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DISEASES OF HUMANS (OTHER THAN CHOLERA) CAUSED BY VIBRIOS[1]

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INTRODUCTION

Vibrio cholerae O-group 1, the organism that causes cholera, has historically been the vibrio of greatest interest to clinicians, microbiologists, public health officials, and epidemiologists. Other vibrios were simply dismissed as "non-agglutinable vibrios" (NAGs) (they did not agglutinate in *V. cholerae* O-group 1 antiserum) or as "non-cholera vibrios" (NCVs). Sometimes these terms were used to indicate all vibrios other than the epidemic strains that cause cholera; in other cases they were used to describe only those vibrios that were biochemically similar to the epidemic strains. Early attempts to create order in the chaotic world of the vibrios included the efforts of Heiberg (34) in 1936 to classify them into six groups by their fermentation of several sugars. However, not until microbiologists began to sort out separate species from the "wastebasket" NCV/NAG terminology did it become apparent that these vibrios included several species that are often pathogenic for humans and that have distinct clinical features, ecologic niches, pathogenic mechanisms, and epidemiologic characteristics. These species now include *V. parahaemolyticus, V. vulnificus, V. alginolyticus,* the non-O group 1 portion of the *V. cholerae* species, and two *Vibrio* species of uncertain pathogenicity for humans—group F (EF6) vibrios and *V. metschnikovii* (enteric group 16) (Table 1). The field is evolving rapidly, and there are still many basic questions to be answered. This review summarizes the current status of knowledge about disease associated with vibrios other than O-group 1 *V. cholerae.*

ISOLATION AND IDENTIFICATION

Isolation

Many cases of gastroenteritis caused by *Vibrio* species are probably missed by bacteriology laboratories that use only routine enteric media for primary isolation of agents of gastroenteritis. Both *Vibrio* species (*V. cholerae* and *V. parahaemolyticus*) that definitely cause gastroenteritis, as well as the other vibrios listed in Table 1, can be isolated from stool specimens by direct inoculation of thiosulfate citrate bile salts sucrose (TCBS) agar. Isolation

Table 1 Summary of features of diseases (other than cholera) caused by vibrios

Organism	Principal reservoirs	Disease	Principal vehicles of transmission	Host risk factors
V. cholerae (non-01)	Unknown	Gastroenteritis	Food, water	None well-established
	? coastal seawater; ? fresh water; ? man, animals	Soft-tissue infections	? water	Wounds
		Septicemia	? seafoods	Cirrhosis and other underlying disease
V. parahaemolyticus	Coastal seawater, seafoods	Gastroenteritis	Seafoods	None well-established
		Soft-tissue infections	Seawater	Wounds
V. vulnificus	Probably coastal seawater and seafoods	Soft-tissue infections	Seawater	Wounds
		Septicemia	Seafoods	Cirrhosis and other underlying disease
V. alginolyticus	Seawater	Soft-tissue infections	Seawater	Wounds
		Ear infections	Seawater	Perforated tympanic membrane, earlier ear infections
Group F (EF6) *Vibrio*	Unknown	Gastroenteritis (?)	Unknown	Unknown
V. metschnikovii (enteric group (16)	Unknown	Gastroenteritis (?)	Unknown	Unknown

of the various *Vibrio* species from extraintestinal sites does not require use of selective media. Most of the media used to culture blood and wounds contain at least 0.5% NaCl, which supports growth of the halophilic organisms. Detailed descriptions of methods for isolating vibrios have been published (29, 31).

Identification

Vibrios are facultative anaerobes that are usually oxidase positive and that ferment glucose without forming gas (95). With the exception of *V. metschnikovii* (56), *Vibrio* species can be differentiated from Enterobacteriaceae by the oxidase reaction. However, the oxidase reaction should be tested on growth from nonselective media because false-negative reactions have been obtained in tests of growth of *V. cholerae* (68) and *Aeromonas hydrophila* (74) from selective media.

V. cholerae can be differentiated from the other species of *Vibrio* and from group F by its lack of a requirement for more than trace amounts of NaCl in growth media. If an organism must have NaCl, it will not grow in a medium such as methyl red–Voges-Proskauer (MR-VP) broth that does

not contain NaCl, but it will grow well on most of the commonly used media such as Trypticase soy agar or heart infusion agar. The requirement for NaCl can be confirmed by obtaining growth on NaCl-deficient media to which NaCl has been added in a final concentration of 0.5 to 1%.

Other oxidase-positive organisms found in clinical specimens, which, like *V. cholerae,* ferment glucose and do not require added NaCl in growth media, include *Aeromonas hydrophila, Plesiomonas shigelloides,* and *Chromobacterium violaceum;* all will grow on MacConkey agar. *V. cholerae* can be differentiated from these species by the arginine dihydrolase reaction (Table 2). Additional characteristics of *V. cholerae* are listed in Table 3.

The NaCl-requiring *Vibrio* species can be divided into two groups by the arginine dihydrolase and ornithine decarboxylase reactions. *V. parahaemolyticus, V. alginolyticus,* and *V. vulnificus* are arginine negative and ornithine positive or variable. *V. anguillarum* (a species common in seawater but not known to be pathogenic for humans), *V. metschnikovii,* and group F are arginine positive or variable and ornithine negative (Table 3). To obtain proper dihydrolase and decarboxylase reactions in the Moeller medium, NaCl must be added to a final concentration of at least 0.5 to 1% (35, 86).

The most useful characteristics for differentiating *V. parahaemolyticus, V. alginolyticus,* and *V. vulnificus* are growth in 10% NaCl; production of acid from lactose, sucrose, and arabinose; and results of O-nitrophenyl-β-D-galactopyranoside (ONPG) and Voges-Proskauer tests (Table 3). The Voges-Proskauer medium must be supplemented with NaCl to a final concentration of 3%.

The most useful reactions for differentiating *V. anguillarum* and group F are acid from arabinose and gas from glucose. *V. anguillarum* is negative in both reactions. Group F strains produce acid from arabinose and vary in production of gas from glucose.

Table 2 Differentiation of *Vibrio cholerae* from three other oxidase-positive, glucose-fermenting, nonhalophilic organisms[a]

Organisms	Reactions		
	L-lysine decarboxylase	L-arginine dihydrolase	L-ornithine decarboxylase
Vibrio cholerae	+[b]	–	+
Aeromonas hydrophila	–[c]	+	–
Plesiomonas shigelloides	+	+	+
Chromobacterium violaceum	–	+	–

[a] Based on data from Special Bacteriology Section, Center for Disease Control, and Hugh & Sakazaki (41).
[b] + = 90% or more positive at 48 hr.
[c] – = 0–9% positive at 48 hr.

Table 3 Characteristics of vibrio species and group F[a]

Test	*Vibrio cholerae*	*Vibrio parahaemolyticus*	*Vibrio alginolyticus*	*Vibrio vulnificus*	*Vibrio anguillarum*[b]	Group F	*Vibrio metschnikovii*[b]
Growth in 0% NaCl	+[c]	–[d]	–	–	–	–	–
Lysine decarboxylase	+	+	+	+	v[e]	–	v
Arginine dihydrolase	–	–	–	–	v	+	+
Ornithine decarboxylase	+	+	v	+	–	–	–
Growth in 10% NaCl	–	–	+	–	–	–	–
Lactose, acid	v	–	–	+ or (+)[f]	–	–	v
ONPG	+[b]	–	–	+	v	+	v
Sucrose, acid	+	–	+	–	+	+	+
Voges-Proskauer	v	–	+	–	v	v	+
Arabinose, acid	–	+	–	–	–	+[b]	–
Gas from glucose	–	–	–	–	–	v	–
Oxidase	+	+	+	+	+	+	–
Nitrate reduction	+	+	+	+	+	+	–
Glucose, acid	+	+	+	+	+	+	+
Maltose, acid	+	+	+	+	v	+	+
Mannitol, acid	+	+	+	v	+	+	+
Xylose, acid	–	–	–	–	–	–	–
Catalase	+	+	+	+	+	v	+
Indole	+	+	+	+	v	v	v
Urease	–	–	–	–	–	–	–
Sensitivity to O/129[g]							
10 μg	+	–	–	+	+	–	+
150 μg	+	+	+	+	+	+	+

[a] Data, except where noted, from the Special Bacteriology Section, CDC: *V. cholerae*, 82 strains; *V. parahaemolyticus*, 160 strains; *V. alginolyticus*, 58 strains; *V. vulnificus*, 47 strains; group F, 25 strains.
[b] Data from Lee et al (56).
[c] + = 90% or more positive at 48 hr.
[d] – = less than 10% positive at 48 hr.
[e] v = 10–89% positive at 48 hr.
[f] + or (+) = 90% or more positive or delayed positive, 3–7 days.
[g] 2,4-diamino-6,7-diisopropyl pteridine phosphate; data from J. V. Lee, personal communication, March 7, 1980.
Reactions within heavily lined boxes are considered the most important differential characteristics as indicated in the text.

V. metschnikovii is differentiated from *V. anguillarum* and group F by the fact that it is oxidase negative and does not reduce nitrate.

NON-O GROUP 1 *VIBRIO CHOLERAE*

Introduction

Vibrios that are biochemically similar to the epidemic strains of *V. cholerae* but do not agglutinate in *V. cholerae* O-group 1 antiserum have been referred to in the past as non-agglutinable vibrios (NAGs) or as non-cholera vibrios (NCVs). They are now included in the species *V. cholerae* (40), and in this review they will be referred to as non-O group 1 *V. cholerae* or non-O1 *V. cholerae*. These organisms have caused outbreaks and sporadic cases of gastrointestinal illness and in a few instances have been isolated from persons with extraintestinal disease.

Clinical Features

The characteristic features of gastrointestinal illness caused by non-O1 *V. cholerae* cannot be described with any confidence; two or more syndromes may be associated with these organisms. There have been only two reported outbreaks in which the clinical features were described. In an outbreak in Czechoslovakia in 1965 (1), 100% of the patients had diarrhea, 25% had vomiting, 11% had low-grade fever, and none had blood or mucus in their stools. The illness lasted 1–2 days. In an outbreak among airplane passengers arriving in Australia in 1973, most had diarrhea, vomiting, and severe abdominal cramps, and a few had low-grade fever (22). The illness usually lasted 18–24 hr.

In the reported descriptions of sporadic cases, one cannot be certain that the organism isolated caused the illness. In 1965 McIntyre et al (59) reported on 19 persons with sporadic cases of diarrheal illness in Bangladesh; stool cultures of these patients yielded a heavy growth of non-O1 *V. cholerae*. Some of the patients had severe dehydrating diarrhea, and 50% required intravenous fluid therapy. In 1979 Spira et al in Bangladesh (102) published a detailed description of 14 cases of diarrheal illness in adults from whom non-O1 *V. cholerae* were the only potential pathogens isolated. In addition to diarrhea, the patients had vomiting (100%), abdominal pain (71%), temperatures higher than 100°F (43%), and muscle cramps (21%). The mean duration of diarrhea was 42.1 hr, the mean stool volume was 60 cc/kg, and quantitative cultures of the patients' stools found a mean of 3–4 X 10^8 vibrios/ml. None of the stools studied from the 14 had more than 5 fecal leukocytes/hpf, and only 1 had more than 5 fecal red blood cells/hpf.

