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Temporal Relationship of *Vibrio parahaemolyticus* in Patients and the Environment

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We prospectively compared the occurrence of *Vibrio parahaemolyticus* in patients and the environment in the Pacific Northwest. Inpatient and outpatient stool and wound specimens and water samples from 10 estuarine sites were cultured for *V. parahaemolyticus* over a period of 3 years. *V. parahaemolyticus* infections were detected in 13 patients (8 with gastroenteritis; 5 with wound infections), and all of the infections were found in outpatients in physicians' offices. Ten of the infections were locally acquired, and three occurred in patients returning from tropical travel. *V. parahaemolyticus* was isolated from 11 to 33% of the environmental samples, and each sampling site yielded the organism at some time during the study. *V. parahaemolyticus* was found in the environment only during the summer months, when water temperatures were ≥17°C and salinities were ≤13‰ (parts per thousand), and locally acquired infections were detected only when the organism was present in large numbers in the environment. We conclude that *V. parahaemolyticus* causes locally acquired gastroenteritis and wound infections, as well as traveler's diarrhea, in the Pacific Northwest, that patients with *V. parahaemolyticus* infections are likely to be seen in physicians' offices rather than hospitals, that locally acquired *V. parahaemolyticus* infections occur only when the organism is present in the environment, and that the organism is likely to be present during the summer months, when warm, low-salinity water conditions prevail in the coastal marine environment.

Vibrio parahaemolyticus is a well-recognized cause of gastroenteritis related to consumption of raw or improperly cooked seafood (1, 7). The organism has also occasionally been reported as a cause of extraintestinal infections (2, 7). *V. parahaemolyticus* is found in temperate marine environments around the world, and many infections due to this organism are thought to be of environmental origin. Occurrence of the organism is favored by warm-water conditions, and the organism is not found in the water column when temperatures are low (3). The present studies were undertaken to compare prospectively the occurrence of *V. parahaemolyticus* in human infections and estuarine environments. The results indicate that occurrence of human infections due to *V. parahaemolyticus* is closely related to the presence of the organism in the marine environment.

MATERIALS AND METHODS

Clinical studies. Specimens from inpatients in a general hospital and outpatients in physicians' offices were included in the study. Stool specimens were cultured on sheep blood agar for detection of *Vibrio* and *Aeromonas* spp. and on MacConkey, sorbitol-MacConkey, cefsulodin-Irgasan-novobiocin, and Hektoen enteric agars for detection of other enteric pathogens. Stool specimens were also cultured on thiosulfate-citrate-bile salts-sucrose agar (TCBS) for 6 months at the beginning of the study, but no vibrios were recovered from the medium, and it was not used routinely for the duration of the study. Wound specimens were cultured on blood, chocolate, and MacConkey agars. These media were used because *V. parahaemolyticus* grows well on them (4, 7), and it was not cost effective to culture all wounds on TCBS routinely. Colonies growing on blood agar were screened for oxidase, and oxidase-positive colonies were identified by classical biochemical testing as described below. For patients who yielded *V. parahaemolyticus*, clinical information was obtained from the referring physicians and from interviews with the patients.

Environmental studies. Surface water samples were obtained from 10 sites in and around Vancouver, British Columbia, Canada. Samples were collected throughout the year. The temperature and salinity of each site were determined by using portable instruments at the time of each sample collection. One-liter water samples were collected from each site in sterile Whirl Pak bags. The samples were maintained at ambient temperature and transported to the laboratory for processing within 2 h of collection. Duplicate volumes of 100, 10, and 1 ml of each water sample were filtered through 0.2-μm-pore-size membrane filters, and the filters were cultured on the surface of TCBS and tryptic soy agar plus tetrazolium (TSAT) (6). TSAT has been shown to be effective for recovery of *V. parahaemolyticus* from environmental sources (6). The cultures were incubated overnight at 35°C, and green colonies on TCBS or red colonies on TSAT were subcultured to sheep blood agar. After overnight incubation, the growth of each isolate was tested for oxidase, and oxidase-positive colonies were identified by classical biochemical testing. The number of colonies identified as *V. parahaemolyticus* was recorded, and the total number of *V. parahaemolyticus* per liter of the original sample was calculated.

