SPECIAL ARTICLE

The human pathogenic vibrios – A public health update with environmental perspectives

INTRODUCTION

Members of the genus *Vibrio* are widespread in many natural aquatic environments often forming a major component of microbial populations associated with recycling of organic compounds such as chitin (Baumann & Baumann, 1981). A few species are economically important pathogens of fish and shellfish (Colwell & Grimes, 1984; Cameron *et al.* 1988). Most of the species are found in estuarine and marine environments. Some organisms are more commonly established ecologically in freshwaters but can be transported to saline environments by water flow.

Evidence from epidemiological and ecological studies in the last 20 years has firmly established that some environmental strains of *Vibrio* sp. are also etiologic agents of a wide range of diarrhoeal and systemic disease in man (Table 1; Janda *et al.* 1988). So recent has been this recognition that many of the pathogenic vibrios have only been characterized within the last decade. Perhaps the most provocative outcome has been recognition that the etiologic agent of cholera, *Vibrio cholerae*, is commonly found as a natural resident of aquatic environments in cholera-free areas and that its presence is not necessarily associated with faecal contamination or sporadic human infections (Rogers *et al.* 1980; Feacham, Miller & Drasar, 1981). The traditional concepts that cholera is a tropical disease most commonly associated with developing countries and that *V. cholerae* has limited potential for survival outside the human intestine, are being radically revised as the ecology of the etiologic agent is investigated in cholera endemic regions (Glass *et al.* 1983) as well as areas free from the influence of endemic human infections (Anon, 1980; Levine, 1980; Miller, Feacham & Drasar, 1985).

Most research and information on the human pathogenic vibrios has, for several years, involved *V. cholerae* due to the severity of infection and to the emotive reaction this organism evokes with medical authorities and the public. Emergence of fulminating systemic infections associated with other *Vibrio* species in the environment, such as *Vibrio vulnificus*, is increasingly attracting more attention (Morris, 1988). These infections can range from self-limiting gastroenteritis and wound infections to severe necrotizing infections of soft tissues and fatal septicaemia in patients with underlying debilitation (Armstrong, Lake & Miller, 1983). In virtually every instance, infection can be associated with consumption of seafood or contact with natural aquatic environments (Bonner *et al.* 1983; Janda *et al.* 1988).

The occurrence of infections with pathogenic vibrios in the United Kingdom is relatively infrequent compared with other developed countries such as the United

States. Most cases in the United Kingdom arise after the victim has returned having acquired the infection abroad.

However, some cases may arise in the European Community but pass unreported possibly due to lack of awareness for these pathogens. Accordingly, the spectrum of recently reported infections associated with human pathogenic vibrios is reviewed here along with a brief update on aspects of pathogenicity. Infection by human pathogenic vibrios is usually inadvertent and results through a complex series of ecological interactions before contact by the victim with aquatic environments, either by exposure or by consumption of seafood. The underlying environmental conditions which may lead to initiation of infection with human pathogenic vibrios are described in more detail.

TAXONOMIC DETAILS

Vibrios are members of the genus *Vibrio* containing Gram-negative, rod-shaped bacteria which utilize glucose fermentatively so distinguishing them from the Pseudomonadaceae whose members do not ferment glucose. Most *Vibrio* species produce cytochrome oxidase unlike the fermentative Gram-negative bacteria of the family Enterobacteriaceae (Tison & Kelly, 1984a).

Significant changes in nomenclature have recently occurred within the genus *Vibrio*. Assignment of several pathogenic *Vibrio* species to the genus *Beneckea* is particularly important due to the resulting confusion. Most criteria distinguishing these genera are now considered inappropriate so the genus *Beneckea* has been abolished and most of its members reallocated in the genus *Vibrio*. Thus, the species *Beneckea parahaemolytica* named in earlier publications is considered identical to *Vibrio parahaemolyticus* and so on (West & Colwell, 1984). The application of molecular taxonomic techniques may result in reassignment of some *Vibrio* species to other newly-named genera (MacDonnell & Colwell, 1985; Nearhos & Fuerst, 1987).

The genus *Vibrio* presently contains eleven species pathogenic for man (Table 1). Those of prime medical concern are *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. Other organisms implicated as opportunistic pathogens are *V. alginolyticus*, *V. damsela*, *V. fluvialis*, *V. furnissii*, *V. hollisae*, *V. mimicus*, *V. metschnikovii* and *V. cincinnatiensis* (Morris & Black, 1985; Brayton et al. 1986).

Discussions of taxonomic criteria for speciation within the genus *Vibrio* such as nucleic acid homology and methods to establish phenotypic traits are beyond the scope of this article. These have been reviewed by Sakazaki & Balows (1981), Tison & Kelly (1984a), West & Colwell (1984), Farmer, Hickman-Brenner & Kelly (1985); West et al. (1986), and Bryant et al. (1986). Caution is needed when using commercial Gram-negative identification systems for identifying pathogenic *Vibrio* species as some brands yield inaccurate results (Overman et al. 1985).

Of particular importance is the classification of *V. cholerae*. Serological and taxonomical studies have shown that this species contains over 70 serotypes, based on somatic (0) antigen, with the etiologic agent of cholera designated as serotype 01 (Sakazaki & Donovan, 1984). Two biovars, named classical and El Tor, exist within the 01 serotype of *V. cholerae* and are differentiated by their sensitivity to polymyxin, some vibriophages and by ability to agglutinate chicken

Table 1 *Pathogenic* Vibrio *species associated with human infections*

Species	Gastrointestinal tract	Wound	Ear	Primary septicaemia	Bacteremia	Lung	Meninges
V. cholerae 01	+ +	(+)	*	*	*	*	*
V. cholerae non-01	+ +	+	+	(+)	(+)	*	(+)
V. parahaemolyticus	+ +	+	(+)	*	(+)	(+)	(+)
V. vulnificus	+	+ +	*	+ +	+	(+)	(+)
V. fluvialis	+ +	*	*	*	*	*	*
V. alginolyticus	*	+ +	+	*	(+)	*	*
V. damesela	*	+ +	*	*	*	*	*
V. furnissii	(+)	*	*	*	*	*	*
V. hollisae	+ +	*	*	(+)	*	*	*
V. mimicus	+ +	+	+	*	*	*	*
V. metschnikovii	(+)	*	*	(+)	*	*	*
V. cincinnatiensis	*	*	*	*	(+)	*	(+)

+ +, most common site of infection; +, other sites of infection; (+), rare sites of infection; *, infection remains to be firmly established.

erythrocytes (Farmer, Hickman-Brenner & Kelly, 1985). The El Tor biovar is currently predominant throughout the globe (Barrett & Blake, 1981).

The so-called 'NAG' (non-agglutinable) and 'NCV' (non-cholera vibrios) strains in historic publications and which biochemically resemble *V. cholerae* serotype 01, have been included in the species definition and are collectively known as non-01 *V. cholerae* serotypes (West & Colwell, 1984).

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON SURVIVAL OF PATHOGENIC VIBRIOS

Since human pathogenic vibrios are naturally-occurring in aquatic environments of areas free from endemic disease, the microbial ecology of these pathogens becomes important because this significantly dictates the occurrence and epidemiology of human infections. Review of the major ecological studies involving *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* indicates that, in general terms, water temperature, concentration of organic material in the water, salinity, and the potential for association with sediments or the surfaces of higher organisms, significantly influence the occurrence and number of these pathogens in aquatic environments. Studies on the other pathogenic vibrios are however often limited to infrequent isolations from the aquatic environment or their implication in diseases which arise after contact with freshwater or saline aquatic environments.

Water temperature

Water temperature appears to be the single most important factor governing the incidence and density of pathogenic vibrios in natural aquatic environments. Pathogenic vibrios are found more frequently in environments whose water temperature exceeds 10 °C for at least several consecutive weeks (Bockemuhl *et al.* 1986; Rhodes, Smith & Ogg, 1986; Chan *et al.* 1989). In some regions this threshold temperature may be higher (De Paola *et al.* 1983; Clarke & Doyle, 1985).