In reports from the United States, however, blood and mucus were often found in stools. Hughes et al (43) reported on 13 persons with sporadic cases of diarrhea in the United States from whom non-O1 *V. cholerae* were isolated; associated symptoms included abdominal cramps (92%), nausea (77%), vomiting (69%), chills (64%), fever (58%), mucus in stools (30%), and bloody stools (8%). The illness lasted an average of 7 days. Since these cases were reported to the Center for Disease Control (CDC), they probably represented the most severe end of a spectrum of disease. Two of three persons subsequently reported on from Florida (18) had bloody stools. Thus, while some persons with non-O1 *V. cholerae* gastroenteritis have a severe dehydrating cholera-like illness, many have fever and some have bloody diarrhea, both of which are unusual for adults with cholera.

Treatment of severe dehydrating non-O1 *V. cholerae* gastroenteritis should focus on oral and/or intravenous rehydration. Antibiotics (particularly tetracycline) have been shown to decrease the severity and duration of cholera, but the value of antibiotic therapy of non-O1 *V. cholerae* gastroenteritis has not been determined.

In contrast to toxigenic *V. cholerae* O1 strains that do not cause extraintestinal disease, at least 18 instances of isolating non-O1 *V. cholerae* from human sources other than feces have been reported (2, 28, 33, 43, 79, 84). The sources have included bile or gallbladder (two), blood (five, including a patient with isolates from blood, a wound, and cerebrospinal fluid), infected wounds (four), ears (four), sputum (two), an appendix (one), peritoneal fluid (one), and cerebrospinal fluid (one). Although the pathogenic significance of the organisms is uncertain in some of these cases, they appear to have had a pathogenic role in at least seven cases—the five persons with septicemias (43, 79, 84) and two with wound infections (43). Those with septicemias had preexisting underlying disease [cirrhosis (two), kwashiorkor, leukemia, and severe peripheral vascular disease]. The ear infections included three cases of otitis media and one of otitis externa.

Ecology

Non-O1 *V. cholerae* strains are widely distributed in the environment in Europe, Asia, and the United States. They have been found in sewage, sewage-contaminated surface water, estuarine waters (both sewage-contaminated and apparently free from fecal contamination), seafoods, and animals (9, 88, 94).

Studies of the ecology of non-O1 *V. cholerae* in Germany, the United States, and England have generally demonstrated that the organisms are found in brackish surface waters, are more numerous during the warmer months, and are not necessarily associated with sewage contamination. They appear to be autochthonous estuarine bacterial species. In German

rivers, there were more non-O1 *V. cholerae* in the summer, and the organisms were associated with water that had a pH greater than 7.5 and a salinity of 0.01% (100 mg NaCl/liter) or greater (70). In another German study, the organisms were detected in 22% of 100-ml samples of seawater collected from the Baltic Sea over a 3-year period and were present even when the coliform count was very low (69). The incidence was lowest during the first two quarters of the year (15%) and highest during the third quarter (36%). In the United States, Kaper et al detected non-O1 *V. cholerae* organisms of many serotypes throughout much of Chesapeake Bay (51). Most came from water with a salinity of 0.4–1.7%; they were not apparently correlated with the presence of fecal coliforms and were found throughout the year in low numbers, generally 1–10 cells per liter. In England, Bashford et al (6) found large numbers of non-O1 *V. cholerae* in surface waters in Kent, including a drainage ditch where the possibility of sewage contamination was considered to be negligible. As in Germany, more of the organisms were apparently present in the summer. Thus, at least some non-O1 *V. cholerae* appear to be free-living in the environment, but the pathogenicity of such free-living strains for humans is unknown.

Pathogenicity

The most comprehensive study of the pathogenic mechanisms of non-O1 *V. cholerae* was performed by Spira et al (102). They used a battery of assays to characterize the biologic activity of 110 isolates from patients with diarrheal illness, persons with non-diarrheal illness, and the environment. These assays were (*a*) production of diarrhea and death in the infant rabbit by whole culture, (*b*) ability to cause fluid accumulation in the rabbit ileal loop using 1. whole culture and 2. culture filtrates, (*c*) rabbit skin permeability activity, (*d*) induction of morphologic changes in Chinese hamster ovary (CHO) and adrenal cells, and (*e*) induction of fluid accumulation in the suckling mouse assay by culture filtrates. Four patterns of biologic activity were observed:

1. Production of a cholera toxin (CT)-like toxin [positive infant rabbit, ileal loop (both whole culture and filtrate), rabbit skin, and CHO/adrenal cell assays].
2. Production of a heat-stable toxin [positive ileal loop (both whole culture and filtrate) and infant mouse assays].
3. "Enteritis" (positive infant rabbit and/or ileal loop with whole culture assays) without production of either toxin.
4. No activity in any of these assays.

Spira & Daniel (101) subsequently compared the patterns of biologic activity of 18 strains from feces of persons with diarrheal illness in Bangladesh and 18 presumably free-living strains from relatively unpolluted portions of the aquatic environment in Bangladesh. All of the fecal isolates had some activity in these assays (33% produced CT-like toxin and 67% were in the "enteritis" group), and 67% of the environmental isolates had no activity (33% were in the "enteritis" group, and none produced CT-like toxin) ($p < .0001$, Chi-square test). Thus, there were considerable differences between human and environmental strains, but there was also some overlap in the "enteritis" group. None of these 36 strains had heat-stable toxin-like activity, but 2 strains from Chesapeake Bay discussed in Spira et al's earlier report (102) had such activity.

The CT-like toxin produced by some strains of non-O1 *V. cholerae* is very similar but not identical to CT (101, 114, 115). Some strains appear to produce more than one toxin (115).

The ability of non-O1 *V. cholerae* to produce CT-like toxin appears to be clinically relevant. Spira & Daniel found that 6 patients with toxigenic isolates had more severe illness than 12 with nontoxigenic isolates: the weight loss of members of the former group was greater (mean 5.6 vs 1.7%), their admission serum specific gravity was higher, their diarrhea lasted longer (mean 50 vs 35 hr), and their stool volume was greater (mean 103 vs 41 cc/kg) (101). Significant antitoxin titer rises were found in two of three patients with toxigenic isolates, but none were found in the eight patients with nontoxigenic isolates ($p = 0.055$) (Fisher's 2-tailed exact test).

Using the rabbit brush border assay, Spira et al (102) found that, in the 18 strains from persons with diarrhea in Bangladesh, the mean number of vibrios adhering per brush border was roughly but significantly related to the stool volume of the patient from whom the strain was isolated. Toxigenic isolates demonstrated relatively high brush border adherence.

A number of investigators have described a hemorrhagic effect of filtrates from some non-O1 *V. cholerae* in the skin of guinea pigs and rabbits (72). This hemorrhagic factor can usually be diluted sufficiently to permit detection of the vascular permeability factor (PF) in rabbit skin tests. Its significance for human disease is unknown.

Robins-Browne et al have described a non-O1 *V. cholerae* isolate from the blood of a child in South Africa with fever but no gastroenteritis (84). This organism was positive in the CHO cell assay and positive in the rabbit skin test. Although the organism was not invasive in the Sereny test, it invaded the rabbit ileal mucosa when the ligated rabbit ileal loop test was performed. Furthermore, it was cultured from the blood, liver, and spleen of two rabbits enterally infected with the organism. We do not know what

proportion of non-O1 *V. cholerae* strains have this invasive property or its significance for human disease.

The studies by Spira and others demonstrate that non-O1 *V. cholerae* can produce diarrhea not only by producing toxin similar to that produced by *V. cholerae* O1 but also by other as yet ill-defined mechanisms. Currently, there is no assay (other than human feeding experiments) or group of assays with which to determine confidently the potential of a given strain to be an enteric pathogen for humans.

Epidemiology

Non-O1 *V. cholerae* have been isolated from the stools of persons with diarrheal illnesses who were infected in Asia, Africa, Europe, North America, and South America. The proportion of diarrheal illness associated with these organisms in these areas is unknown. No non-O1 *V. cholerae* were isolated from 385 persons with diarrhea in Mexico City in 1966–1967 (106), although they have been isolated from travelers returning from Mexico. In Bangladesh, non-O1 *V. cholerae* were isolated on direct plating from 3% (34/1120) of persons hospitalized at the Cholera Research Laboratory because of diarrheal illness during the early 1960s and from only .01% (1/6951) of well persons (59). Gastrointestinal disease caused by non-O1 *V. cholerae* probably occurs in many countries where it has not been reported. Outbreaks and sporadic cases have occurred, but large epidemics and pandemics like those caused by *V. cholerae* O1 have not been reported.

The non-fecal isolates from humans have been reported from the United States, Europe, and Africa (2, 28, 33, 43, 79, 84).

Little is known about the seasonality of non-O1 *V. cholerae* disease. In the United States, most domestically acquired cases occur during the warmer months.

Studies of sporadic cases of diarrheal illness associated with isolation of non-O1 *V. cholerae* from stools in the United States (1972–1979) have found that the patients frequently have a history of consumption of mollusks, especially raw oysters; the incubation period is usually less than 48 hr. Oysters were shown epidemiologically to be the vehicle of transmission on the only occasion when a case-control study was done (18); in that instance, at least some of the oysters came from beds with elevated fecal coliform counts. Mollusks are theoretically a good vehicle of transmission because they are filter-feeding organisms and can concentrate bacteria.

Studies of three outbreaks [Czechoslovakia (1), the Sudan (110), and Australia (22)] showed that the vehicle of transmission was food in two instances (potatoes and an egg and asparagus salad) and water (polluted well water) in one. The incubation periods in the three outbreaks were 20–30 hr, less than four days, and 5–38 (mean of 11.5) hr.

Most likely, transmission is almost exclusively by contaminated food or water. In the outbreak in the Sudan in 1968 (110), despite poor sanitary conditions and large numbers of infected persons, no further cases occurred four days after the well suspected of causing the epidemic was closed. No secondary cases were observed and no person-to-person transmission was evident. Non-O1 *V. cholerae* organisms multiply readily in a variety of foods and thus can reach the presumably high numbers needed to cause infection (83).

The source of infection is unknown for most cases of extraintestinal disease. Some persons have a history of recent exposure to seawater.

The reservoir of non-O1 *V. cholerae* strains that cause human disease is unknown. The organisms appear to survive and multiply in the environment, but whether or not such free-living strains are pathogenic for man is unknown. Humans may be the principal reservoir, and the organisms in food and water that cause disease may have come from an infected human. A human chronic carrier state for non-O1 *V. cholerae* may occur; in the United States the organism has been isolated from bile (43), raising the possibility of chronic biliary tract infections, and a study of sailors arriving in the Soviet Union showed that the carrier state could last 60 or more days (116).

A question of considerable epidemiologic significance is whether "transformation" of somatic antigen can occur in the natural environment, i.e. can non-O1 *V. cholerae* become *V. cholerae* O1? This was claimed in a preliminary report (8), but no other investigators have been able to reproduce such changes (32), and no such changes have been known to occur in nature.