Identification of *V. parahaemolyticus*. Clinical and environmental isolates were subjected to a battery of classical biochemical tests (4, 7). The test media were supplemented with 1% NaCl unless otherwise stated. Organisms with the following reactions were identified as *V. parahaemolyticus* fermentative reaction in triple-sugar-iron agar; growth in nutrient broth plus 3% NaCl; no growth in nutrient broth without NaCl; positive reactions for lysine, ornithine, arab-

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TABLE 1. *V. parahaemolyticus* infections

Case no.	Date	Site of infection	Travel history
1	August 1985	Foot wound	None
2	August 1985	External otitis	None
3	August 1985	Knee wound	None
4	September 1985	Leg wound	None
5	August 1986	Gastroenteritis	None
6	August 1986	External otitis	None
7	August 1986	Gastroenteritis	None
8	September 1986	Gastroenteritis	None
9	October 1986	Gastroenteritis	Thailand
10	May 1987	Gastroenteritis	Jamaica
11	July 1987	Gastroenteritis	None
12	August 1987	Gastroenteritis	None
13	September 1987	Gastroenteritis	Florida

inose, and indole; and negative reactions for arginine, sucrose, *o*-nitrophenyl-β-D-galactopyranoside, Voges-Proskauer, and salicin (4, 7).

RESULTS

***V. parahaemolyticus* in patients.** Thirteen patients with *V. parahaemolyticus* infections were identified between October 1984 and October 1987 (Table 1). All were diagnosed as outpatients in physicians' offices, and none required hospitalization. *V. parahaemolyticus* was the only potential pathogen isolated in all cases, except for patient 9, who also had *Campylobacter* sp. and *Plesiomonas shigelloides* isolated from stool cultures after returning from Thailand. Eight patients had intestinal infections, and five had extraintestinal infections, including wound infections and external otitis. Three patients acquired their infections while traveling, and ten acquired their infections locally. Four of the patients who had gastroenteritis gave a history of raw seafood consumption within 2 days of the onset of illness. Patient 5 had severe diarrhea (12 stools per day) for 2 weeks, resulting in a 3.2-kg weight loss. Patient 11 developed diarrhea 1 day after ingestion of raw oysters, and her stool cultures yielded heavy, light, and scant growth of *V. parahaemolyticus* on days 1 to 3 of her illness, respectively. The cases of external otitis were associated with swimming in seawater, and the other extraintestinal infections occurred in wounds exposed to seawater.

Isolation of *V. parahaemolyticus* from the environment. Water samples were collected at intervals throughout the year from 10 sites on the coast of southern British Columbia. The sites included areas likely to be used for water sports (beaches and parks) and areas subject to organic pollution (sewage treatment plant outfall, marinas, and an industrial area; Table 2). Overall, 187 samples were collected and 40 (21%) of the samples yielded a total of 215 isolates of *V. parahaemolyticus*. *V. parahaemolyticus* was isolated from each of the sites at some time during the study, but it was isolated from none of the sites more than one-third of the time. No sites were identified that yielded the organism significantly more often than the others, and the organism was detected as often in relatively pristine areas as in polluted areas.

Seasonal variation in the occurrence of *V. parahaemolyticus* in patients and the environment. The average number of *V. parahaemolyticus* isolated from all of the 10 sites each month from October 1984 to October 1987 was compared with the number of patients with locally acquired *V. parahaemolyticus* infections each month and with the average monthly temperature and salinity of the seawater at the sites (Fig. 1). The organism was not detected in samples from any of the sites between November and May, when water temperatures were less than 14°C and salinities were greater than 13‰ (parts per thousand). *V. parahaemolyticus* was first detected in low counts in June, when the average temperature was 17°C and the salinity was the lowest average value recorded (6.5‰). The highest counts of the organism were detected in July, when the average water temperature was 20°C and the average salinity remained low. Thereafter, the number of *V. parahaemolyticus* in the water samples steadily declined as the salinity increased. The organism was no longer detectable in November.

TABLE 2. Isolation of *V. parahaemolyticus* from 10 estuarine sampling sites

Site no. (description)	Temp range (°C)	Salinity range (o/oo)	No. of samples positive/no. tested (% positive) for *V. parahaemolyticus*
1 (sewage plant outfall)	4–29	5–19	7/22 (32)
2 (mouth of river)	5–21	0–12	5/22 (23)
3 (creek outflow)	10–22	0–19	3/21 (14)
4 (beach in residential area)	6–22	5–22	5/22 (23)
5 (beach in city)	6–22	6–24	4/20 (20)
6 (beach in remote area)	7–22	9–23	5/18 (28)
7 (marina in city)	7–22	10–20	2/18 (11)
8 (marina in residential area)	6–22	7–22	4/22 (18)
9 (city park)	6–22	8–22	2/11 (18)
10 (industrial area)	6–22	8–20	3/11 (27)