Seasonal and geographical variations, dependent on water temperature, of bacterial counts in waters and sediment have been reported and reviewed for *V. cholerae* (Roberts *et al.* 1982; West & Lee, 1982; Nair *et al.* 1988; Perez-Rosas & Hazen, 1989), *V. parahaemolyticus* (Ayres & Barrow, 1978; Kaneko & Colwell, 1978; Watkins & Cabelli 1985; Kelly & Dan Stroh 1988*b*) *V. fluvialis* (Barbay, Bradford & Roberts, 1984), and *V. vulnificus* (Kelly, 1982; Tamplin *et al.* 1982; Oliver, Warner & Cleland, 1983). Pathogenic vibrios are less frequently isolated from natural aquatic environments when water temperatures exceed 30 °C (Seidler & Evans, 1984; Williams & La Rock, 1985).

Sediments

  Most pathogenic vibrios rapidly disappear from the water column at temperatures below 10 °C but can persist in the environment in sediments. Under more favourable environmental conditions the vibrios can proliferate and re-emerge in the water (Williams & La Rock, 1985). This has been demonstrated principally with *V. cholerae* (West & Lee, 1982; Hood & Ness, 1982) and *V. parahaemolyticus* (Kaneko & Colwell, 1978; El-Sahn, El-Banna & El-Tabey Shehata, 1982; Kumazawa & Kato 1985) and may well occur for all pathogenic vibrios.

Salinity and nutrient concentration

  Pathogenic *Vibrio* species have halophilic characteristics and occur most frequently in water ranging in salinity from 5 ‰ to 30 ‰ so significantly limiting their presence to estuarine and inshore coastal areas (West & Lee 1982; Seidler & Evans, 1984; Bockemuhl *et al.* 1986; Tison *et al.* 1986; Kelly & Dan Stroh, 1988*a*). Pathogenic vibrios may be isolated from some freshwaters (less than 5 ‰ salinity) where it is possible that the interaction of high water temperature and elevated organic nutrient concentration overcomes the deleterious effect of low salinity (De Paola *et al.* 1983; Tacket *et al.* 1984; Sarkar *et al.* 1985; Rhodes, Smith & Ogg, 1986; Nair *et al.* 1988). This conclusion arises from the important ecological studies reported by Singleton *et al.* (1982*a*, *b*) and Miller, Drasar & Feacham (1984). The influence of selected environmental conditions on a toxigenic strain of *V. cholerae* 01 were investigated using microcosms (laboratory microecosystems). The prolonged survival of the organism was possible in low salinity and nutrient environments. The effect of low salinity was spared if sufficient organic nutrient was available. It appears that, without additional work, these ecological observations have been used to explain why other pathogenic *Vibrio* species occur in natural aquatic environments outside the optimum range of salinity for survival (Sarkar *et al.* 1985). In contrast, Rhodes, Smith & Ogg (1986) suggested that inland water could also become sufficiently brackish to support pathogenic vibrio survival where water is stagnant and evaporates during summer months so increasing the salt content.

  In an unusual case a wound infection due to *V. parahaemolyticus* was acquired at an inland pond. The salinity could have been raised sufficiently to support growth of this halophilic vibrio as a result of spillages of crude oil nearby. The oil originated from the Gulf of Mexico and would contain some salt. In addition, the crude oil was thought to be the source of the inoculum of *V. parahaemolyticus* into the pond (Tacket, Barrett Sanders & Blake, 1982).

Association with higher organisms

Most pathogenic vibrios appear to maintain high numbers and prolong their existence by association with a variety of higher organisms in the aquatic environment including plankton, shellfish and fish. In particular the chitin component in plankton appears to enhance significantly this phenomenon of prolonged survival (Huq *et al.* 1984, 1986; Karunasagar, Venugopal, Karunasagar & Segar, 1987). Studies have established such an association between chitinous zooplankton and *V. cholerae* (Huq *et al.* 1984), *V. parahaemolyticus* (Kaneko & Colwell, 1978; Sarkar *et al.* 1985; Watkins & Cabelli, 1985) and *V. vulnificus* (Oliver, Warner & Cleland, 1983). It is likely that, at some stage, all pathogenic vibrios become associated with chitinous parts of planktonic material to both increase numbers of cells in the aquatic environment and to prolong survival in unfavourable conditions.

Bivalve molluscan shellfish may become rapidly contaminated when filter-feeding on planktonic material colonized by pathogenic vibrios and so are often subsequently incriminated as vectors in food-poisoning incidents (Salmaso *et al.* 1980; De Paola, 1981; Kelly & Dinuzzo, 1985). Association with the flesh of filter-feeding bivalve molluscs after harvesting prolongs the survival of pathogenic vibrios outside aquatic environments. Storage of contaminated shellfish at inappropriate temperatures can then lead to rapid proliferation of pathogenic vibrios (Eyles, Davey & Arnold, 1985; Karunasagar, Karunasagar, Venugopal & Nagesha, 1987). Marked seasonal variations of pathogenic vibrios in filter-feeding bivalve shellfish flesh are often seen since the frequency of contamination is influenced by the numbers of bacteria in the surrounding water column (Hackney, Ray & Speck, 1980; Sobsey *et al.* 1980; El-Sahn, El-Banna & El-Tabey Shehata, 1982; Fletcher, 1985; Tison *et al.* 1986; Kelly & Dan Stroh, 1988*a*; Chan *et al.* 1989).

Crustacean shellfish can also become colonized with pathogenic vibrios; this appears dependent on high counts of bacteria in the surrounding water column so is more commonly observed in warmer climates (Palasuntheram & Selvarajah, 1981; Davis & Sizemore, 1982; Huq *et al.* 1986).

Fish from inshore coastal waters and estuaries can be expected to be colonized with low numbers of human pathogenic vibrios. Ecological studies have largely centred on *V. parahaemolyticus* since this organism commonly causes gastro-enteritis after consumption of raw fish (Binta *et al.* 1982; Sarkar *et al.* 1985).

Animal and birdlife reservoirs

There is no clear evidence that animals in countries endemic for cholera act as a significant reservoir for *V. cholerae* 01 (Sanyal *et al.* 1974; Miller, Feacham & Drasar, 1985). However, non-01 serotypes of *V. cholerae* have been isolated from domestic animals, waterfowl and a variety of wildlife in nearshore habitats of non-endemic cholera regions (De Paola, 1981). The role of land animals in maintaining this pathogenic vibrio in the aquatic environment, and transmitting disease, however, remains unclear. Lee *et al.* (1982) failed to detect *V. cholerae* in the droppings of sheep grazing next to ditchwater in the United Kingdom from which this organism could be regularly isolated. In contrast, Rhodes, Schweitzer & Ogg

(1985) reported non-01 *V. cholerae* associated with enteric disease in horses, lambs and bison in Western Colorado where the organism occurs in some freshwater environments.

Evidence is, however, accumulating to suggest that aquatic birds serve as carriers to disseminate *V. cholerae* over wide areas not endemic for cholera. At the ditchwater site studied by Lee *et al.* (1982), non-01 *V. cholerae* was isolated from the freshly voided droppings of swans nesting nearby. The serotype of the swan isolate was identical to that of the first isolate to be detected in the ditchwater that year. This study also reported a low frequency of intestinal carriage of non-01 *V. cholerae* in seabirds caught in the United Kingdom but it was concluded at that time that their role in maintenance and transmission of *V. cholerae* in aquatic environments was equivocal. More recent evidence, presented by Ogg, Ryder & Smith, (1989) revealed the carriage in the guts of aquatic birds of toxigenic strains of *V. cholerae* 01 and non-01 serotypes in central regions of the United States. Interestingly, no other pathogenic *Vibrio* species appear to be harboured by aquatic birdlife despite use of methods in various studies for their isolation.

TECHNIQUES FOR DETECTING PATHOGENIC VIBRIOS IN THE AQUATIC ENVIRONMENT

Traditional laboratory methods for culture and enumeration of pathogenic vibrios from aquatic environments involve either plating of samples on selective agar media or enrichment in broth followed by isolation on a selective agar medium (West, 1989). Water or sewage samples may require filtration in order to concentrate bacteria (Barrett *et al.* 1980; Kaysner *et al.* 1987*a*).