Two serotyping systems for non-O1 *V. cholerae* based on the O antigen are widely used. In both systems, the epidemic strain of *V. cholerae* is designated O-group 1. The system developed by Sakazaki et al included 60 serotypes by 1976 (96). The system developed by Smith & Goodner at the Vibrio Research Laboratory (VRL) recognized 72 serotypes by 1979 (98). Studies at CDC showed that each system included some serotypes not included in the other, and many serotypes in each system appeared to correspond to serotypes in the other (23). Neither system has thus far determined any marked differences in the serotypes of isolates from human and non-human sources (89, 98). However, comprehensive systematic studies on this subject have not yet been published.

In 1936, Heiberg (34) classified vibrios into six groups by their fermentation of sucrose, mannose, and arabinose; two more groups were added by Smith & Goodner in 1965 (99). Non-O1 *V. cholerae* meeting the minimal criteria for vibrios fall into at least four of the eight groups (98). There is no apparent correlation between serotypes of the non-O1 *V. cholerae* and their Heiberg groups (98). Since the serotyping systems were developed,

Heiberg grouping has been of little practical value in studying non-O1 *V. cholerae.*

VIBRIO PARAHAEMOLYTICUS

Introduction

V. parahaemolyticus was first recognized as a cause of food poisoning in Japan in the early 1950s. In the past decade it has been found to be an important cause of diarrheal illness in many parts of the world, and it has occasionally been associated with extraintestinal disease.

Clinical Features

Two clinical syndromes have been described for patients with *V. parahaemolyticus*-associated gastroenteritis. The cardinal manifestation of the most commonly described syndrome is watery diarrhea. In eight *V. parahaemolyticus* outbreaks in the United States (3), the clinical manifestations included diarrhea (98%), abdominal cramps (82%), nausea (71%), vomiting (52%), headache (42%), fever (27%), and chills (24%). Temperature rarely exceeded 38.9°C. The illness was usually self-limited, with a median duration of three days. In severe cases, dehydration, hypotension, and acidosis may occur (58). A dysenteric syndrome with mucoid or sanguinous stools has been described in several countries. In Calcutta, India, one study showed that one quarter of 60 hospitalized patients with *V. parahaemolyticus*-associated gastroenteritis had blood and mucus in their stools (97). All eight persons involved in an outbreak in Bangladesh (42) had liquid stools, severe abdominal cramps, nausea, and vomiting. The six most severely ill persons had gross blood in their stools; on microscopic exam, these stools contained numerous red blood cells and polymorphonuclear leukocytes. The median duration of illness was 2.5 days.

Although severe cases of *V. parahaemolyticus*-associated gastroenteritis requiring hospitalization do occur, the illness is usually mild or moderate. Deaths have not been reported in the United States; however, in Japan, of 81,534 cases reported between 1965 and 1974, 31 (0.04%) deaths were recorded (62). Most patients do not require antimicrobial therapy, but those with severe or protracted cases may benefit from treatment with tetracycline (4).

The first published indication that *V. parahaemolyticus* might cause extraintestinal infections appeared in 1969, when Twedt et al reported that 14 strains of halophilic vibrios isolated from extraintestinal sites of patients in the United States and submitted to CDC were *V. parahaemolyticus* or closely related organisms (105). Zen-Yoji et al subsequently found that 6

of the 14 organisms were indeed *V. parahaemolyticus* (111). By 1976 CDC had received 18 *V. parahaemolyticus* isolates from extraintestinal sites (35). Between 1970 and mid-1979, at least nine case reports of extraintestinal infections associated with isolation of *V. parahaemolyticus* were published (14, 60, 73, 78, 85, 103, 108, 113). However, two of these (85, 113) were subsequently reported to be caused by *V. vulnificus* (lactose-fermenting *Vibrio*) (35, 109).

In the seven reported instances in which *V. parahaemolyticus* was isolated from extraintestinal sites, five isolates were from wounds of the feet or legs, one was from an ear, and one was from blood. The patient with septicemia had preexisting underlying disease (cirrhosis); he had diarrhea followed by chills and fever, and three blood cultures all yielded *V. parahaemolyticus* (103). The pathogenic significance of the organism in some of the other cases is uncertain, but it probably caused some of the illnesses. It was isolated from pus from an infected foot laceration in pure culture (60) and from synovial fluid from a patient with synovitis of the knee who had suffered a puncture wound near the patella (78). All of the patients recovered except for the one with septicemia; he died of hepatic failure, which probably was not directly related to his infection.

Ecology

V. parahaemolyticus is part of the normal flora of estuarine and other coastal waters throughout most of the world. It has been isolated from seawater, sea mud, or seafoods in Asia, North America, Australia, New Zealand, Africa, Hawaii, and Europe. It has also been isolated from fresh water and freshwater fish in India (24).

V. parahaemolyticus absorbs onto chitin and onto copepods (minute crustacean animals) (49); most of the organisms in water are associated with zooplankton. In three temperate regions—Japan (64), Korea (19), and the United States (48)—large numbers of *V. parahaemolyticus* organisms can be isolated from seawater in summer but not in winter. However, the organism can be isolated from sediment even in winter (48). *V. parahaemolyticus* apparently passes the winter in sediment, is released from the bottom in the spring and becomes attached to zooplankton, and then proliferates as the water temperature rises (50).

Pathogenicity

In 1968, Sakazaki et al (90) reported that the ability of *V. parahaemolyticus* to cause hemolysis on Wagatsuma agar (the Kanagawa phenomenon) was associated with gastrointestinal illness. They found that 96.5% of isolates from patients with diarrhea were hemolytic (Kanagawa-positive), com-

pared to only 1% of isolates from seafoods and seawater. Subsequent experience has confirmed this very close association between Kanagawa positivity and gastrointestinal illness. However, isolates from extraintestinal infections tested at CDC have been found to be Kanagawa-negative. To our knowledge, the only reported extraintestinal isolate that proved to be Kanagawa-positive was from a patient with septicemia whose illness began with diarrhea (103).

The Kanagawa reaction is caused by a heat-stable direct hemolysin with a molecular weight of approximately 42,000 (38). The heat-stable hemolysin is cytotoxic to human FL cells in cell culture (92), cardiotoxic in vivo for mice (36), and cardiotoxic in vitro for mouse heart cells (36). Although changes have been described in the electrocardiograms of acutely ill patients (39), the clinical importance of the heat-stable hemolysin is unknown. Live *V. parahaemolyticus* does not induce intestinal fluid accumulation in infant rabbits (15). Sakazaki et al (91) found that 14 (88%) of 16 living cultures of Kanagawa-positive strains produced dilation and inflammation in the ligated rabbit ileal loop test, whereas only seven (22%) of 32 Kanagawa-negative strains were positive in the test. Sections of the gut loop of a positive test revealed mucosal disarray with necrosis, ulceration, and hemorrhage. Although they and others found that cell-free filtrates from both Kanagawa-positive and Kanagawa-negative strains concentrated by lyophilization produced positive rabbit ileal loops, this response has been attributed to the high (>20%) concentrations of NaCl produced by the concentration method (45). Culture filtrates and whole-cell lysates did not produce positive adult rabbit ileal loop responses when tested by Johnson & Calia (45).

Donta & Smith (25) found that filtrates from cultures of *V. parahaemolyticus* strains from patients with gastroenteritis did not effect either morphologic changes or steroidogenesis in Y-1 adrenal cells. However, Honda et al detected a heat-labile factor in culture filtrates of Kanagawa-positive *V. parahaemolyticus* organisms that produces a reaction in CHO cells similar to that produced by cholera toxin (37). The reason for the disparate findings is unclear.

There is some evidence that *V. parahaemolyticus* invades the intestinal tissue of humans; ulceration of the rectosigmoid was observed in one US patient (13), and leukocytes and blood have been observed in the stools of patients in Bangladesh (42). Kanagawa-positive, but not Kanagawa-negative, organisms can penetrate the intestinal epithelium of infant rabbits (15). All are Sereny test negative (15). Carruthers observed that while both Kanagawa-positive and Kanagawa-negative *V. parahaemolyticus* can cause cytotoxic effects on HeLa cell cultures, Kanagawa-positive organisms act more rapidly (16). Nonviable preparations have no effect on HeLa cells.

Ohashi et al (71) developed a reversed passive hemagglutination test which they found to be a more sensitive method than Wagatsuma agar to detect the heat-stable hemolysin responsible for the Kanagawa reaction. They found that all Kanagawa-positive isolates from humans with diarrheal disease, 32 (51%) of 63 Kanagawa-negative isolates from feces of humans with diarrhea, and none of 96 Kanagawa-negative isolates from seafoods and kitchen swabs produced the hemolysin.

Carruthers found that Kanagawa-positive *V. parahaemolyticus* adheres rapidly to HeLa cells and to human fetal intestinal (HFI) cells, whereas Kanagawa-negative *V. parahaemolyticus* does not adhere to HeLa cells and adheres to HFI cells at a much slower rate (17).

Although it is possible to detect antihemolysin antibodies (antibodies against the thermostable direct hemolysin produced by Kanagawa-positive strains of *V. parahaemolyticus*) in specimens from many patients 5–10 days after admission, many patients with typical cases have no increase in titer, and this test is not currently used for diagnosing illness (67).

Despite extensive study of the pathogenic mechanisms of *V. parahaemolyticus,* we do not yet understand how it causes gastrointestinal disease in humans.

Epidemiology

During the last decade, *V. parahaemolyticus*-associated gastroenteritis has been reported in North America, Central America, Africa, Europe, and Asia. Extraintestinal infections have been reported in North America, Europe, Asia, and Australia. In only a few countries have surveys given any indication of the importance of the organism as a cause of gastrointestinal illness (63). In Calcutta, India, in a one-year study of 3,433 diarrheal stools, *V. parahaemolyticus* was isolated from 378 (11%). In two studies in Indonesia, the organism was isolated from 2.6 and 3.7% of patients with gastroenteritis. In Thailand, 10.7% (850/7930) of stools from patients with diarrhea at an infectious disease hospital yielded the organism. In Vietnam, *V. parahaemolyticus* was isolated from 8.5–15% of patients with diarrheal illnesses. In Korea, 3 (1.5%) of 199 persons with enteric infections had the organism isolated from their stools. In Japan (1965–1974), 24% (81, 534/345,738) of reported cases of food poisoning were attributed to *V. parahaemolyticus.* The organism was isolated from only 0.3% of 2,000 healthy Japanese (112). In the United States, except in occasional large outbreaks (3, 55), *V. parahaemolyticus* is rarely isolated from patients with gastroenteritis; however, the incidence of the disease is unknown because most laboratories do not use culture media appropriate for isolating vibrios from stools.

Marked seasonality of the disease has been noted in the United States (3), Thailand (63), and Japan (63). In the United States and Japan, outbreaks of the disease occur almost exclusively during the warm summer and early fall months; in Bangkok, Thailand, the proportion of persons with diarrheal illness from whom *V. parahaemolyticus* is isolated varies from 3.9% in the relatively cool month of January to 22.6% in the warm month of September. This seasonal variation may reflect both enhanced opportunity for *V. parahaemolyticus* to multiply in unrefrigerated foods during the summer months and the increased prevalence of *V. parahaemolyticus* in the environment during warm weather. Extraintestinal isolations also appear to occur during the warm months: the four cases with known dates occurred in June–October.