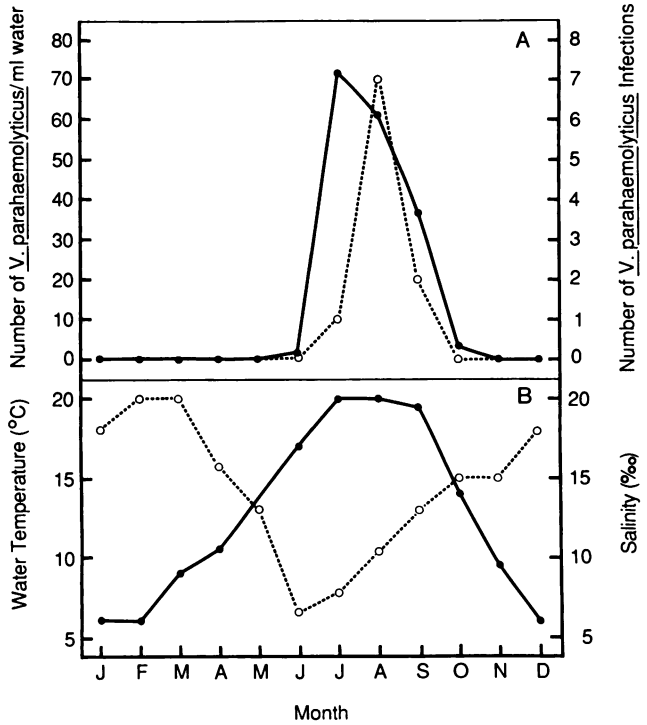

FIG. 1. Seasonal variation in recovery of *V. parahaemolyticus* from patients and the environment. (A) Monthly recovery of *V. parahaemolyticus* from 10 environmental sampling sites (●) (average number of CFU per milliliter of water) and recovery of *V. parahaemolyticus* from patients (○). (B) Average monthly temperature (●) and salinity (○) for 10 sampling sites.

The number of locally acquired *V. parahaemolyticus* infections was closely associated with the occurrence of the organism in the environment. Infections were detected in July, August, and September, and 70% of the infections were detected in August, when large numbers of the organism were present in the environment. No infections were detected in October through June, when *V. parahaemolyticus* was not present or was present in very low numbers in environmental water samples.

DISCUSSION

These findings demonstrate that *V. parahaemolyticus* is a cause of locally acquired wound infections and gastroenteritis, as well as traveler's diarrhea, and locally acquired *V. parahaemolyticus* infections were detected only under warm, low-salinity water conditions, when the organism was present in large numbers in the coastal marine environment. The only *V. parahaemolyticus* infections detected when the organism was not present in the environment were in patients who acquired their infections while traveling. All of the *V. parahaemolyticus* infections encountered in the study were in outpatients visiting physicians' offices. Although some of the infections demonstrated substantial morbidity, none of the patients required hospitalization, and the infections would not have been detected if only specimens received in the hospital laboratory had been included in the study.

Previous studies in the Chesapeake Bay have demonstrated that *V. parahaemolyticus* is found in water samples only under warm-water conditions and the organism disappears from the water column during the winter (3). The Northern Pacific Ocean is a cold-water environment, and the previous findings indicate that *V. parahaemolyticus* would seldom be found there. However, the coastal regions of the Pacific Northwest have many protected environments with restricted tidal flow, and summer temperatures above 20°C are common in these areas (Table 2). Therefore, it is not surprising that *V. parahaemolyticus* can be found in these environments, and our finding that the organism is found in Pacific Northwest environments only during the summer are consistent with previous observations made in other areas.

The present results also suggest that salinity is important in regulating the occurrence of *V. parahaemolyticus* in the environment. The estuarine environments analyzed in the present studies are of relatively low salinity because of abundant rainfall and influx of fresh water from rivers. Previous studies with a similar organism, *V. vulnificus*, indicated that low-salinity conditions, in addition to warm temperatures, were favorable to the occurrence of the organism (5). The present studies also suggest that low-salinity conditions are favorable to the occurrence of *V. parahaemolyticus*. The organism did not appear in the environment until water temperatures were 20°C, but low-salinity conditions were also present when maximum numbers of *V. parahaemolyticus* were detected (Fig. 1). After maximum numbers of the organism were detected under high-temperature, low-salinity conditions, the numbers declined, despite continued warm-water conditions, as the salinity increased. These findings suggest that once permissive water temperatures are reached, salinity becomes the controlling factor in the occurrence of *V. parahaemolyticus* in the environment. These findings indicate that additional in vitro and in situ studies should be done on the effect of salinity on *V. parahaemolyticus* occurrence in the environment.

V. parahaemolyticus infections have been assumed to be of environmental origin, but little work has been done to confirm this assumption. In the present studies, *V. parahaemolyticus* infections were closely associated with the presence of the organism in the environment, and no locally acquired *V. parahaemolyticus* infections were detected when the organism was not present in the local environment. These findings support the assumption that *V. parahaemolyticus* infections are of environmental origin, and studies comparing the biochemical characteristics, plasmid profiles, and Kanagawa reactions of clinical and environmental *V. parahaemolyticus* isolates are in progress.

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