Reviews of the variety of media for isolating pathogenic *Vibrio* species have been provided by Sakazaki & Balows (1981), Joseph, Colwell & Kaper (1982) and West (1989). Alkaline peptone water at pH 8·6 is the most satisfactory general enrichment medium for all pathogenic *Vibrio* species (Farmer, Hickman-Brenner & Kelly, 1985). A two-step enrichment method has been successfully used to prevent overgrowth by other bacteria in the alkaline peptone water broth (Rhodes, Smith & Ogg, 1986).

Thiosulphate-Citrate-Bilesalts-Sucrose (TCBS) agar is the most commonly used selective agar medium for isolation of pathogenic *Vibrio* species (Bolinches, Romalde & Toranzo, 1988). There is some concern that TCBS agar fails to grow recently described pathogens (Hickman *et al.* 1982). Considerable variation occurs between commercial brands of TCBS agar, and within each brand lot. Media should be regularly monitored to ensure at least 70 % recovery of the species under study (West *et al.* 1982).

The 'viable but non-culturable' phenomenon

Many studies have described the survival characteristics of *V. cholerae* 01 in natural aquatic environments (Barua & Burrows, 1974; Feacham, Miller & Drasar, 1981; Singleton *et al.* 1982*a, b*). The viability of *V. cholerae* in these historic investigations was determined by isolation on various solid culture media with changes in colony counts used to ascertain the rate for survival in aquatic environments.

More recent laboratory-based studies have recognized that *V. cholerae* 01 can enter a state of 'dormancy' under unfavourable aquatic environmental conditions (Xu *et al.* 1983; Roszak & Colwell, 1987). In this state, *viable* cells of *V. cholerae* 01 remain demonstrable as shown by fluorescent microscopy, but *non-culturable* on conventional culture media. Furthermore, animal studies have indicated that 'viable but non-culturable' toxigenic *V. cholerae* 01 retain virulence and can induce fluid accumulation in ligated intestinal loop preparations (Colwell *et al.* 1985).

The impact of the 'viable but non-culturable' phenomenon on the ecology of *V. cholerae* 01, and the epidemiology of cholera, is gradually emerging. Brayton *et al.* (1987) used monoclonal antibody staining combined with fluorescence microscopy to demonstrate that *V. cholerae* 01 cells persist in aquatic environments of cholera endemic areas between epidemic periods of disease. Previously, the organism was thought to disappear from natural aquatic environments as each cholera epidemic subsided (Barua & Burrows, 1974).

It has been postulated that toxigenic *V. cholerae* 01 can persist permanently in natural aquatic environments, if necessary by entering the 'viable but non-culturable' state under adverse conditions of water salinity and organic nutrient concentration. Under favourable, seasonally-dependent, conditions numbers of *V. cholerae* 01 can increase in the aquatic environment and may provide a source for infection in local communities.

This could explain the regularity of cholera epidemics in endemic areas as well as the persistence of foci of cholera in developed countries such as the Gulf coast of the United States even though conventional techniques show that *V. cholerae* 01 is not continuously present in these aquatic environments (Miller, Feacham & Drasar, 1985).

Application of molecular biology techniques

Techniques such as chromosomal restriction endonuclease digest profiling have provided unequivocal evidence to establish finally natural aquatic environments as the epidemiological reservoir for toxigenic *V. cholerae* 01 in non-endemic cholera regions. Molecular epidemiology studies in the Gulf coast region of the United States reported by Kaper *et al.* (1982), Shandera *et al.* (1983) and Lin *et al.* (1986) all conclude that a single strain of toxigenic *V. cholerae* 01 was responsible for a widely distributed series of cholera outbreaks over a decade with the organism maintained throughout in the aquatic environment. Extensions to these studies demonstrated further that a secondary reservoir of cholera toxin genes also resided in a few strains of non-01 *V. cholerae* from the same area (Kaper *et al.* 1986). Such observations make eradication of cholera from these developed regions of the world a formidable challenge to public health agencies.

CHARACTERISTICS OF INFECTIONS WITH PATHOGENIC *VIBRIO* SPECIES AND THE INTERACTIONS WITH THE AQUATIC ENVIRONMENT

Vibrio cholerae serotype 01

Symptoms of infection

V. cholerae 01 is responsible for the disease cholera (Barua & Burrows, 1974). In overt cases, victims effortlessly produce voluminous amounts of 'rice water' stool

and characteristically appear dehydrated with poor skin turgor and sunken eyes. The symptoms of cholera range over a wide spectrum and asymptomatic infections often occur (Blake *et al.* 1980). Extraintestinal infections due to *V. cholerae* 01 have not been commonly reported (Johnston *et al.* 1983). Where these do arise, the isolate often does not produce cholera toxin indicating other, as yet ill-defined, virulence factors.

Pathogenicity

Strains of *V. cholerae* 01 possess a number of virulence factors expressed as extracellular products. The pathophysiologic characteristics of cholera are mediated by a single enterotoxin which stimulates secretion of isotonic fluid by enterocytes of the small intestine (Holmgren, 1981; Mekalanos, 1985). The pathogenicity of *V. cholerae* 01 has been investigated at the molecular genetic level and comprehensively reviewed by Levine *et al.* (1983) and Mekalanos *et al.* (1988). The increasing recognition that *V. cholerae* 01 is naturally resident in aquatic environments has focussed attention on the effects of salinity and other parameters on toxigenicity. Tamplin & Colwell (1986) found a significant increase in cholera toxin production with increasing water salinity whereas Miller *et al.* (1986) could not detect such a relationship.

More recent evidence, acquired during development of live oral vaccines for cholera, indicate that a second enterotoxin different from cholera toxin in antigenic nature, receptor site, mode of action and genetic homology can be produced by environmental strains of *V. cholerae* 01 (Saha & Sanyal, 1988). This may explain the observations made by Morris *et al.* (1984) who isolated a strain of *V. cholerae* 01 from a patient with severe gastroenteritis after oyster consumption but which failed to produce detectable amounts of cholera toxin.

The ability of *V. cholerae* 01 to elicit disease after ingestion by a susceptible host is not only dependent of toxin production but also on resistance to non-specific host defence mechanisms such as gastric acid and mucus linings in the small intestine (Levine *et al.* 1983) and on colonization of the intestines (Peterson & Mekalanos, 1988). Strains of *V. cholerae* 01 unable to produce detectable amounts of cholera toxin *in vitro* are rarely associated with diarrhoeal disease (Levine *et al.* 1982), although the cases reported by Morris *et al.* (1984), Batchelor & Wignall (1988) and Honda *et al.* (1988) are significant exceptions. This is an important observation as, for many years, possession of the 01-specific antigen by strains of *V. cholerae* was considered to correlate with ability to produce toxin and cause epidemics. Possession of the 01-specific antigen, while historically associated with epidemic virulence, should not therefore be taken as sole indication of toxigenicity in *V. cholerae* (Kaper, Moseley & Falkow, 1981).

The minimum infective dose of toxigenic *V. cholerae* 01 is in the range 10^3–10^5 live organisms. Stomach acidity significantly influences the severity of infection and dose required to cause overt disease. Hypochlorohydric persons are more susceptible to cholera due to reduced protection afforded by gastric acid (Levine, Black & Clements, 1984).

Ecological interactions and epidemiology

Epidemiological and ecological studies strongly implicate environmental reservoirs such as foodstuffs and the natural aquatic environment as sources of *V.*

cholerae 01 in sporadic and epidemic disease outbreaks (Blake *et al.* 1980; Barua, 1983; Glass *et al.* 1983; Johnston *et al.* 1983; Miller, Feacham & Drasar, 1985; Klontz *et al.* 1987; Hunt *et al.* 1988).

