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Epidemiological studies on *Vibrio cholerae* from the San Lorenzo River, Santa Cruz County, California : correlations to water quality

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EPIDEMIOLOGICAL STUDIES ON VIBRIO CHOLERAE FROM THE
SAN LORENZO RIVER, SANTA CRUZ COUNTY, CALIFORNIA:
CORRELATIONS TO WATER QUALITY

A Thesis

Presented to

The Faculty of the Department of Biological Sciences
San Jose State University

In Partial Fullfillment
of the Requirements for the Degree
Master of Arts

By

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August, 1990

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ABSTRACT

EPIDEMIOLOGICAL STUDIES ON VIBRIO CHOLERAE FROM THE SAN LORENZO RIVER, SANTA CRUZ COUNTY, CALIFORNIA: CORRELATIONS TO WATER QUALITY

by Brett Austin

This thesis represents a year-long study of the prevalence of the bacterium Vibrio cholerae in a freshwater river. Gauze filtration of samples was coupled with enrichment at 42°C to yield a 44% prevalence rate of the organism. A positive correlation was found between point prevalence and mean sample temperature. A seasonal variation was observed in prevalence of V. cholerae.

Site prevalence was positively correlated with three water quality factors: elevated conductivity, elevated fecal coliform counts, and elevated ratios of fecal coliforms to fecal streptococci.

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Thanks to T.S. Eliot, for the words:

"We shall not cease from exploration. And the end of all our exploring will be to arrive from whence we came, and know the place for the first time."

Special thanks must be given to my entire family for their support, love, and encouragement.

Final thanks to my son Tyler, who gives it all meaning, and to my wife, Jennifer. Without her nothing ever would have gotten done.

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INTRODUCTION

Vibrio cholerae, the causative agent of cholera, has a long history of study by the medical community. The present definition of the genus Vibrio includes organisms that are Gram-negative and oxidase-positive rods. They are facultatively anaerobic chemoorganotrophs capable of respiratory and fermentative metabolism. The organisms are motile by sheathed, polar flagella when grown in liquid media. The cells are generally single, straight or curved, and do not form endospores ¹.

The morbidic potential of V. cholerae has been recognized since Robert Koch isolated the organism in 1883. The majority of cholera victims present a cardinal symptom of "rice water stool," a copious discharge of watery diarrhea. This symptom is induced by cholera toxin, an exotoxin produced by proliferating cells whose cellular production is chromosomally linked ². Additional symptoms include nausea, vomiting, muscular cramping, and weakness. Six world-wide epidemics of cholera occurred between 1817 and 1947. The seventh pandemic of cholera began in 1961 and spread from Indonesia to Asia, the Middle East, Eastern Europe and Africa, and continues to this day ³.

In the United States, no cases of indigenous cholera were reported between 1911 and 1972. Since then, sporadic cases have occurred, and V. cholerae has been isolated from a number of patients and a variety of environmental sources. In 1983, a case of cholera-like disease in Santa Cruz County, California, yielded V. cholerae from the patient's stools ⁴. An environmental survey produced V. cholerae isolates from a variety of Santa Cruz County's coastal marine sites ⁵. The highest number of organisms per volume of sample was associated with the outflow point of the San Lorenzo River, a popular site for public bathing. To determine the possibility of a threat to the public health, the following research project was designed.

The purpose of this research was to conduct a survey of the San Lorenzo River in Santa Cruz County. It was hypothesized that the presence of environmental strains of V. cholerae in the river would be demonstrated using the methodologies to be described.

The proposed research was designed to achieve three specific goals:

- (1) to detect the bacterium V. cholerae in water samples taken from the San Lorenzo River;
- (2) to describe the distribution of the organism along the watercourse; and
- (3) to correlate water quality data with the isolation of V. cholerae.

LITERATURE REVIEW

Historical perspectives:

John Snow's famous example of early epidemiological methods was founded on his research concerning the incidence of cholera in populations served by various wells in London, England ⁶. He discovered that a water system contaminated with sewage produced a cholera death rate eight times higher than the rate produced by unpolluted water ⁷.

While in Egypt during the cholera epidemic of 1883, Robert Koch became convinced that poor sanitation was responsible for the disease. Because previous pandemics of cholera had begun in India, the German government allowed Koch to travel to Calcutta in December of that year. His work in India produced the cultivation of the "comma bacilli" and helped to clarify the germ theory of disease ⁸.

The taxonomy of the members of the genus Vibrio remains in a changing state, but the medically important species are well established. V. cholerae has been meticulously characterized, both biochemically and serologically. A complete biochemical protocol for identifying pathogenic Vibrio spp. has been described by Farmer et al. ⁹.

An overview of the classification of the Vibrionaceae by West and Colwell ¹⁰ asserts that the seventh edition of Bergey's Manual ¹¹ placed the genus Vibrio in the family Spirillaceae chiefly because of the Gram-negative staining

reaction and the curved appearance of the stained cell. This description also included other genera, notably Pseudomonas and Campylobacter. They further assert that research by Shewan et al. in the 1950's and early 1960's led the International Association of Microbiological Societies Subcommittee on the Taxonomy of Vibrios to establish a description of the genus Vibrio that, based on the mode of glucose metabolism, differentiates the organism from Pseudomonas and related genera. The eighth edition of Bergey's Manual created the family Vibrionaceae, which included the genera Aeromonas, Lucibacterium, Photobacterium, Plesiomonas, and Vibrio ¹². The genus Beneckea was included as a genus of uncertain taxonomic position. Bergey's Manual of Systematic Bacteriology ¹³ reduced the number of genera in the family to four: Vibrio, Photobacterium, Aeromonas, and Plesiomonas.