V. parahaemolyticus—associated gastroenteritis appears to be transmitted exclusively by food, usually raw or cooked seafood, although sometimes other foods presumably cross-contaminated by raw seafood have been thought to transmit the infection. In India, a study showed that one third of 60 patients with *V. parahaemolyticus*-associated gastroenteritis denied having eaten fish or shellfish during the previous seven days (97).

Several mechanisms have contributed to *V. parahaemolyticus*-associated food poisoning (3). In outbreaks involving raw seafoods, food naturally contaminated with small numbers of vibrios was held unrefrigerated long enough to allow organisms to proliferate to very large numbers. Outbreaks involving boiled shrimp have been attributed to failure to cook the shrimp at temperatures high enough to kill vibrios. Other outbreaks have been attributed to contamination of cooked seafood by raw seafood, followed by inadequate refrigeration.

The generation time of *V. parahaemolyticus* has been reported to be as short as nine minutes under ideal conditions (52), enabling the organism to multiply very rapidly in mishandled foods and quickly reach the rather large infecting dose; the ID_{50} has been determined in volunteer studies to be about 10^5–10^7 organisms for persons given antacids (93). The optimum temperature for growth is 37°C, although it will grow well at 25–44°C.

Five of the six persons with wound and ear infections had a history of recent exposure to seawater, and the sixth person was in an airplane crash near a seacoast. No history of exposure was given for the person with diarrhea and septicemia (103), who was presumably infected by the oral route.

The principal reservoir for *V. parahaemolyticus* is seafoods and seawater, usually in coastal areas. However, in Calcutta, India, *V. parahaemolyticus* has been isolated from river water, pond water, and freshwater fish (24).

Although persons with asymptomatic infections have been found, there are no reports of long-term carriage of the organism, and there is no evidence that infected foodhandlers have been a source of the organism in disease outbreaks.

In most common-source outbreaks of *V. parahaemolyticus*-associated gastrointestinal disease in Asia and the United States in which patients did not have bloody stools, the incubation period has ranged from 4 to 96 hr, with a mode of 15 hr (3, 66). However, the incubation period may be shorter for the dysenteric illness caused by *V. parahaemolyticus;* in a small outbreak in Bangladesh, incubation periods ranged from 20 min to 9 hr, with a median of 2.5 hr (42). The reason for the apparently shorter incubation period for dysenteric illness has not been explained. For extraintestinal infections, the incubation period is usually one to two days (12).

By 1976, 12 O-antigens (heat-stable somatic antigens) and 59 K-antigens (capsular or envelope antigens) had been identified (65). Many different serotypes have been found throughout the world, and the most common serotypes vary from place to place. No particular serotypes have been strongly associated with the Kanagawa phenomenon or with human illness. Thus, for practical purposes, determining *V. parahaemolyticus* serotypes is useful chiefly in epidemiologic studies of outbreaks.

VIBRIO VULNIFICUS

Introduction

Beginning in 1964, the Special Bacteriology Section of CDC occasionally received isolates from extraintestinal sites that were at first thought to be variants of *V. parahaemolyticus* but were later differentiated from *V. parahaemolyticus* by several biochemical reactions, including fermentation of lactose, and were referred to as the lactose-fermenting (L+) *Vibrio* (35, 109). This *Vibrio* has been shown by deoxyribonucleic acid reassociation experiments to be a separate species (20). Baumann et al studied 14 L+ *Vibrio* strains from CDC, assigned them to a group they designated C-2 (7), and then named them *Beneckea vulnifica* (82). This name has not been widely used, and Farmer (27) has proposed that the species be called *Vibrio vulnificus,* the name that we will use here. By mid-1979, reports of 41 cases of *V. vulnificus*-associated infection had been published (11, 30, 57, 61, 85, 104, 113), including two cases that were initially identified as *V. parahaemolyticus*-associated infections (85, 113) and four that were identified only as halophilic vibrios (cases 1 and 2 of Reference 104; cases 2 and 3 of Reference 30).

Clinical Features

Data for 39 of the cases mentioned above were included in our description of the clinical characteristics and epidemiology of *V. vulnificus* isolates submitted to CDC between 1964 and 1977 (11). The cases had two distinctly different clinical presentations. Fifteen illnesses began with signs of infection in a preexisting wound or ulcer (the "wound-infection" group). Twenty-four illnesses initially had no apparent primary focus of infection and began with systemic symptoms suggestive of septicemia—the abrupt onset of chills, fever, and/or prostration (the "primary-septicemia" group).

The patients with wound infections rapidly developed swelling and erythema around a recent wound or, in one case, a chronic stasis ulcer. The lesions often extended to involve adjacent areas, vesicles or bullae appeared (33%), and necrosis (27%) sometimes ensued. *V. vulnificus* was isolated from all cultured wounds. Some patients had fever (80%) and chills (46%) after signs of a wound infection appeared, and three had positive blood cultures. One third of the patients with wound infections had underlying disease—diabetes (two), alcoholism (one), chronic congestive heart failure and stasis ulcers (one), and leukemia and diabetes (one). All were treated with antibiotics to which *V. vulnificus* is susceptible (35), and many had their lesions debrided or incised and drained. Most were recovering within several days, and only one death occurred; the patient with leukemia developed lymphangitis, bacteremia, high fever, and shock, and died 36 hr after onset.

The patients with "primary septicemia" usually first noticed malaise followed by chills (82%), fever (92%), and prostration. Shortly after the onset of chills and fever, some had vomiting (21%) and diarrhea (17%). One third had hypotension (systolic blood pressure $\leq$ 80 mm Hg) at some point in the first 12 hr after admission. Metastatic cutaneous lesions appeared in 75%, usually within 36 hr after the onset of illness. Most commonly, erythematous or ecchymotic areas appeared on one or more extremities (usually the lower), bullae or vesicles formed, and necrotic ulcers later formed. A few patients had generalized papular or maculopapular eruptions. *V. vulnificus* was isolated from the blood of most (83%) of the patients with the primary septicemia syndrome; it was also isolated from the lesions of many (55%) of those with secondary cutaneous lesions, suggesting that the lesions were probably caused by hematogenous seeding of vibrios to the affected areas. Preexisting hepatic disease (including four cases of hemochromatosis) was noted for 75% of these patients, and five of the other six had known underlying disease: alcohol abuse or alcoholism (three), thalassemia major (one), and diabetes (one). Forty-six percent died, including all six patients with hypotension on admission and all four with hemochromatosis.

Thirty-eight percent of the patients with primary septicemia underwent surgical procedures—debridement (four), incision and drainage (one), fasciotomy (one), and leg amputation (three)—and all were treated with antibiotics to which *V. vulnificus* is susceptible. Most of those who survived became afebrile within several days. Most of the deaths were of persons who were already desperately ill when antibiotic therapy was begun; the median interval from onset of therapy to death was one day. The deaths appeared to be caused by intractable shock secondary to Gram-negative sepsis.

Ecology

Little is known of the geographic distribution and ecology of this organism. CDC has received isolates from water from a bay in Guam and from an oyster thought to have been harvested from the Gulf of Mexico. The reservoir of the organism in nature is apparently seawater, where it is probably part of the normal marine flora like the closely related *V. parahaemolyticus* (48).

Pathogenicity

The pathologic findings associated with wound infections caused by *V. vulnificus* have not been well described. However, a detailed description of the autopsy findings for a person with an apparent case of primary septicemia in Japan has been published (57). Microscopic examination of bullous skin lesions showed acute necrosis in the upper dermis without inflammatory cellular response, acute inflammation and necrosis of the fibroadipose tissue in the lower dermis and around the vessels, and transmural acute necrotizing vasculitis in subcutaneous tissue without thrombosis. The patient also had acute necrotizing epididymitis and orchitis, and acute splenitis with Gram-negative bacterial invasion. Necrotizing vasculitis was also noted in a leg amputated from one of the patients in the United States (30).

V. vulnificus has been studied in animal models by Poole & Oliver (77). They found that subcutaneous injections of the organisms into mice caused severe local infections with gross edema followed by tissue necrosis and death. The edema appeared to be caused by massive vascular leakage of plasma proteins, and death apparently was caused by generalized loss of intravascular fluid with hemoconcentration and hypotension. Although force-feeding *V. vulnificus* to mice caused no disease, injection into ligated rabbit and rat ileal loops caused bacteremia and death.

Epidemiology

V. vulnificus infections have been reported from Japan (57), Belgium (61), and 16 states in the United States (11). All but three of the US cases

occurred in the period May to October, the warmer half of the year. Most of the patients were males over 40 years of age.

The US wound infections appeared to have been caused by contamination by seawater (12 patients) or saltwater crabs (two patients); only one of the patients had no known history of such contacts. Significantly more patients with wound infections than patients with septicemic onset had been in recent contact with seawater ($p<0.001$). Many patients with septicemic onset appeared to have been infected by eating raw oysters; significantly more patients with primary septicemia than patients with wound infections were known to have eaten raw oysters within one week of onset of illness ($p<0.001$), and all 19 patients who had septicemic onset and known oyster-eating habits often ate raw oysters (11). The median incubation periods were 12 hr for wound infections and 16 hr for primary septicemias.

VIBRIO ALGINOLYTICUS

Introduction

V. alginolyticus was not thought to be pathogenic for humans until 1973, when six isolates from tissue infections that had been reported by Twedt et al (105) in 1969 to be *V. parahaemolyticus* were found by Zen-Yoji et al (111) to be *V. alginolyticus.* In the same year, Von Graevenitz & Carrington (108) published the first case report, that of an ear infection apparently caused by *V. alginolyticus.* By mid-1979, at least 20 case reports of infections associated with *V. alginolyticus* isolates had been published (26, 33, 60, 73, 75, 76, 86, 87, 100, 108). In addition, in 1978 Prociv reported isolating *V. alginolyticus* from 20 wound infections in Western Australia (80). Hollis et al at CDC reported in 1976 that they had received 42 strains of *V. alginolyticus* from human extraintestinal sources for identification (35), but some of these strains came from patients whose case reports had already been published.

In some of the reported incidents, as when *V. alginolyticus* was isolated in pure culture from blood or wounds, the organism probably caused disease. However, when chronic ulcers and external otitis were present and when multiple potential pathogens were isolated, it is not clear whether *V. alginolyticus* caused the disease.

Clinical Features

Little detail about clinical features is given in most of the 20 case reports. The isolates came from wounds (nine), cutaneous ulcers (two), a burn, and ear drainage (eight); the patient with isolates from burns also had an isolate from blood.

The wounds were of the legs and feet (six), fingers (two), and scalp (one). Seven of the injuries occurred while swimming or at the seashore. The lesions, when described, were generally swollen and erythematous. Prociv wrote that most of 20 wound infections in Australia were "associated with a varying degree of cellulitis and a seropurulent exudate" (80). Five of the nine case reports of wound infections stated that *V. alginolyticus* was isolated from the wound in pure culture. None of the patients were reported to have chronic underlying disease, and none were seriously ill. Most of the patients were treated with a systemic antibiotic, usually one to which the organism was resistant, and with debridement or incision and drainage of the wound; all recovered. Of Prociv's 20 patients with wound infections, none were treated with antibiotics and most infections had cleared within one or two days after becoming apparent. Thus, it is not possible to determine from the published reports the effect of treatment with antibiotics to which the organism is susceptible.