In regions of poor sanitation where drinking water supplies are not protected from contamination by copius infectious discharges from cholera victims, the classic waterborne transmission of epidemic disease can predominate (Feacham, 1981, 1982). The concept that *V. cholerae* 01 only occurs in natural aquatic environments through close association with cholera cases in surrounding communities was developed from historic studies of endemic disease in areas of poor sanitation where sources and routes of infection were not clear, due to the ·ubiquity of the organism, unless associated with explosive foodborne or waterborne disease outbreaks. Thus the tradition formed that the human intestine was the exclusive reservoir of *V. cholerae* 01 and the presence of organisms in foods or aquatic environments was *solely* due to continuous recontamination from infectious faecal discharge since survival of *V. cholerae* 01 outside the intestine was considered limited (Pollitzer, 1959).

Sporadic outbreaks of cholera in non-endemic regions, where living standards are relatively high, such as the United States, Australia and Italy have provided opportunities to apply novel epidemiological and molecular ecological techniques for study of disease transmission in the absence of a ubiquitous occurrence of organisms in the environment (Anon, 1980). Data quickly established that disease was not imported from endemic areas and that significant natural aquatic reservoirs existed for *V. cholerae* 01 in freshwater and saline environments. These were the source of toxigenic strains in outbreaks of disease (Blake *et al.* 1980, Rogers *et al.* 1980; Salmaso *et al.* 1980; Feacham, 1981, 1982; Kaper *et al.* 1982; Johnston *et al.* 1983; Klontz *et al.* 1987; Hunt *et al.* 1988).

However, the counts of free-living *V. cholerae* 01 in the water column of freshwater and saline environments are typically several magnitudes less in number than required to induce cholera. This implies that numbers must increase sufficiently to a minimum infective dose before contact with a susceptible host. Improper handling of shellfish and seafood contaminated with low numbers of *V. cholerae* 01 would allow multiplication leading to the required infective dose but this appears to be a minor contributory factor in cholera outbreaks (Blake *et al.* 1980; Feacham, 1981; Barua, 1983). More plausible is the multiplication of *V. cholerae* 01, in association with seasonal blooms of chitinous zooplankton, to numbers likely to trigger infection in surrounding local communities using the aquatic environment (Huq *et al.* 1984; Miller, Feacham & Drasar, 1985).

Biological concentration of naturally occurring *V. cholerae* 01 by fish and shellfish, especially bivalve molluscs, in the aquatic environment may in fact represent the first stages of increase in organism numbers towards an infective dose (Salmaso *et al.* 1980; Feacham, Miller & Drasar, 1981; Huq *et al.*, 1984).

Despite the scepticism which surrounded original suggestions by Colwell, Kaper & Joseph (1977) that *V. cholerae* 01 was a naturally-occurring organism resident in natural aquatic environments, the epidemiological role of natural aquatic reservoirs has been established in cholera endemic and non-endemic areas (Miller, Feacham & Drasar, 1985). Under conditions of increasing water temperature and blooms of chitinous zooplankton, there is a proliferation of *V. cholerae* 01 to high

enough numbers and which may trigger infection in communities living nearby and using water for drinking, oral hygiene or harvesting seafood and shellfish (Huq et al. 1984). The likely seasonality of these ecological events provides a compelling explanation for the seasonality and regularity of cholera outbreaks. The environmental factors leading to increases in numbers of V. cholerae 01 are established but there is a need to investigate the behavioural habits of communities, especially in endemic areas, which predispose initiation of human infections in index cases (Miller, Feacham & Drasar, 1985).

In non-endemic areas, infections in communities cease once the bloom of organisms in water fades. There is little opportunity for secondary person-to-person spread of infection due to good hygienic practices (Miller, Feacham & Drasar, 1985). In cholera endemic regions, unsanitary conditions and poor hygienic practices allow spread of cholera to many cases through communities after the index case(s) acquire disease initially from contact with the aquatic environment (Hughes et al. 1982; Umoh et al. 1983; Tauxe et al. 1988). For example, a single worker on an oil rig in the Gulf coast of the United States developed cholera. The rig's drinking water became contaminated and was used to rinse cooked rice. This food subsequently acted as the vehicle for transmission of the disease to other rig workers (Johnston et al. 1983).

Vibrio cholerae non-01 serotypes

Symptoms of infection

There are several detailed reports of clinical presentation of diseases associated with this group of organisms (Blake, Weaver, & Hollis, 1980; Morris et al. 1981; Safrin et al. 1988). Invariably all persons with diarrhoea had a history of shellfish consumption or foreign travel whereas patients with systemic infections had a history of recent occupational or recreational exposure to natural saline environments.

Features which are common to reports of gastrointestinal disease include diarrhoea, sometimes bloody, occasional vomiting with abdominal cramps and, less frequently, low-grade fever. Some symptoms may be so severe as to mimic cholera (Hughes et al. 1978; Piergentili et al. 1984).

Extraintestinal infections with non-01 V. cholerae are frequently reported. Usual sites for infection are wounds and ears following exposure to aquatic environments (Thibaut, Van de Heyning & Pattyn, 1986). Cases of bacteraemia caused by non-01 V. cholerae have been reported with a high rate of fatality especially with immunocompromised victims (Hughes et al. 1978; Siegel & Rogers, 1982; Safrin et al. 1988).

An unusual case of neonatal meningitis was reported by Rubin et al. (1981). In this, the only significant link with the aquatic environment was an infant's bottle kept in a cooler along with live crabs caught from a nearby estuary. The most likely spread of the organism was from the crabs to the milk feed thence to the infant's gut.

Pathogenicity

The spectrum of virulence factors elaborated by non-01 V. cholerae is considerably broader than those associated with V. cholerae 01. As yet, the precise role of most of these factors in disease is not known (Robins-Browne et al. 1977;

Shehabi & Richardson, 1985). Non-01 *V. cholerae* strains can elaborate a wide range of biologically active extracellular toxins, including a cholera-like toxin, other enterotoxins, cytotoxins and haemolysins whose properties have been reviewed by Nishibuchi & Seidler, (1983) and Datta-Roy *et al.* (1986). In addition, a few strains have been shown to produce a haemolysin related to the thermostable direct haemolysin produced by *Vibrio parahaemolyticus* (Yoh, Honda & Miwatani, 1986*a*). A toxin resembling shiga toxin from *Shigella dysenteriae* has been isolated from some strains. O'Brien *et al.* (1984) speculate this may be responsible for the bloodly diarrhoea associated with some infections. A small percentage of non-01 serotypes of *V. cholerae* possess genes to make cholera toxin (Kaper *et al.* 1986) and cause diarrhoeal disease indistinguishable from cholera (Arita *et al.* 1986). As with *V. cholerae* 01, the ability to colonize intestinal surfaces appears to be critical in strains of non-01 *V. cholerae* in order to elicit gastroenteritis (Spira, Fedorka-Cray & Pettebone, 1983).

Ecological interactions and epidemiology

Non-01 serotypes of *V. cholerae* occur more frequently, and in higher numbers, than *V. cholerae* 01 since the 01 serotype is but one of the many serotypes which can be found in the aquatic environment (De Paola *et al.* 1983). Counts of non-01 *V. cholerae* as high as 10^4 per 100 ml have been reported in waters (West & Lee, 1982; Roberts, Bradford & Barbay, 1984) but more frequently numbers are much lower (De Paola *et al.* 1983; Kenyon *et al.* 1983; Kaysner *et al.* 1987*a*; Perez-Rosas & Hazen, 1989). Non-01 serotypes of *V. cholerae* may undergo similar bio-concentration, as *V. cholerae* 01, in the aquatic environment in association with zooplankton and shellfish.

Most human infections have a history of previous exposure to natural saline environments (Hughes *et al.* 1978) or recent consumption of seafood or shellfish (Wilson *et al.* 1981; Morris *et al.* 1981).

Curiously, some infections do not appear to have a readily established link with freshwater or saline aquatic environments (Thibaut, Van de Heyning & Pattyn, 1986; Safrin *et al.* 1988). It is possible that other environmental niches are yet to be fully elucidated.

