIPATED TO DECLINE IN THE FUTURE?

There are several reasons why shellfish-vectored outbreaks and related cases of gastroenteritis are not projected to decline.

- The faecal coliform count, which is the main standard indicator for waterborne faecal contamination, is not reliable in determining the quality of water at shellfish harvesting sites (Rippey 1994; Anonymous 1996; Wilson and Moore 1996). The transmissive stages of enteropathogens can persist in aquatic environments for greater lengths of time than the
enteric indicator bacteria (Graczyk and Schwab 2000; Richards 2003). Thus, waters considered to be “safe” based on the faecal coliform standards can be contaminated by enteropathogens of anthropogenic or human origin (Rippey 1994; Anonymous 1996; Wallace et al. 1999; Graczyk and Schwab 2000). Monitoring techniques are discussed in more detail in chapter 7 of this publication.

- Animal operations such as individual farms, industrial animal production facilities, or concentrated animal feeding operations located near shores can generate enormous surface runoff, particularly under adverse weather conditions, and can cause water pollution (Freire-Santos et al. 2000; Gomez-Bautista et al. 2000).
- Deficiencies at sewage treatment plants such as volume limitations related to designed capacity of a plant under adverse weather conditions (e.g., heavy rainfall), allow the discharge of large amounts of unprocessed waste waters. In addition, the periodic breakdown in particle removal, or inadequate disinfection can deliver human enteropathogens into shellfish-harvested waters (Rippey 1994).
- Transmissive stages of human enteropathogens are resistant to environmental degradation (including heat, sunlight, temperature fluctuations) and may even remain infectious after exposure to chemical water treatment processes such as chlorination (McDonnell et al. 1997; Graczyk and Schwab 2000). These pathogens can still be infectious even after the oyster meat has been processed (McDonnell et al. 1997; Graczyk and Schwab 2000) and are also only slowly depurated (removed) from molluscan shellfish tissue (Graczyk and Schwab 2000).
- Increased faecal pollution determined by the faecal coliform counts has decreased the total area of coastal habitats approved for harvesting of molluscan shellfish (particularly oysters) for human consumption in some areas. Thus, there is evidence that large and very productive areas have been closed, resulting in illegal harvesting of oysters from unapproved or closed, but profitable waters (Rippey 1994). Such criminal activity unavoidably affects public health when contaminated shellfish enter the market (Rippey 1994).
- Improper post-harvest handling and transportation of molluscan shellfish (such as inappropriate temperature control) affects oysters directed for consumption in a raw form (Rippey 1994). Holding of oysters at temperatures greater than 4°C in transit or in the market place can contribute to multiplication of bacterial enteropathogens (Rippey 1994).
- Many shellfish-related outbreaks have more than one contributing factor (Wallace et al. 1999). For example, contaminated ingredients added to
raw or lightly cooked molluscs have also been reported as contributing factors for foodborne infections (Wallace et al. 1999). Development of new molecular techniques which can be applied to a wide variety of food items has dramatically increased the sensitivity and specificity of detection of human enteric parasites in the tissue of molluscan shellfish (see citations in Table 3.1).

3.8 CONCLUSIONS

Molluscan shellfish can efficiently filter, retain and accumulate in their tissues environmentally derived human enteropathogens without affecting their infectivity. Therefore, such shellfish can cause human foodborne illnesses if consumed raw or after inadequate processing. Information derived from epidemiologic investigations and surveillance systems indicates an upward trend in foodborne illnesses in some areas linked with consumption of molluscan shellfish.

Worldwide, the majority of outbreaks have been linked to oysters followed by clams and mussels, and most of the reports originate from the United States, followed by Europe, Asia and Australia (Potasman et al. 2003). HAV virus caused the largest shellfish associated outbreak, but NLV caliciviruses have caused the highest number of outbreaks (Potasman et al. 2003). Vibrio species are the most common bacterial pathogens in shellfish. Foodborne illnesses following consumption of molluscan shellfish continue to occur throughout the world despite the fact that:

1. testing of waters for faecal coliforms from which oysters are harvested for human consumption often demonstrates that the water quality meets the criteria of national standards or guidelines;
2. oysters harvested from such waters are considered as “safe” with regards to faecal pollution;
3. sanitation procedures at oyster-harvesting facilities meet national or local standards; and
4. in most instances, neither confirmed evidence of improper handling or processing of outbreak-implicated oysters nor the environmental source(s) of pollution are detected.

These facts indicate that the monitoring of water for faecal coliforms at molluscan shellfish-harvesting sites may not be sufficient for indicating the presence of human enteropathogens of anthropogenic or agricultural origin.

Reducing the number of outbreaks and cases of foodborne diseases due to bivalve molluscs will require the coordinated efforts of different agencies with
responsibility for water quality assessment, shellfish harvesting and processing, disease surveillance and consumer education (Rippey 1994; Anonymous 1996; Wallace et al. 1999). It may be useful to reduce or eliminate economic incentives for illegal harvesting of shellfish from unapproved or prohibited waters that result in contaminated shellfish reaching the marketplace (Rippey 1994).

Prevention of foodborne gastroenteritis caused by consumption of molluscan shellfish requires consumer education to ensure thorough cooking of shellfish (Rippey 1994; Wallace et al. 1999; Freire-Santos et al. 2000; Graczyk and Schwab 2000). Education should be particularly focused on populations that are predisposed to serious illness after consumption of contaminated shellfish, including people with pre-existing liver or kidney diseases, diabetes and suppressed immune systems (Rippey 1994; Wallace et al. 1999). Several factors may impede consumer education campaigns about the risk of raw shellfish consumption (Altekruse et al. 1999). For example, point-of-sale warning signs may not reach vulnerable populations (Altekruse et al. 1999). This occurred in New York, USA where victims of a foodborne outbreak of *V. vulnificus* infection who suffered from liver diseases, did not know that they should avoid consumption of raw oysters (Wallace et al. 1999).

It may be useful to issue guidelines for sanitization of molluscan shellfish to reduce pathogen counts in molluscan shellfish or inactivate potential pathogens, via cold shock, heat shock, pasteurization, relaying, depuration or irradiation (Altekruse et al. 1999).

Continued surveillance for outbreaks and cases of gastroenteritis associated with consumption of raw shellfish are needed to assess the efficacy of current practices designed to prevent human illnesses. Public health officials should consider consumption of raw shellfish as a possible source of infection during the evaluation of a gastroenteritis outbreak (Rippey 1994; Wallace et al. 1999; Graczyk and Schwab 2000).

### 3.9 REFERENCES