One of the isolates from cutaneous ulcers came from a man with sickle cell anemia and chronic leg ulcers (86). When examined, he had serosanguinous fluid draining from bilateral malleolus ulcers and fever (temperature of 37.8°C); a culture of one of the ulcers yielded *V. alginolyticus* and *Staphylococcus aureus.* The other isolate from a cutaneous ulcer came from a man with chronic venous insufficiency of the right leg (*75*). When examined, he had a tender, erythematous 1.5-cm leg ulcer of four weeks' duration but he had no history of trauma to the area. *V. alginolyticus, Staphylococcus aureus,* and *Klebsiella pneumoniae* were isolated in culture. The effect of antibiotic therapy on these two patients was unclear.

The isolate from blood came from a woman who sustained severe burns in a boat explosion and was subsequently immersed in seawater (26). Despite intensive care, she died five days later. On the third day after the accident, some of her burns became macerated and she developed "clinical symptoms indicating a septic condition" (26). Although cultures of the surface of the burns yielded multiple organisms, *V. alginolyticus* was isolated in pure culture from biopsy specimens of burns and from blood.

Three of the isolates from ears came from patients with acute otitis media (60, 73, 108). All three patients had perforated tympanic membranes and developed purulent discharges. *V. alginolyticus* was isolated in pure culture from two patients (60, 73), and a few colonies of anaerobic diphtheroids and *Staphylococcus epidermidis* were also isolated from the third. They were treated with erythromycin (108) or tetracycline (60, 73), antibiotics to which these organisms were susceptible in vitro, and recovered uneventfully. Two isolates came from patients with external otitis (33, 87). One patient had had previous acute attacks; at the time of the reported acute

attack, culture of a swab of the ear yielded a profuse pure growth of *V. alginolyticus* (87). The other patient had a draining chronic external otitis; an atypical *V. alginolyticus* was only one of several species isolated (33). No clinical details are given for three patients from whom isolates were obtained from ears (76); *V. alginolyticus* was the only organism isolated from two of these persons.

Ecology

Seawater is the normal habitat for *V. alginolyticus,* and it has been isolated from seawater and seafood in many parts of the world (5, 47, 54, 81, 107). Studies by Baross & Liston of oysters in Washington State showed that *V. alginolyticus* was rarely found in winter, but the counts rose rapidly with increasing water temperature, and the organism was abundant in summer (5). The minimum growth temperature for *V. alginolyticus* is 8°C (5).

Pathogenicity

The pathogenic mechanisms of *V. alginolyticus* have received little attention. To our knowledge, no studies have been done to determine if isolates from human infections can be differentiated from environmental isolates.

Epidemiology

V. alginolyticus is reported to have been isolated from extraintestinal sites from persons exposed in Australia (60, 80), Europe (33, 73, 87), Mexico (100), Hawaii (76), and all three coasts of the United States (26, 75, 86, 108). The infections with reported dates occurred in months when seawater is relatively warm. Seventeen (85%) of the 20 patients were male. The 18 patients whose ages were known ranged in age from 8 to 44, with a median age of 22.

All 11 persons with cutaneous infections and known exposures had been exposed to seawater shortly before the onset of clinical signs of infection; the incubation period is stated for only one patient, for whom it was 24 hr (86). The incubation period in Prociv's series of 20 *V. alginolyticus* wound infections in Australia was also 24 hr (80). Of the seven patients with ear infections and known exposures, six had been swimming in the sea shortly before onset, and one denied any recent exposure to seawater (108). The incubation period (5 days) was reported for one patient (60).

OTHER VIBRIOS POSSIBLY PATHOGENIC FOR MAN

Group F (EF6) Vibrios

Lee et al have described a group of *Vibrio*-like organisms that probably constitute a new species, and they have designated it group F (56). They report that these organisms are widely distributed in the marine and estua-

rine environment around Britain, and they have received similar strains from patients with diarrhea who were infected in Bangladesh, Bahrain, and Jordan. These organisms appear to be similar or identical to organisms designated group EF6 at CDC.

The clinical features and epidemiology of disease associated with isolation of this group of organisms have not yet been well defined, but some information is available from the International Center for Diarrhoeal Disease Research in Bangladesh (ICDDR,B) (formerly the Cholera Research Laboratory). In 1976–1977, hundreds of cases of diarrheal illness associated with isolation of group F vibrios occurred in Dacca and in the area around Matlab in rural Bangladesh. The organism was rarely isolated before and has rarely been isolated since this period. Approximately half the patients were children less than 5 years of age (46). Studies of family members of infected persons showed that group F vibrios were present in the stools of less than 1%. The clinical symptoms were similar to those of cholera, except that some patients had blood and mucus in their stools and some had abdominal pain and fever (46).

Nine strains from ICDDR,B tested at CDC were negative in the adrenal cell, infant mouse, and Sereny tests. Other investigators have reported that the organism kills mice when injected intraperitonially and produces a heat-labile toxin that causes fluid accumulation in ligated rabbit ileal loops (44). Currently, it is not certain that the organism is a pathogen.

Vibrio metschnikovii (Enteric Group 16)

Lee et al (56) state that the organism called *Vibrio metschnikovii* by Lee, and Enteric Group 16 by CDC, is widely distributed in rivers, estuaries, and sewage and that it has been isolated from the intestines of humans and other animals. However, there is currently no published evidence that the organism causes disease in humans or other animals. CDC has received Enteric Group 16 organisms for identification that were isolated from shrimp and and from the blood of an elderly woman with gallbladder disease.

CONCLUSION

Vibrio infections cannot be prevented unless we know how they are transmitted. Although gaps in our knowledge about the transmission of these infections remain, seafoods eaten cooked or raw and/or seawater are clearly important vehicles of transmission for non-O1 *V. cholerae, V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus.*

When seafoods are eaten cooked, preventing infection is simple; regardless of *Vibrio* content, such foods will be safe if they are cooked at high enough temperatures to sterilize them and if they are protected from cross-

contamination after cooking and then held until eaten at temperatures too cold (≤4°C) or too hot (≥60°C) to permit multiplication of vibrios. A common problem is that seafoods that are considered cooked by traditional criteria are not sterile (10, 53).

When seafoods are eaten raw, prevention of *Vibrio* infections is difficult, and it may be impossible to guarantee that any raw seafood is totally safe. Current programs to minimize the risk from eating raw shellfish, particularly oysters, attempt to prevent consumption of shellfish that have been subjected to fecal contamination. When successful, such programs should prevent hepatitis A, typhoid fever, viral gastroenteritis, and other diseases caused by contamination by human feces. However, this approach is probably ineffective against organisms that are pathogenic for man and that are probably part of normal estuarine flora: *V. vulnificus, V. parahaemolyticus,* and possibly non-O1 *V. cholerae.* It may be prudent for persons with underlying disease such as cirrhosis or leukemia to avoid raw seafood altogether, since these persons seem to be far more likely than others to have septicemia after eating raw seafood. The clustering of *V. vulnificus* and non-O1 *V. cholerae* disease during warm weather suggests that the risk is greatest in those months, whether because of greater numbers of vibrios in warm seawater or multiplication of vibrios in mishandled seafoods after harvesting. Perhaps even healthy persons anxious to minimize the risks of foodborne *Vibrio* infections should not eat raw seafoods during the warm summer and fall months. Direct measurement of the numbers and types of vibrios in shellfish and shellfish waters as suggested by Colwell & Kaper (21) may eventually be a useful adjunct to fecal coliform counts in determining when raw shellfish from a growing area present relatively little hazard to consumers. However, much more work on the ecology, epidemiology, and pathogenicity of *Vibrio* species must be done before such *Vibrio* counts can be considered for incorporation into shellfish regulations.

Prevention of *Vibrio* wound infections may be impossible, since injuries frequently occur during recreational and occupational exposures to seawater, and most seawater contact occurs when the water is warm. Thorough cleansing of wounds after exposure to seawater or raw seafoods might prevent disease, but the effectiveness of this measure has not been evaluated. Swimmers with preexisting ear disease could probably prevent otitis by keeping seawater out of their ears.

The importance of *Vibrio* infections as a cause of morbidity and mortality in the United States, and thus the importance of taking preventive measures, is still unknown. Certainly the small numbers of vibrios isolated from persons with infections and submitted to CDC for identification represent only a small fraction of the number of infections that occur. The numbers of strains of non-O1 *V. cholerae, V. vulnificus, V. alginolyticus,* and *V.*

parahaemolyticus submitted to CDC each year have increased dramatically in the past decade, a pattern which must represent primarily increased ascertainment rather than increased incidence of disease. Heightened awareness of clinicians, microbiologists, and epidemiologists about the existence of these pathogens and the settings in which they should be suspected, and collaboration between these professions, can lead to better understanding and control of *Vibrio* infections in the coming decade.

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observed in human acute promyelocytic leukemia cell lines, is the 20–30-fold amplification in the number of copies of *Myc* per cell. This amplification is correlated with over-production of apparently normal *Myc* mRNA.

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Forthcoming Events

13–15 NOVEMBER 1985

Principles and Mechanisms of Neurotoxicity: The Johns Hopkins University, School of Hygiene and Public Health, Department of Environmental Health Sciences, offers a course dealing with the principles and mechanisms of toxicity of chemicals whose target organ is the nervous system.

Further information may be obtained from: Dr Jacqueline Corn, Director, Continuing Education Program, Department of Environmental Health Sciences, The Johns Hopkins School of Hygiene and Public Health, 615 North Wolfe Street, Room 1101, Baltimore, Maryland 21205, USA (tel.: (301) 955-2609).

27–30 AUGUST 1986

III World Congress of the World Federation of Associations of Clinical Toxicology and Poison Control Centres

and the

XII International Congress of the European Association of Poison Control Centres (EAPCC)

will be held in the Brussels Congress Centre on 27–30 August 1986. The main topics of the Congress will be: toxicokinetics and pharmacokinetics in clinical toxicology; new investigation techniques, immunotoxicology – immunotherapy, paediatric toxicology, new solvents, medical aspects of environmental pollution, standardization and criteria for data of poison control centres and, ethics, deontology and responsibility of poison control centres.

Further information can be obtained at the Administrative Secretariat: SDR Associated, Rue Vilain XIIII, 17 a, B-1050 Brussels, Belgium.

Book Review

RITA R. COLWELL (ed)
Vibrios in the Environment
John Wiley & Sons, New York, 1984; 634 pp., $59.85

This volume includes virtually every aspect of the biology of the Vibrionaceae. Sections are included on molecular genetics, serology, pathogenicity, taxonomy and ecology of this diverse group of bacteria. A great deal of emphasis is placed on the biology of *Vibro cholerae*, the causitive agent of cholera, but the pathogenic potential of vibrios such as *V. parahaemolyticus* is not overlooked.

Several chapters may prove to be especially significant. Siebeling, Adams, Yusef and Larson present a newly developed serological typing scheme to identify *V. cholerae* collected from the United States. This 'local' serological system may supplement that of the Sakazaki system. Several chapters deal with the molecular taxonomy of *Vibrio* spp. Especially interesting is the similarity between the cholera toxin and the heat-labile enterotoxin of *Escherichia coli*. The possibility is discussed by Mekalanes that the toxin gene is on a transposable element that may provide a mechanism for infection of non-toxinogenic strains of vibrios. West and Colwell provide an exhaustive discussion on the identification and classification of the Vibrionaceae.