Non-01 serotypes appear more capable than *V. cholerae* 01 for survival and multiplication in a wide range of foods (Roberts & Gilbert, 1979), suggesting that this group of vibrios can also be transmitted by multiple routes other than water and seafood in non-endemic cholera regions. It is likely that any outbreak of foodborne illness caused by non-01 *V. cholerae*, but not involving seafood, would therefore demonstrate the epidemiological characteristics typical of illness caused by commoner enteric pathogens.

Vibrio parahaemolyticus

Symptoms of infection

Two disease syndromes are associated with infections caused by *V. para-haemolyticus*. The organism is an important cause of diarrhoeal disease, in particular associated with consumption of raw seafood dishes prepared in traditional Japanese culinary style (Joseph, Colwell & Kaper, 1982). Diarrhoeal disease may also occur with seafood which is contaminated with *V. para-haemolyticus* and not cooked sufficiently (Mihajlovic *et al.* 1982).

Symptoms include mild diarrhoea with abdominal cramps and occasional bloody tinge, nausea and vomiting, and some fever. Hosptialization is rare, unless severe fluid loss has occurred, and the illness is generally self-limiting after a few days (Blake, Weaver & Hollis, 1980).

A few extraintestinal infections have been reported, in particular from wounds. Recovery from extraintestinal infections is generally uneventual unless pre-existing underlying debilitation is present (Blake, Weaver & Hollis, 1980; Sautter *et al.* 1988). A rare pneumonic form of *V. parahaemolyticus* infection has been reported (Yu & Uy-Yu, 1984).

Pathogenicity

Unique amongst the pathogenic vibrios is the correlation between pathogenicity of *V. parahaemolyticus* and beta haemolysis of human erythrocytes in an agar medium. This reaction is known as the 'Kanagawa phenomenon'. The association between the ability of an isolate to produce the thermostable direct haemolysin (TDH) responsible for the Kanagawa phenomenon (KP) and its ability to cause gastroenteritis is well established (Sakazaki & Balows, 1981; Nishibuchi & Kaper 1985; Honda *et al.* 1987). The role of the TDH in clinical disease is not fully understood since strains also produce a range of biologically active toxins which may also be virulence factors (Joseph, Colwell & Kaper, 1982; O'Brien *et al.* 1984; Sarkar *et al.* 1987). Virtually all strains of *V. parahaemolyticus* associated with gastro-intestinal disease are KP$^+$ whereas isolates from extraintestinal infections are usually KP$^-$ (Blake, Weaver & Hollis 1980; Tacket *et al.* 1982; Johnson *et al.* 1984; Sautter *et al.* 1988).

Ecological interaction and epidemiology

There is a ubiquitous distribution of *V. parahaemolyticus* in fish and shellfish caught in estuaries and inshore coastal waters (Sakazaki *et al.* 1979; Hackney, Ray & Speck, 1980; Abeyta, 1983; Chan *et al.* 1989). Gastroenteritis due to *V. parahaemolyticus* is almost exclusively associated with consumption of contaminated seafood and shellfish (Bryan, 1980; Beauchat, 1982; Nolan *et al.* 1984). There is a pronounced seasonal incidence of gastroenteritis with outbreaks occurring mostly during warmer months when *V. parahaemolyticus* is most prevalent in the aquatic environments from which seafood and shellfish are harvested (Kelly & Dan Stroh, 1988*b*). There is a significant risk of contracting gastroenteritis, due to *V. parahaemolyticus*, from raw seafood prepared and eaten in Japanese-style restaurants unless careful hygienic practices prevent multiplication of the organisms to the high levels (10^6/g or more) necessary to cause food poisoning (Blake, Weaver & Hollis, 1980).

Extraintestinal infections always occur after exposure to natural saline aquatic environments or after handling seafood and shellfish contaminated with *V. parahaemolyticus* (Blake, Weaver & Hollis, 1980; Armstrong *et al.* 1983; Nolan *et al.* 1984).

Such infections are most likely to occur when water temperatures are sufficiently high to permit multiplication of *V. parahaemolyticus* in aquatic environments to high enough numbers to initiate extraintestinal infections (Clarke & Doyle, 1985).

A rare case of pneumonia was acquired from aerosols laden with *V. para-*

haemolyticus and generated by pressure-hosing a hold in a boat used to catch fish in the warm waters of the Gulf of Mexico (Yu & Uy-Yu 1984). In this, it is presumed that the waste and liquor in the hold from the fishing activity was contaminated with *V. parahaemolyticus*.

Curiously, the KP reaction of environmental strains of *V. parahaemolyticus* is predominantly negative (Palasuntheram & Selvarajah, 1981; Binta *et al.* 1982; Sarkar *et al.* 1985; Watkins & Cabelli, 1985) whereas isolates from diarrhoeal disease are almost exclusively KP$^+$. Selective multiplication of those few strains of KP$^+$ *V. parahaemolyticus* in the aquatic environment probably occurs in the human intestine during infection and these predominate in faecal samples once diarrhoeal symptoms develop (Joseph, Colwell & Kaper, 1982). There is some additional evidence of selection for KP$^+$ isolates in the aquatic environment. Kumazawa & Kato (1985) considered that the main reservoirs for KP$^+$ isolates were sediments and shellfish. It is not clear why KP$^+$ isolates may occur here rather than be equally distributed in the aquatic environment.

Vibrio vulnificus

Symptoms of infection

Infection by *V. vulnificus* was probably first reported in 1970 occurring in a previously healthy man who developed a leg infection and diarrhoea after bathing and collecting shellfish in seawater (Wickbodt & Sanders, 1983). At that time, infection was thought to be due to *V. parahaemolyticus* (Roland, 1970). Recognition of the severity and frequency of *V. vulnificus* infections did not become apparent until the late 1970's (Blake *et al.* 1979).

Three distinct clinical syndromes associated with infections by *V. vulnificus* have emerged from detailed review of nearly 100 clinical cases (Johnston, Becker & McFarland, 1985; Hoffmann *et al.* 1988; Klontz *et al.* 1988). The major forms involve rapidly progressive infections with few diarrhoeal symptoms so making them unique amongst the pathogenic vibrios (Blake, Weaver & Hollis, 1980). One disease syndrome is characterized by rapid onset (often less than 24 h) of fulminating septicaemia followed by appearance of cutaneous lesions. The disease has a high mortality rate (more than 50% of persons with primary septicaemia die) and is invariably associated with consumption of raw bivalve shellfish, contaminated with *V. vulnificus* (Morris & Black, 1985). It is thought that *V. vulnificus* gains entry into the bloodstream via the portal vein or the intestinal lymphatic system (Poole & Oliver 1978; Johnston, Andes & Glasser 1983; Pollak *et al.* 1983; Chin *et al.* 1987). Elderly males with underlying defects in liver function linked to alcohol abuse, or other metabolic disorders involving iron metabolism appear to be most susceptible to this type of infection (Blake *et al.* 1979; Pollak *et al.* 1983; Tacket, Brenner & Blake, 1984; Brady & Concannon, 1984, Sacks-Berg, Strampfer & Cunha, 1987). Another significant contributing factor to *V. vulnificus* septicaemia appears to be deficiencies in the host immune system (Johnston, Andes & Glasser, 1983; Chin *et al.* 1987).

The other major form of *V. vulnificus* infection is characterized by a rapidly progressing cellulitis following contamination of a wound sustained during activities associated with saline aquatic environments. Infection can occur in healthy, as well as debilitated, persons and is characterized by wound oedema,

erythema and necrosis which may progress occasionally to septicaemia (Blake, Weaver & Hollis, 1980; Tacket, Brenner & Blake, 1984; Woo *et al.* 1984; Tyring & Lee, 1986; Hoffman *et al.* 1988). Both forms of infection can be fatal within 24 h of the onset of the symptoms.

The third distinct form of *V. vulnificus* infection is less common but appears to produce acute diarrhoeal symptoms. Johnston, Becker & McFarland, (1986) recently described gastroenteritis caused by *V. vulnificus* after consumption of raw oysters. There was no accompanying blood infection and the gastroenteritis was self-limiting although prolonged. All the victims had underlying a variety of mildly debilitating conditions, such as alcohol abuse, which were possible predisposing factors. Mortality is rare if infection is limited to gastroenteritis (Klontz *et al.* 1988).

More rarely, *V. vulnificus* has been associated with meningitis (Katz, 1988) and pulmonary infection (Sabapathi, 1988).

Pathogenicity

Virulence mechanisms in *V. vulnificus* have attracted much attention due to severity of infections. Strains produce several toxins which may feature as virulence factors in infection (Morris, 1988).

Animal models confirmed the invasive properties of this species soon after its recognition in clinical cases (Poole & Oliver, 1978). Virulence in animal models was later shown to be strongly associated with the presence of a polysaccaride capsule (Simpson *et al.* 1987). Invasiveness is also assisted by an ability to resist the bactericidal effects of human serum (Carruthers & Kabat, 1981) as well as the phagocytic action of polymorphonuclear leukocytes (Kreger, Dechatelet & Shirley, 1981; Simpson *et al.* 1987). Resistance to killing by human serum is mediated by the presence of excess iron (Wright, Simpson & Oliver, 1981; Helms, Oliver & Travis, 1984). Additionally, virulent capsulated strains can use transferrin-bound iron for growth and demonstration of virulence but crucially not at the normal level of transferrin saturation in humans (Morris *et al.* 1987*b*; Simpson *et al.* 1987; Zakaria-Meehan *et al.* 1988). This may explain the association of infections with persons with pre-existing liver or blood disorders which result in impaired iron metabolism and subsequent elevated serum iron levels (Reyes *et al.* 1987; Katz, 1988).

The extensive tissue damage which is characteristic of infections suggests involvement of several specific toxins. Production of collagenase by *V. vulnificus* may contribute to the ability to invade body tissues (Smith & Merkel, 1982). Gray & Kreger (1985), Oliver *et al.* (1986), and Kothary & Kreger (1987), have reported production of compounds by *V. vulnificus* which exhibit a wide range of cytolytic and proteolytic activities and may correlate with various pathological features of infection in man. Failure of diffusible toxins to elicit disease implies that direct contact between host cells and virulent *V. vulnificus* cells is required (Bowdre, Poole & Oliver, 1981). A vascular permeability factor has recently been recently characterized from this pathogenic *Vibrio* species (Miyoshi & Shinoda, 1988).

Ecological interactions and epidemiology

It is noteworthy to mention that taxonomic identification of *V. vulnificus* requires examination of several important phenotypic traits. This has not always been adequately undertaken in some reported studies and the opportunity for mis-identification of this pathogenic vibrio for related species, notably *V. para-haemolyticus*, should be borne in mind when reviewing historic data on *V. vulnificus* (West *et al.* 1986). Additionally, there is an isolated report from Oliver *et al.* (1986) that *V. vulnificus* may rarely possess the unusual trait of bioluminescence which could confuse further the identification of this species, particularly with *Vibrio harveyi*.

There is striking trend towards a greater frequency of infection during warmer months of the year when *V. vulnificus* is most abundant in the aquatic environment (Tilton & Ryan, 1987). Infections are more likely to occur in areas where water temperature remains high most of the year so the distribution of reported disease is predominantly in the mid-Atlantic and Gulf coast states of North America (Kelly, 1982; Tamplin *et al.* 1982; Bonner *et al.* 1983; Kaysner *et al.* 1987*b*). Wound infection syndromes are associated with trauma and exposure to saline aquatic environments whilst septicaemic infections are significantly associated with consumption of raw molluscan shellfish (Blake, Weaver & Hollis, 1980; Johnston, Andes & Glasser, 1983; Johnston, Becker & McFarland, 1985).

Other types of illness may occur less frequently but will be commonly linked to exposure to the aquatic environment (Tacket, Brenner & Blake, 1984). For example, Tison & Kelly (1984*b*) reported a case of *V. vulnificus* endometritis acquired during sexual intercourse submerged in seawater from which the organism was frequently isolated. Katz (1988) reported a case of meningitis in a boy with thalassemia that developed 3 days after he ate raw oysters. A case of pulmonary infection with *V. vulnificus* in a patient who fell into a harbour after a heart attack was described briefly by Sabapathi (1988). Curiously a few infections have no apparent direct association with natural aquatic environments (Chagla *et al.* 1988) suggesting a possible secondary but minor environmental reservoir.

Strains of *V. vulnificus* isolated from the aquatic environment have been shown to possess virulence factors equivalent to those demonstrated in isolates from clinical sources (Tison & Kelly, 1986; Morris *et al.* 1987*a*). This clearly demonstrates that the likelihood of acquiring infection is dependent on the presence and magnitude of numbers of the organism in the aquatic environment.

Vibrio fluvialis

Symptoms of infection

V. fluvialis has emerged as a potential enteropathogen in natural aquatic environments since its involvement in diarrhoeal disease was first reported in 1977. A notable outbreak of diarrhoeal disease involving *V. fluvialis* in Bangladesh was described by Huq *et al.* (1980). Most patients were under 10 years of age and experienced diarrhoea. Abdominal pain, fever and dehydration were also commonly seen in most cases. Other details associated with sporadic infections have been described (Tacket *et al.* 1982; Hickman-Brenner *et al.* 1984; Yoshii *et al.*

1984). The report of a case of gastroenteritis possibly due to *V. fluvialis* after raw oyster consumption was reported by Spellman *et al.* (1986). The incidence of gastroenteritis due to *V. fluvialis* in developing countries appears low (Batchelor & Wignall, 1988).

Pathogenicity

The mechanisms through which *V. fluvialis* causes gastroenteritis are not completely established. Gastroenteritis is most likely mediated through toxins whose identity require elucidation but have been shown to cause fluid accumulation in rabbit ileal loops (Seidler *et al.* 1980; Huq, Aziz & Colwell, 1985) and suckling mice (Nishibuchi & Seidler, 1983, 1984).

Similar toxins with a possible role in pathogenicity have been described (Lockwood, Kreger & Richardson, 1982; Payne, Siebeling & Larson, 1984).

Ecological interactions and epidemiology

The studies of Seidler *et al.* (1980) and Khan & Shadidullah (1982), and a report of isolations of *V. fluvialis* in Louisiana coastal waters, seafood and shellfish (Barbay, Bradford & Roberts, 1984) represent early information on the epidemiology and ecology of this organism. Buck, Spotte & Gadbaw, (1984) reported the isolation of *V. fluvialis* from the teeth of a shark species indicating an unusual but potential source of this pathogenic vibrio in wound infections.

More recently, Gianelli *et al.* (1984) noted the presence of low numbers of *V. fluvialis* in shellfish from the Adriatic sea and local retail outlets. Similarly low numbers of *V. fluvialis* occurred in fish caught in warm waters (Schandevyl, Van Dyck & Piot, 1984). Chan *et al.* (1986, 1989) described the isolation of *V. fluvialis* from seawater and shellfish around Hong Kong but few ecological conclusions can be drawn from these studies. Based on these limited observations, it appears in general that low numbers of *V. fluvialis* are likely to be found in warmer waters. In cooler climates, isolation of *V. fluvialis* from aquatic environments is rare (Bockenmuhl *et al.* 1986). Curiously, *V. fluvialis* has been isolated from the gut of deep-water (300–400 m) invertebrates; the public health significance of this is not clear (Dilmore & Hood, 1986).

Vibrio alginolyticus

Symptoms of infection

V. alginolyticus is considered weakly pathogenic for man and often occurs as an opportunistic pathogen in mixed bacterial infections of extraintestinal wounds (Bonner *et al.* 1983). Most reports of wound infected with *V. alginolyticus* list mild cellulitis and varying amounts of seropurulent exudate as clinical features (Schmidt, Chmel & Cobbs, 1979). Most infections are self-limiting and opportunistic (Blake, Weaver & Hollis, 1980; Wagner & Crichton, 1981). Curiously, the organism has a predisposition for hosts with ear disorders (Pien, Lee & Higa, 1977; Prociv, 1978).