The section on ecology emphasized the distribution of *V. cholerae* in aquatic habitats. Interestingly the presence of *V. cholerae* and fecal choliforms are negatively correlated, indicating that the vibrio maybe a native of many environments. Lastly, several chapters investigate the prevention of disease in seafood.

The emphasis on *V. cholerae* is understandable in the light of its potential pathogenicity but the ecology and molecular biology of the other vibrios would have been interesting from a comparative viewpoint. The study of this family of bacteria is also exciting from the aspect of following the movement of a toxicogenetic element among strains of related organisms. Currently a great deal of discussion is revolving around the fate and effects of genetic elements introduced into the environment. The vibrios may serve as a naturally occurring model of such a system.

The majority of the book is produced from camera-ready typed copy but is, in general, very readable. An extensive index is provided. I would certainly recommend this book to not only those interested in the causal organism of cholera but also to microbiologists in general.

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Vibrio cholerae in Marine Foods and Environmental Waters: A Literature Review

ANGELO DePAOLA

ABSTRACT

Seafoods have been implicated in a number of cholera outbreaks in the United States and abroad. The incidence of *Vibrio cholerae* in the environment may be attributed to its extended survival in seawater or to short- or long-term carriers. Strains of *V. cholerae* which do not agglutinate in O group 1 antiserum (NAG) have been associated with a number of gastroenteritis, septicemia, and meningoencephalitis cases in recent years, and genetic studies illustrate a close relationship between the deoxyribonucleic acid (DNA) of NAG and *V. cholerae*. The ability of NAGs to elicit responses in toxigenicity testing systems similar to those caused by *V. cholerae* has also been demonstrated. The incidence of NAGs and *V. cholerae* in U.S. coastal waters and seafoods, their survival, and the conditions that promote genetic changes and increase NAG virulence must be fully evaluated to determine the public health significance of these organisms. These studies are needed to reevaluate the effectiveness of the National Shellfish Sanitation Program (NSSP) growing area standards and criteria so that consumers may be protected against shellfish-associated vibrio diseases.

INTRODUCTION

AN OUTBREAK of cholera occurred after the consumption of home-cooked crabs in Louisiana in 1978; a cholera-like disease caused by strains of *Vibrio cholerae* which do not agglutinate in O group 1 antiserum (NAG) also occurred after the consumption of raw oysters in the same area. Recent data indicate that NAG strains of *V. cholerae* may be ubiquitous in estuarine waters and are not necessarily associated with domestic sewage.

These situations have resulted in a renewed interest in the public health significance of the vibrio group as a cause of seafood-associated gastroenteritis in the United States and an urgent need to reevaluate the effectiveness of National Shellfish Sanitation Program (NSSP) standards and guidelines for the classification of shellfish growing area waters.

CHOLERA IN SEAFOOD AND THE ENVIRONMENT

Epidemiology of seafood-related outbreaks

Seafood products have been increasingly incriminated in cholera outbreaks since the 1960s, especially in the Western Hemisphere. An explosive outbreak of *Vibrio cholerae* El Tor in the Philippines during 1961 and 1962 was reported by Joseph et al. (1965), who suggested that the initial infection was transmitted principally by shrimp that were consumed raw. Dutt et al. (1971) investigated the role of shellfish in vibrio infections during the 1969 cholera outbreak in Kelantan, Malaysia. El Tor and NAG strains were isolated from water and shellfish and from the stools of suspected patients and contacts. In 1973 a cholera epidemic caused by the El Tor biotype began in Naples, Italy, and resulted in 25 deaths among the 278 bacteriologically confirmed cases (Baine et al., 1974). The suspected vehicle of transmission was raw seafood, particularly mussels washed with dockside water that may have been contaminated with *V. cholerae*. One year later in Portugal, 2467 bacteriologically confirmed cases in which 48 deaths occurred were reported (Blake et al., 1977). *V. cholerae* biotype El Tor was isolated from 42% of the shellfish samples tested, and consumption of raw or partially cooked cockles was significantly more common among cholera patients than paired controls. During the same period six cases of cholera occurred in Guam and were associated with home-preserved fish (Merson et al., 1977). In the United States the first case of cholera since 1911 was reported in Texas in 1973. Although the source of the infection was undetermined, the individual had consumed raw oysters (Weissman et al., 1974). In 1977 a similar case occurred in Alabama in an individual who had eaten large quantities of raw oysters for many years (Cameron et al., 1977). *V. cholerae* El Tor Inaba was isolated from the stools of 11 persons who displayed 8 symptomatic and 3 asymptomatic infections in Louisiana in the fall of 1978 (Bradford and Caraway, 1978). A relationship was noted between infected individuals and consumption of boiled and steamed crabs, and *V. cholerae* was isolated from some leftover crab. A single raw shrimp sample was also found to be positive for El Tor organisms. In a Food & Drug Administration (FDA) investigation, samples of blue crab from the suspect areas revealed numerous nonagglutinable strains of *V. cholerae*, but O-1 serotypes were not found (Twedt, personal communication). In October 1978, FDA began monthly monitoring of shellfish, water, and muds from 14 sampling sites from Florida to Texas for the presence of *V. cholerae* (Hunt, 1978).

A summary of these cholera outbreaks (Table 1) suggests a westward migration of El Tor from Asia to Europe and finally to North America. If this trend continues future outbreaks can be predicted in North and South America. Most of the infections were caused by ingestion of raw or partially cooked shellfish and crustacea; however, a fin fish was incriminated in the Guam outbreak. In the Eastern Hemisphere cholera has usually infected impoverished people who live in unsanitary conditions. However, in the West, cholera often occurs among the middle class and is usually more severe in individuals with gastric disorders. Most of the outbreaks occur in the late summer and peak in August. Although isolates of *V. cholerae* from the environment were difficult to find during the outbreaks in Italy and Louisiana, they were common in Portugal (42% in shellfish; 24% in water), Guam, Malaysia, and the Philippines. The difficulty in determining the source of *V. cholerae* in outbreaks suggests the organism's prolonged survival periods or perhaps the presence of asymptomatic carriers.

SURVIVAL AND PROLIFERATION IN SEAFOOD AND ENVIRONMENT

THE SURVIVAL of *V. cholerae* under varying conditions has been investigated by a number of researchers. Pollitzer (1959) reviewed the literature on *V. cholerae* and reported a wide range of survival periods depending on experimental conditions. Research conducted in India

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illustrated greater viability in stool samples collected at cooler ambient temperatures. Pollitzer (1959) cited a number of investigations conducted on the survival of *V. cholerae* in seawater during the early part of the century. In general, survival was enhanced by intermediate salinities, lower temperature, high organic content, near neutral pH, dark storage, and absence of competing microflora. Studies were made on the survival of *V. cholerae* in artificially contaminated ocean water and bay water from New York. Survival time in unsterilized bay water was 47 days compared to 7 days for ocean water, but survival for more than 285 days was observed when both ocean and bay water were sterilized before being inoculated with *V. cholerae*.

Pollitzer (1959) also reviewed investigations of the survival of *V. cholerae* in seafoods. Japanese researchers reported uptake of *V. cholerae* by oysters and clams kept in cholera-polluted seawater. The vibrios entered the gastrointestinal tract of the shellfish and survived for 1.5 months at 0-5°C and 15-20 days at 22°C. In shucked oysters smeared with cholera vibrios and stored at 20°C, the number of organisms first decreased, then increased, with the maximum number occurring after 68 hr. A gradual decrease followed and most vibrios disappeared by 171 hr. Vibrio survival increased to 20 days on oysters and clams that had been sterilized or boiled before contamination. *V. cholerae* survived only a few days on fish stored at room temperature but persisted for more than 3 wk when refrigerated. Felsenfeld (1963) compared the survival time of classical and El Tor biotypes in shallow well water and in the stomachs of fish. Mean survival time for El Tor biotypes was 19.3 days, while classical cholera organisms survived for 7.5 days in water; both biotypes disappeared from fish stomachs within a mean time of 5.3 days. Felsenfeld (1965) determined survival under "natural" contamination conditions by inoculating various foods with vibrio-free stool material to which a saline suspension of cholera organisms was added. Survival periods of less than 1 wk in raw and cooked catfish stored at room temperature increased up to 3 wk when the product was stored at 2°C. Pesigan et al. (1967) contaminated various foodstuffs and water directly with stool material from cholera patients and carriers to determine viability of El Tor vibrios. Vibrios in raw seafoods survived 2-4 days when stored at 30-32°C and 4-9 days when stored between 5 and 10°C. Likewise, El Tor vibrios survived in seawater for 10-13 days at 30-32°C and 58-60 days at 5-10°C. Venkatraman and Ramakrishnan (1941) reported that *V. cholerae* survived for 198 days in 2% sea salt solution. Miyaki et al. (1967) observed a survival time for El Tor vibrios of more than 1 month in various foodstuffs frozen at −20°C and much longer in foods frozen at −72°C. Optimum conditions for growth and survival were pH 6-8 and osmotic pressure 250-700m Osm.

V. cholerae strains other than those agglutinable in cholerae O group 1 antiserum were reported by Colwell et al. (1977) in water samples from the Chesapeake Bay. In a later publication Kaper et al. (1979) reported that these vibrios are indigenous to Chesapeake Bay waters, sediments, and shellfish and usually occur when the salinity is between 5 and 15 ppt. Kaneko and Colwell (1973) reported that vibrio species and related bacteria associate with zooplankton in summer months and that chitinase activity in vibrios plays a role in this association. The chitinase activity may increase the affinity to crustacea, thus explaining why *V. cholerae*, a chitin digester, is found more frequently in crustacea (crabs and shrimp) than in other seafoods. The chitinase activity may also prolong the survival of *V. cholerae* by providing a maintenance or growth substrate. Some strains of *V. cholerae* can survive for long periods in estuarine environments with moderate salinities (5-15 ppt) where most seafood is harvested. Survival is further enhanced in cool seasons and when the amount of organic matter is high.

Cholera carrier state

The presence of *V. cholerae* organisms in waters of areas which have been free from cholera infection for decades could be explained by the existence of asymptomatic carriers. Sayamov (1963) injected El Tor and classical cholera organisms into the gall bladder of rabbits and noted that the cholera multiplied in the bile system throughout the experiment (15 wk). Cholera carrier studies in the Philippines showed a prevalence rate of 21.7% among household contacts of cholera patients and 8.4% for next door neighbors compared to 0.35% in the general population (Dizon et al., 1967). During the carrier state of 5-19 days, the vibrio concentration in stool samples was between 10^2 and 10^5/ml in carriers compared to 10^6-10^9/ml in cholera cases. Azurin et al. (1967) reported a long-term carrier of El Tor cholera who excreted vibrios intermittently from 1962 through 1966. Duodenal intubation demonstrated that the vibrios were lodged in the biliary tract. Barui et al. (1972) compared detection methods of *V. cholerae* in inapparent infections collected by purgation and multiple rectal swabs. The detection rate was 94% with rectal swab cultures and only 54% with a single purge.