Pathogenicity

Pathogenic mechanisms of *V. alginolyticus* have not been elucidated due to the obscure role of this organism in human disease. Larsen, Farid & Dalsgaard, (1981)

have reported minor biochemical differences between environmental and clinical strains of *V. alginolyticus* but their significance is not clear. Hare, Scott-Burden & Woods (1983) reported production of extracellular protease and collagenase by *V. alginolyticus* but their relevance as virulence factors remains unclear.

Examination of histories of infections with *V. alginolyticus* suggests that this vibrio does not exhibit any significant virulence even with severely immuno-compromised patients (Taylor *et al.* 1981; Janda *et al.* 1986).

Ecological interactions and epidemiology

Despite the ability to recover this species, often in high numbers, from marine environments and seafoods, *V. alginolyticus* is only infrequently associated with human infections. Like most pathogenic *Vibrio* species, this organism occurs in high numbers in warm waters (Larsen & Willeberg, 1984; Chan *et al.* 1986). Counts are often reported as a related observation in ecological studies primarily associated with *V. parahaemolyticus* (Sakazaki *et al.* 1979; Binta *et al.* 1982; Schandevyl, Van Dyck & Piot, 1984; Molitoris *et al.* 1985; Williams & La Rock, 1985; Chan *et al.* 1989). Infections are invariably associated with recent exposure to saline aquatic environments (Taylor *et al.* 1981; Wagner & Crichton, 1981). For example, Opal & Saxon (1986) reported an infection of a head injury with a pure culture of *V. alginolyticus* 3 months after a diving accident in warm seawater. In another case, *V. alginolyticus* was isolated from the fatal infection of a burn patient who was immersed in seawater to douse flames after a boating accident (English & Lindberg, 1977). Infections after swimming or handling seaweed have been recorded (Wagner & Crichton, 1981). A tenuous link with the marine environment was made by Lessner, Webb & Rabin (1985) who reported conjunctivitis with *V. alginolyticus* in an elderly patient who handled seashell fragments before admission to hospital for emergency heart surgery.

Vibrio damsela

This organism is a recently described marine bacterium associated with traumatic wound infections acquired in warm tropical and semi-tropical coastal areas. In many instances of infection, the wounds were exposed to warm seawater or brackish water at the time of injury. Typically, these would be lacerations of the foot or leg sustained while swimming or handling fish (Coffey *et al.* 1986). The occurrence of this species in the aquatic environment appears seasonal and primarily dependent on water temperature as well as the possible interaction with certain fish species which may serve as an environmental reservoir in freshwater environments (Morris *et al.* 1982; Schandevyl, Van Dyck & Piot, 1984; Coffey *et al.* 1986).

In one incident, a fatal wound infection was acquired by an elderly man during the cleaning of a catfish caught in a freshwater lake near Houston, USA. The predeliction of pathogenic vibrios for persons of poor health was demonstrated here since the victim was over 60 years old, alcoholic and suffered from diabetes (Clarridge & Zighelboim-Daum, 1985).

Virulent isolates of *V. damsela* produce large amounts of very potent extracellular, heat labile cytolysin, active against rabbit erythrocytes, which may contribute to the pathogenicity of this vibrio (Kothary & Kreger, 1985).

Vibrio furnissii

This species was formerly classified as the aerogenic biovar of *Vibrio fluvialis* but further taxonomic study indicated that separate species status was warranted. Reports of the isolation of *V. furnissii* from the aquatic environment are sporadic although it appears widespread being found in the USA and Europe. Despite its implication in a few cases of seafood-associated acute gastroenteritis, including an outbreak on an international aircraft, the epidemiology of this organism is as yet unresolved (Brenner *et al.* 1983; Gianelli *et al.* 1984; Hickman-Brenner *et al.* 1984; Morris & Black, 1985).

Vibrio hollisae

There are few ecological studies reported on this pathogenic *Vibrio* species. Dilmore & Hood (1986) reported isolation from deep sea invertebrates; Nishibuchi *et al.* (1988) recovered *V. hollisae* from healthy coastal fish. Despite the lack of detailed information on the ecology of *V. hollisae*, this recently described pathogen has been associated with blood infections and cases of diarrhoea (Hickman *et al.* 1982).

Many cases of infection have been in the USA, in particularly in warm seawater areas such as the Gulf of Mexico; some cases have occurred in states such as Maryland which are bounded by cooler waters. One case has been associated with consumption of catfish caught in a freshwater river (Lowry, McFarland & Threefoot, 1986).

The clinical features of bacteraemia and diarrhoea in cases involving *V. hollisae* have been described (Morris *et al.* 1982). There is a strong association between *V. hollisae* infections and consumption of raw seafood. Of particular concern is the association between infection and consumption of fish which had been fried (Lowry, McFarland & Threefoot, 1986) or treated by drying and salting (Rank, Smith & Langer, 1988). This indicates a potential for this vibrio to survive some methods of cooking and preservation. Also noteworthy is the observation that some strains of *V. hollisae* fail to grow on TCBS agar and so may not be isolated routinely (Morris *et al.* 1982).

Some studies have investigated the role of haemolysin produced by *V. hollisae* as a virulence factor (Yoh, Honda & Miwatani, 1986b). Uniquely, *V. hollisae* possesses gene sequences homologous with those coding for the thermostable direct hemolysin in *V. parahaemolyticus*. (Nishibuchi *et al.* 1985). A heat sensitive enterotoxin is associated with virulent strains of *V. hollisae* (Kothary & Richardson, 1987).

Vibrio mimicus

Strains of *V. mimicus* were, until recently, considered to be biochemical variants of *V. cholerae* before detailed taxonomic investigation revealed sufficient dissimilarity for creation of a separate species (Davis *et al.* 1981; Sakazaki & Donovan, 1984). These organisms occur in similar environments to *V. cholerae* and other pathogenic *Vibrio* species (Bockemuhl *et al.* 1986; Kaysner *et al.* 1987a). They have been isolated from a variety of clinical disorders associated with exposure to aquatic environments. Shandera *et al.* (1983) reviewed the clinical and epidemiological characteristics of infections associated with *V. mimicus*. The majority of isolates were from stool samples and associated with gastroenteritis

after consumption of raw oysters. A few ear infections arose after exposure to seawater. It is notable that a common feature of all the sporadic incidents was their location at which warm seawater could be expected.

Early studies of the virulence of *V. mimicus* found little evidence of toxin production (Shandera *et al.* 1983). However, virulence mechanisms of *V. mimicus* identified to date include enterotoxin (Nishibuchi & Seidler, 1983) and a toxin almost identical to cholera toxin (Spira & Fedorka-Cray, 1984) strongly suggesting the potential of organisms in this species to cause gastroenteritis. More recently, Chowdhury *et al.* (1987) isolated strains from prawns which produced enterotoxin able to induce fluid accumulation in rabbit ileal loops.

Vibrio metschnikovii

The first published evidence of disease due to *V. metschnikovii* in humans was reported by Jean-Jacques *et al.* (1981) who isolated this organism from the blood and gall bladder of an elderly woman. No epidemiological association with recent exposure to seafood, shellfish or saline aquatic environments was noted. There is little information on the ecology and pathogenicity of this organism other than reports of its isolation from freshwater environments (West & Lee, 1982), estuaries, sewage and seafood and a few isolates from infections (Lee, Donovan & Furniss, 1978; Farmer, Hickman-Brenner & Kelly, 1985; Farmer *et al.* 1988; Urdaci, Marchand & Grimont, 1988).

More recently, Miyake, Honda & Miwatani (1988) reported a clinical case of diarrhoea caused by *V. metschnikovii* and identified a cytolysin as a possible contributory virulence factor produced by this weakly pathogenic organism. Mechanisms by which this cytolysin may elicit pathological effects have been further investigated (Miyake, Honda & Miwatani, 1989).

Vibrio cincinnatiensis

This organism is the most recently described pathogenic vibrio (Brayton *et al.* 1986). In the single case reported to date, the organism was isolated from a case of septicaemia and meningitis in an elderly immunocompetent man who had apparently not been recently exposed to seafood or saltwater (Bode *et al.* 1986). Meningitis caused by pathogenic vibrios is rare but often fatal (Hughes *et al.* 1978). Interestingly, recovery in this case was uneventful.

The environmental reservoir of this newly-recognized pathogenic vibrio should emerge as more cases are reported.

PREVENTION AND CONTROL PROCEDURES

Since pathogenic *Vibrio* species occur naturally in aquatic environments, control of sewage contamination will not completely prevent spread of infection (De Paola *et al.* 1983; Shiaris *et al.* 1987). Exceptions to this involve cholera infections in endemic areas where secondary infections follow contamination of unprotected drinking water supplies or food.

Risks of infection with pathogenic *Vibrio* species are most strongly associated with (i) impaired host resistance factors in susceptible hosts; (ii) occupational or recreational use of natural aquatic environments; and (iii) consumption of

contaminated foodstuffs, particularly seafood. Preventive measures can be implemented at several points in each of these risk areas. In other documented cases, the route of exposure is less identifiable although the majority of these victims did undertake foreign travel to less developed countries before infection.

Host risk factors

There is overwhelming evidence that lack of normal gastric acid production increases the risk of acquiring cholera since the effectiveness of this defence mechanism is diminished. Pre-existing liver disorder and iron overload of blood are significant features in cases of septicaemia due to *V. vulnificus*.

Evidence is increasing that susceptibility to infections with *V. hollisae* may be also related to liver abnormalities (Rank, Smith & Langer, 1988). Persons with these underlying disorders have been advised to consider avoiding consumption of raw seafood and shellfish (Johnston, Becker & McFarland, 1985). People with liver or underlying chronic disease are now being advised by the medical profession to eliminate raw shellfish from their diet to reduce the likelihood of fatal infection due to *V. vulnificus* (Chin *et al.* 1988; Hoffman *et al.* 1988; Katz, 1988; Klontz *et al.* 1988; Morris, 1988). This advice has been endorsed by the United States Food and Drug Administration (Ballentine, 1987).

A plethora of ill-defined debilitating disorders including nutritional status, age, metabolic disorders such as diabetes, and defects in immune systems feature in many sporadic infections associated with pathogenic *Vibrio* species. Correlation of these risk factors with infection by specific pathogens is likely to be forthcoming as more clinical data accumulate (Bonner *et al.* 1983).

Exposure to aquatic environments

The seasonal occurrence and distribution of pathogenic *Vibrio* species in aquatic environments significantly influences the risk of infection in susceptible humans. Pathogenic *Vibrio* species will occur in highest numbers during warmer months of the year and when water temperature is high enough to allow rapid proliferation of organisms to numbers which approach infective doses (Bonner *et al.* 1983; Kelly & Dan Stroh, 1988*b*). Under these circumstances, it is prudent to avoid contamination of puncture wounds, burns and lacerations by simple use of protective equipment. Persons who regularly handle seafood and shellfish could wear protective gloves to avoid splinter injuries and lacerations. There is increasing evidence to suggest avoidance of bites from marine mammals should also be a preventive measure (Buck & Spotte, 1986). It has been recently recommended that persons with underlying debilitating disorders should take preventive measures to avoid contamination of wounds (Hoffman *et al.* 1988). Klontz *et al.* (1988) go so far as to suggest that persons with pre-existing wounds should be warned to avoid contact with seawater of temperatures greater than 20 °C to reduce the risk of *V. vulnificus* wound infection.

Consumption of contaminated foodstuffs

There is convincing epidemiological evidence that consumption of certain foods, especially raw or lightly cooked seafood and shellfish, is associated with outbreaks of diarrhoeal disease due to pathogenic *Vibrio* species. In particular, infections due to *V. cholerae* (01 and non-01 serotypes), *V. parahaemolyticus* and *V. vulnificus*

have been associated with eating raw bivalve shellfish (Salmaso *et al.* 1980; Tacket, Brenner & Blake, 1984). Contamination of these shellfish by pathogenic *Vibrio* species cannot be controlled by restricting faecal contamination of growing water so the rationale of shellfish sanitation programmes has limited application. Raw seafood and shellfish should be kept at refrigeration temperature (less than 5 °C) or placed on ice immediately after harvesting or processing to prevent multiplication to numbers likely to cause disease (Hood, Baker & Singleton, 1984; Fletcher, 1985; Reily & Hackney, 1985; Saxena & Kulshrestha, 1985; Ingham & Potter, 1988). It is noteworthy that although *V. vulnificus* is unable to survive well in whole oysters at near-freezing temperatures (Oliver, 1981), there has been at least one reported case of infection following consumption of refrigerated raw oysters (Johnston, Andes & Glasser, 1983).

Filter-feeding bivalve shellfish are traditionally rendered fit for consumption by self-purification (depuration) in shore-based tanks of clean seawater or by relaying in clean seawater (Richards, 1988). The effect of self-purification on bivalve shellfish contaminated with pathogenic vibrios is not entirely clear. *Vibrio vulnificus* appears quickly eliminated from the flesh under these treatment conditions (Kelly & Dinuzzo, 1985) whereas *V. parahaemolyticus* levels in naturally-contaminated shellfish do not appreciably decrease during depuration (Greenberg, Duboise & Palhof, 1982; Eyles & Davey, 1984). Significantly, Son & Fleet (1980) demonstrated rapid reductions in *V. parahaemolyticus* numbers in depuration systems using artificially contaminated shellfish. These conflicting observations indicate that the mode of contamination of shellfish prior to depuration treatment is an important factor.

Irradiation of seafood with gamma radiation is an increasingly attractive way to destroy pathogenic vibrios (Bandekar, Chander & Nerkar, 1987; Sang, Hugh Jones & Hagstad 1987).

There is invariably evidence of improper cooking and handling when cooked foods, such as seafood or shellfish, are associated with food poisoning outbreaks due to pathogenic *Vibrio* species. Cooking foods to achieve internal temperatures above 60 °C for several minutes appears satisfactory for eradication of pathogenic vibrios (Boutin, Bradshaw & Stroup, 1982; Shultz *et al.* 1984; Saxena & Kulshrestha, 1985; Makukutu & Guthrie, 1986). Cold smoking of seafood is unlikely to be adequate to remove pathogenic vibrios (Karunasagar *et al.* 1986). Recontamination of cooked foods through poor handling and storage can be avoided by adherence to basic food hygiene practices so cross-contamination with raw material is avoided (Paparella, 1984; Bryan, 1988).

SUMMARY

Pathogenic *Vibrio* species are naturally-occurring bacteria in freshwater and saline aquatic environments. Counts of free-living bacteria in water are generally less than required to induce disease. Increases in number of organisms towards an infective dose can occur as water temperatures rise seasonally followed by growth and concentration of bacteria on higher animals, such as chitinous plankton, or accumulation by shellfish and seafood. Pathogenic *Vibrio* species must elaborate a series of virulence factors to elicit disease in humans.

Activities which predispose diarrhoeal and extraintestinal infections include ingestion of seafood and shellfish and occupational or recreational exposure to natural aquatic environments, especially those above 20 °C. Travel to areas endemic for diseases due to pathogenic *Vibrio* species may be associated with infections. Host risk factors strongly associated with infections are lack of gastric acid and liver disorders.

Involvement of pathogenic *Vibrio* species in cases of diarrhoea should be suspected especially if infection is associated with ingestion of seafood or shellfish, raw or undercooked, in the previous 72 h.

Vibrio species should be suspected in any acute infection associated with wounds sustained or exposed in the marine or estuarine environment. Laboratories serving coastal areas where infection due to pathogenic *Vibrio* species are most likely to occur should consider routine use of TCBS agar and other detection regimens for culture of *Vibrio* species from faeces, blood and samples from wound and ear infections.

P. A. WEST
North West Water Authority,
Great Sankey,
Warrington WA5 3LW,
United Kingdom

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