Because of the wide geographical spread of cases and the lack of previous and subsequent cases in the area, it is doubtful that a long-term cholera carrier was responsible for the Louisiana outbreak. Asymptomatic carriers among Southeast Asian immigrants living in the area were considered as a possible source, but no isolates were recovered by Moore swabs in sewers downstream from residences and no clinical cases were reported in the group. The *V. cholerae* strains from Louisiana and Port Lavaca, Texas, were more hemolytic than those from the Eastern Hemisphere, but Ganguly et al. (1966) have reported that the hemolytic ability of *V. cholerae* increases after exposure of the organism to low levels of chlorine, which is used more extensively in the United States than in Southeast Asian countries.

–Continued on next page

Table 1—Epidemiology of seafood related outbreaks of cholera

Location	Year	Month(s)	Strain	Cases	Deaths	Food	Source
Philippines	1961	Nov	El Tor	330	–	Raw shrimp	Unknown
Malaysia	1969	–	El Tor: Inaba Ogawa NAG	135	–	Shellfish	Water
Italy	1973	Aug-Oct	El Tor: Ogawa	278	25	Raw mussels	Tunisia
Portugal	1974	May-Nov	El Tor: Inaba	2467	48	Raw shellfish	African colonies
Guam	1974	Jul-Aug	El Tor: Ogawa Inaba	6	0	Raw fish	Sewage
Texas	1973	Aug	El Tor: Inaba	1	0	Raw oysters	Unknown
Alabama	1977	Apr	El Tor: Inaba	1	0	Raw oysters	Unknown
Louisiana	1978	Apr-Sep	El Tor: Inaba	11	0	Crab	Unknown

VIBRIO CHOLERAE–NON-O GROUP 1

Epidemiology

In the past, isolates of *V. cholerae* which failed to agglutinate in O group 1 antiserum (NAGs) have been considered to be of little public health significance. International quarantine regulations require vaccination against cholera for persons leaving areas infected with *V. cholerae* O group 1. Serotypes within the O group 1 organisms include Inaba, Ogawa, and Hikojima. However, other vibrios have been associated with cholera-like diarrhea (McIntyre and Feely, 1965; Aldova et al., 1968; El-Shawi and Thewaini, 1969; Dutt et al., 1971; Zafari et al., 1973; Dakin et al., 1974; De Gerome and Smith, 1974; Hughes et al., 1978). McIntyre and Feeley (1965) isolated NAGs from cases of diarrhea in East Pakistan and noted a rise in agglutinating antibody titer to these organisms in approximately 1/3 of the cases. Non-O group 1 vibrios in Heiberg group II were implicated in a gastroenteritis outbreak in Czechoslovakia in 1965 (Aldova et al., 1968). French potatoes were the suspected vehicle of 56 of these diarrheal cases in which no fatalities occurred. Zafari et al. (1973) surveyed Iranian pilgrims returning from Saudi Arabia and found less than one percent to be infected with NAGs, although more than half of the infected individuals had mild to severe diarrhea. El-Shawi and Thewaini (1969) studied NAGs isolated from patients with clinical cholera during the 1966 epidemic of cholera in Iraq and reported cultures belonging to Heiberg groups I, II, IV, and V. Dutt et al. (1971) investigated the role of shellfish in the transmission of vibrio infection in the 1969 Kelantan cholera outbreak and found NAGs in shellfish and stools of suspected cholera patients. Sixty-four passengers aboard an international aircraft from London to Sydney suffered acute gastroenteritis shortly after eating the suspected food vehicle, chopped egg on asparagus (Dakin et al., 1974). Examination of stool cultures yielded NAGs in Heiberg groups I and II. De Gerome and Smith (1974) noted the isolation of a NAG from an American who had prolonged diarrhea after eating raw oysters in New Orleans. Between 1972 and 1975, 26 NAG infections were investigated by the Center for Disease Control (CDC) in Atlanta, GA (Hughes et al., 1978). Fifty percent of the isolates were obtained from stool samples of patients with acture diarrhea. Frequently these patients had recently eaten shellfish (Table 2).

NAGs have also been incriminated in cases of septicemia and meningoencephalitis (Back et al., 1974; Fearrington et al., 1974; Prats et al., 1975; Hughes et al., 1978). Back et al. (1974) reported NAG infections in two persons shortly after they were exposed to marine waters in Sweden: vibrios were isolated from a middle ear infection in one and from a leg wound in the other. NAGs were isolated from cultures of blood and cerebrospinal fluid from a North Carolina man who sustained insect bites that were later exposed to polluted river water (Fearrington et al., 1974). This case was fatal, but the patient had a history of hematemesis caused by esophageal varices. A similar case in Spain was reported by Prats et al. (1975) in which a man with hepatic cirrhosis was found to have noncholera vibrio septicemia and meningoencephalitis. Hughes et al. (1978) noted that nine of the NAG infections reported to CDC from 1972 to 1975 were from tissues or fluids other than the gastrointestinal or biliary tracts. Of this figure, which represents 35% of all reported NAG infections in the U.S. during this period, four deaths occurred. Some patients with systemic NAG infections had recently been exposed through occupation or recreation to salt water. Blake et al. (1979) studied the clinical characteristics and epidemiology of disease caused by an unnamed halophilic, lactose-fermenting vibrio from 1964 to 1977. Septicemia in 39 persons was associated with consumption of raw oysters, exposure to seawater, or injury during the handling of crabs. Twelve deaths occurred in this group, usually with preexisting hepatic disease. The NAG outbreaks are summarized in Table 3.

In general, NAG outbreaks are similar to those caused by O-1 *V. cholerae* but are less severe. Consumption of seafood and exposure to salt water are often associated with NAG infections. Outbreaks usually occur during late summer and early fall. Heiberg group II organisms are most commonly isolated from non O-1 cholera infections, but Heiberg groups I, IV, and V have been reported. The implication of sucrose (–) or more specifically Heiberg group V organisms in diarrheal cases illustrates the need for greater consideration of green colonies on thiosulfate citrate bile salt sucrose (TCBS) medium. The reports of NAG septicemia and meningoencephalitis point to a deviation from the clinical picture shown by O-1 *V. cholerae* and raise the question of the invasive potential of NAGs.

LABORATORY STUDIES ON SEROLOGY, TOXIGENCITY, AND GENETICS OF *V. CHOLERAE* AND NAGS

LABORATORY STUDIES have been conducted to determine the public health significance of NAGs. Smith and Goodner (1965) collected 1426 strains of NAGs from diarrheal outbreaks around the world and classified them in 424 catagories based on Heiberg fermentation patterns, serologic evaluation, and "GIN" reactions (gelatin degradation, indole production, and nitrate reduction). They concluded that this group of organisms exhibited extreme changes in morphology which were closely linked to environmental conditions.

In vitro experiments demonstrated conversion of classical *V. cholerae* to El Tor biotypes when the organisms were subjected to repeated exposures of chlorine (Ganguly et al., 1966). Gangarosa et al. (1967) isolated three serotypes of *V. cholerae* (Ogawa, Inaba, and Hikojima) from the stool of a diarrheal patient who lived in an area where only Ogawa had been reported previously. It was suggested that Inaba and Hikojima types were in vivo mutations. This claim was further substantiated by Sheehy et al. (1966) who reported laboratory infections of *V. cholerae* Inaba in two

Table 2–Review of outbreaks of diarrhea caused by V. cholerae-non-O group 1

Location	Year	Season	Strain	Cases	Deaths	Food	Source
East Pakistan	1965	–	Heiberg I, II	19	–	–	–
Czechoslovakia	1965	Fall	Heiberg II	56	0	French potatoes	–
Iran	1965	–	–	96	–	–	Saudi Arabia
Iraq	1966	–	Heiberg I, II, IV, V	22	–	–	–
Malaysia	1969	–	–	10	0	Raw seafood	–
Louisiana	1972	July	Heiberg II	1	0	Raw oysters	–
Iraq	1973	June	Heiberg I, II	64	0	Chopped egg asparagus	–
United States	1972-1975	–	–	13	0	Seafood	Foreign travel

Table 3—Review of nonenteric diseases in man caused by V. cholerae-non-O group 1

Location	Year	Season	Strain	Cases	Deaths	Manifestation	Source	Predisposing factors
Sweden	1972-1973	Aug-Sep	Serotype 107 and 22	2	0	Middle ear and wound infection	Baltic Sea water	Hematemesis
North Carolina	1973	Oct	–	1	1	Septicemia and meningoencephalitis	River water	Esophageal varices
Spain	1973	Aug	Heiberg II	1	1	Septicemia and meningoencephalitis	–	Hepatic cirrhosis
United States	1972-1975	–	–	9	4	Invasion of biliary tract soft tissue and fluids	Foreign travel, salt water	Significant underlying disease

technicians in the United States. As the infections progressed Ogawa types appeared and were the predominant serotype of *V. cholerae* isolated from the stools of one of these patients. Sack and Miller (1969) demonstrated progressive changes of vibrio serotypes in germ-free mice infected with *V. cholerae*, noting reciprocal conversions of both Inaba and Ogawa serotypes and rough to smooth and smooth to rough transformations. Changes in serotype were correlated with the appearance of serum-agglutinating antibody, and serotypic changes seemed to result from the selective effect of antibody within the intestinal lumen. However, Gallut and Quiniou (1970) studied interactions of classical, El Tor, and NAG *V. cholerae* and hypothesized that bacteriophage could be involved in serological and biochemical changes of *V. cholerae*.

The genetic relationship among organisms within the genus *Vibrio* has also been studied. Citarella and Colwell (1970) examined the polynucleotide relationship among vibrio species by means of deoxyribonucleic acid (DNA) reassociation reactions and chromatography hydroxyapatite. While the complementary polynucleotide sequence of *V. cholerae* and some of the NAG strains was nearly identical, there was little relationship between these organisms and *V. parahaemolyticus*, *V. alginolyticus*, or *V. marinus*. In serological studies of the proteases and alkaline phosphatases of NAGs, Hsieh and Liu (1970) found that these enzymes were indistinguishable from those produced by classical strains of *V. cholerae*, but were distinct from those produced by *V. metschnikovii* and *Aeromonas* species.

The public health significance of NAGs should be determined by their ability to cause disease in humans. Dutta et al. (1963) used the infant rabbit model to determine toxicity of El Tor and NAG vibrios isolated from clinical cholera cases and from cholera-like water vibrios isolated from tanks or rivers. Diarrheal symptoms were noted in rabbits infected intraintestinally by some of the strains in each of these groups, but only the El Tor biotype caused death. However, when these strains were inoculated intraintestinally in rabbits and recovered from the large intestine (rabbit passaged), then used to infect infant rabbits, the virulence was enhanced. The infective dose of "rabbit passaged" strains was reduced from 10^9 to 10^6, and NAGs or water vibrios were able to cause death in infant rabbits. This work was further substantiated by McIntyre et al. (1965) who noted that low inoculum levels of NAGs isolated from diarrheal cases in Pakistan produced diarrhea in in infant rabbits. In comparing the toxins produced by *V. cholerae* and NAGs, Ohashi et al. (1972) found that both toxins evoked positive ileal loop reactions in rabbits, caused fatal diarrhea in suckling mice, and increased permeability in guinea pig skin. Although volume to length ratios in ileal loops were less for NAGs than *V. cholerae*, higher toxicity was produced by NAG toxin in suckling mice. A heat stable "hemorrhagic principle" elaborated by some serotypes of *V. cholerae* and NAGs was also reported. Zinnaka and Carpenter (1972) noted that NAGs isolated from human diarrheal disease caused fluid accumulation in the rabbit ileal loop test and increased vascular permeability in the rabbit skin. Kaper et al. (1979) reported that most NAGs isolated from the Chesapeake Bay produced fluid accumulation in rabbit ileal loops and a positive response in Y-1 mouse adrenal cells.

Although laboratory studies have illustrated similarities between O-1 and non-O-1 *V. cholerae*, the public health significance of the non-O-1 organisms is still controversial. Their ability to produce toxin raises questions about the mechanisms involved. A number of environmental factors, such as elevated temperature or animal passage, may be involved. Although other possibilities may not involve toxigenicity, they could be linked to virulence factors such as motility and adhesion to the gut wall. Finkelstein et al. (1977) that a cell-bound hemagglutinin plays a role in attachment of *V. cholerae* to the epithelial surface of the small intestine. They hypothesized that El Tor vibrios, which have cell-bound hemagglutinin, are better able to establish infection in man than classical biotypes, which produce more toxin but have only a soluble hemagglutinin. The literature does not indicate that animals serve as reservoirs for *V. cholerae* or NAGs. However, a large concentration of wildlife exists in southern Louisiana, and many of these animals, such as birds and raccoons, eat seafood and would therefore offer a mechanism for survival and proliferation as well as a route for serotype changes and increased toxicity.

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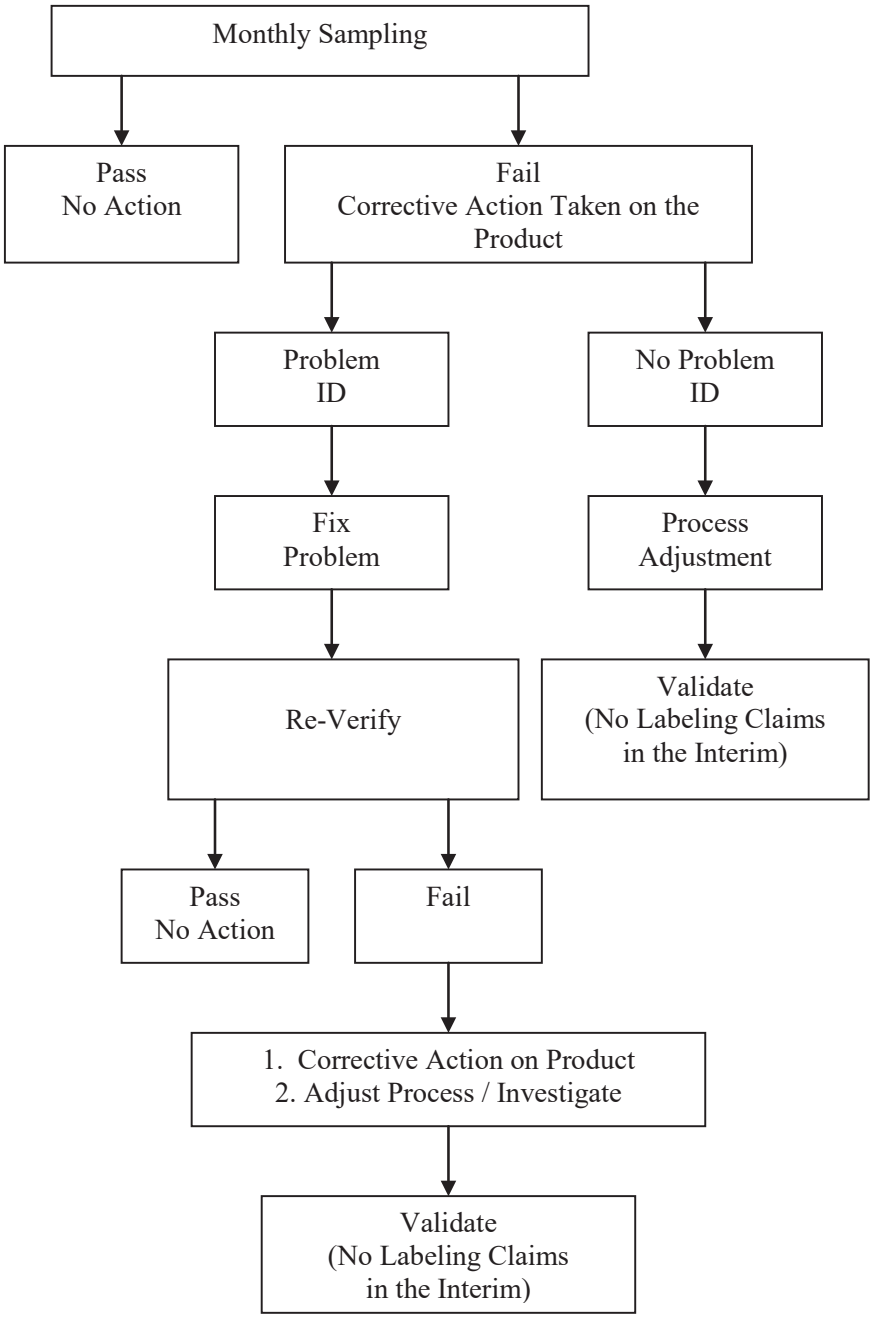
Ms received 4/10/80; revised 6/21/80; accepted 6/28/80.

Section IV. Guidance Documents

Chapter IV. Naturally Occurring Pathogens

Verification Sampling Protocol Decision Tree



Note: When a monthly verification fails, the verification must be reported within one week of failure

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Description: Flow chart showing the post harvest processing verification sampling protocol and decision making process.

Collect monthly shellfish meat samples for process verification.

If the monthly samples pass, no action is required.

If the monthly samples fail, take the following measures; (1) Identify the problem, (2) Fix the problem, (3) re-verify the process by sampling. If the re-verification samples pass, no further action is required. If the re-verification samples fail, then; (1) Corrective action must be taken on the product, (2) The process must be investigated, (3) Any problems identified must be adjusted, and (4) The process shall be revalidated. No labeling claims can be made during the interim revalidation process.

If the monthly samples fail and no problem can be identified then; (1) Adjustments shall be made to the process, and (2) The process shall be revalidated. No labeling claims can be made during the interim revalidation process.

Table 1. Overview of pathogenic *Vibrio*-species associated with human infections.

	Vibrio -species	Site of infection						
		GI tract	Wound	Ear	Primary septicaemia	Bacteremia	Lung	Meninges
1	*V. cholerae* O1/O139	++	(+)	?	?	?	?	?
	V. cholerae non O1/ non O139	++	+	+	(+)	(+)	?	(+)
2	*V. parahaemolyticus*	++	+	(+)	?	(+)	(+)	(+)
3	*V. vulnificus*	+	++	?	++	+	(+)	(+)
4	*V. fluvialis*	++	?	?	?	?	?	?
5	*V. alginolyticus*	?	++	+	?	(+)	?	?
6	*V. damsela*	?	++	?	?	?	?	?
7	*V. furnissii*	(+)	?	?	?	?	?	?
8	*V. hollisae*	++	?	?	(+)	?	?	?
9	*V. mimicus*	++	+	+	?	?	?	?
10	*V. metschnikovii*	(+)	?	?	(+)	?	?	?
11	*V. cincinnatiensis*	?	?	?	?	(+)	?	(+)
12	*V. carchariae*	?	++	?	?	?	?	?

GI tract: gastro intestinal tract, Primary septicaemia: septicaemia with no apparent infectious focus, ++ : most common site of infection, + : other sites of infection, (+) : rare sites of infection, ?: infection site remains to be firmly established. (Table redrawn from West, 1989, and updated with information from Oliver and Kaper, 1997)

This overview might give the false impression that all these twelve species are equally important as human pathogens. In fact, three *Vibrio* species represent a serious and growing public health hazard: *V. cholerae* (toxigenic strains belonging to serogroups O1 or O139 causing cholera), *V. parahaemolyticus* and *V. vulnificus*. Human infections with the remaining species are less common and usually less severe, although deaths have been reported (Oliver and Kaper, 1997). Four *Vibrio* species, *V. cholerae* (strains belonging to serogroups other than O1 or O139), *V. fluvialis, V. hollisae* and *V. mimicus* have been associated with a significant number of infections arising from contaminated seafood elsewhere in the world. Most of these infections presents as gastroenteritis (Table 1). They should be considered if further risk assessments are undertaken on pathogenic vibrios in the future.

In contrast to most other foodborne pathogens, bacteria belonging to the genus *Vibrio* have the aquatic habitat as their natural niche. The growth of all *Vibrio* species is stimulated by concentrations of Na^+ greater than those of unsupplemented

laboratory media indicating the marine origin of these bacteria (Varnam and Evans, 1991). Another characteristic of these bacteria, showing their adaption to aquatic conditions, is their close interaction with protozoa.

The prevalence and density of human pathogenic vibrios in the environment and also in seafood products are shown to be highly dependent on the ambient temperature with the largest numbers occurring at high sea water temperatures (Baffone *et al*., 2000; Høi *et al.*, 1998; Oliver and Kaper, 1997; O'Neill *et al.*, 1992; West, 1989). This may at least in part be explained by the ability of these bacteria to respond to adverse environmental conditions by entering a viable, but non-culturable phase (Linder and Oliver, 1989; Oliver and Kaper, 1997; Varnam and Evans, 1991). In such a state cells are viable, but it is not possible to obtain growth on the media routinely employed for their isolation.

4. VIBRIO VULNIFICUS

4.1. Hazard identification

V. vulnificus infections seems to be rare in Europe but little data exist on their true incidence. However, *V. vulnificus* infections are usually severe and they will be diagnosed even without having this organism specifically in mind because the organism will grow on most ordinary media. Thus, if these infections were more common in Europe than they seem to be, this higher incidence would probably be reflected by more reports in the literature.

In the USA, most cases of infections with *V. vulnificus* are associated with the consumption of raw oysters. The major form of the disease is primary septicaemia, i.e., septicaemia with no apparent infectious focus (Levine and Griffin, 1993; Oliver and Kaper, 1997). Other presentations are wound infections or gastrointestinal infection. Gastrointestinal infection is very rare, only accounting for 3 % of the reported cases involving *V. vulnificus* in the USA (Evans *et al*., 1999). There are no reports of any gastrointestinal infections with *V. vulnificus* as the etiological agent from Europe.

The majority of publications on infections by *V. vulnificus* involves sporadic cases. Thus, this bacterium is usually not involved in typical foodborne disease outbreaks. There are so far no reported cases of more than one person developing infection with *V. vulnificus* after consumption of raw oysters from the same batch (Oliver and Kaper, 1997). However, Bisharat *et al.* (1999) report on the development of bactaeremia and wound infection in over 60 patients after contact with pond cultured fish. This is, according to the authors, the first documented case of an outbreak of invasive *V. vulnificus* infection from a single common source.

The number of reported cases of foodborne infections involving *V. vulnificus* in the USA reaches 50 on an annual basis (Linkous and Oliver, 1999). These figures only involve cases serious enough to require hospitalisation. According to Linkous and Oliver (1999), the number of unreported cases in the USA might on an annual basis be as high as 41000, indicating that most infections with *V. vulnificus* might be self-limiting.