One of the earliest recognized characteristics of V. cholerae was that isolates from patients involved in a cholera epidemic would agglutinate with sera harvested from animals infected with earlier isolates. In 1935, the known members of the V. cholerae group were arranged into six groups defined by their heat-stable cell wall antigens (O antigens) ¹⁴. By definition, the O1 epithet was appended to those organisms that exhibited the biochemical characteristics of V. cholerae and were capable of producing

epidemic disease. The remaining members were collectively referred to as "non-cholera vibrios" or "Non-Agglutinating Vibrios" (NAG). NAG is a misnomer, as these organisms will agglutinate with specific antisera. Current terminology refers to this group as non-O1 Vibrio cholerae.

Pathogenesis:

A severely afflicted cholera patient will suffer extreme, rapid loss of water and electrolytes ¹⁵. Fluid loss can equal one liter per hour, and the untreated patient may die in twelve to twenty-four hours. These symptoms occur when cholera toxin is released into the small intestine by multiplying cells of V. cholerae. Cholera toxin is a heat-labile toxin composed of two subunits. One subunit is responsible for the attachment and penetration of the host gut mucosal epithelium. The other subunit stimulates adenyl cyclase, leading to a buildup of cyclic adenosine monophosphate (cAMP) with concomitant release of isotonic fluid ¹⁶.

Both O1 and non-O1 V. cholerae are capable of carrying the gene that codes for the production of cholera toxin ¹⁷, but toxin production alone does not account for the entire spectrum of V. cholerae morbidity. Non-O1 V. cholerae gastroenteritis occurs much more frequently than

O1 V. cholerae enteric infection in the United States ¹⁸, and most non-O1 isolates lack the cholera toxin gene ¹⁹.

Other factors that may potentially enhance infectivity and disease expression include cell-associated factors such as the bacterial flagellum and adhesins, and a variety of extracellular factors, including proteases, mucinases, enterotoxins, and cytotoxins ²⁰. Despite the recognition of these factors, only the elaboration of cholera toxin has been firmly linked to a specific gastrointestinal syndrome ²¹.

V. cholerae infects other body sites as well. A review by Janda et al. ²² of isolates submitted to the Centers for Disease Control indicates that extraintestinal recovery of V. cholerae occurs primarily from ear infections, blood, wounds, respiratory and biliary tracts, and cerebrospinal and peritoneal fluids. These authors also state that "persons found to harbor V. cholerae at extraintestinal body sites are much more likely to have underlying diseases, and infection is almost always due to V. cholerae non-O1."

Vibrios in the Environment:

Vibrios in general, and V. cholerae in particular, have been isolated from a number of environmental sources. It has been proposed that V. cholerae be included among the autochthonous flora of temperate zone coastal estuaries, salt marshes, and brackish waters ²³. Recent work has revealed the

presence of non-O1 V. cholerae in coastal shore-breaks and river-mouths of California, Washington, and Oregon ²⁴. Research by Singleton et al. ²⁵ demonstrated that long-term survival of V. cholerae requires a brackish environment. Other researchers have recovered V. cholerae from waters with salinity as low as two parts per thousand ²⁶.

Seasonal fluctuation appears to be a common occurrence for V. cholerae populations, with salinity and water temperature being the two most important environmental factors ^{27,28}. Distinctive ecological associations have been described linking both O1 and non-O1 V. cholerae to copepods and plankton and V. cholerae O1 biotype El Tor to water hyacinths ^{29,30}.

Isolation of vibrios from fresh water has attracted little attention outside of endemic areas of cholera in India. Low numbers of V. cholerae were isolated from fresh water ditches in Kent, England during 1978 and 1979 ³¹. In the United States, non-O1 V. cholerae has been isolated from fresh and brackish water in Colorado ³² and from standing water in Louisiana ³³.

Collection Techniques:

A variety of collection techniques has been developed to isolate vibrios from environmental sources. Direct detection in water sources using fluorescent-antibody staining has been

demonstrated ³⁴. The most successful methods for water samples have involved some means of concentrating the sample. Filtration of one to ten liters of sample through membrane filters, diatomaceous earth or epoxy resin-bonded glass microfibers is recommended by the 15th edition of Standard Methods ³⁵. Moore swabs, folded lengths of cheesecloth tied to wires, are recommended for streams, lakes, and estuaries. An effective method utilizing ten liters of sample filtered through cotton gauze was developed for use with standing and slow-flowing water sources ³⁶. Hranitzky et al. ³⁷ utilized antibody-bound beads in a glass column to isolate *V. cholerae* from polluted water. Larson et al. ³⁸ modified this technique by mixing one-liter water samples from oyster bays with five grams of antibody-coated beads for an unspecified period of time.

Whatever the method employed, expeditious processing of collected samples will maintain the sensitivity of the chosen method. Standard Methods protocol ³⁹ requires cooling of water samples to below 10°C to reduce microbial changes; this is not recommended for vibrios ⁴⁰. Reducing the temperature of samples after collection places additional stress on vibrios, leading to reduced numbers of viable organisms.

Identification:

Some form of enrichment is needed to detect the low numbers of V. cholerae present in environmental sources. Because these organisms can readily grow in an alkaline medium (pH 8.5-9.0) and tolerate salt, the standard enrichment broth is alkaline peptone water ⁴¹. Other enrichment procedures ⁴² have utilized the bile resistance of V. cholerae and the organism's elaboration of proteases.

Incubation temperatures and duration of enrichment usually fall in the range of 35°C to 37°C for six to eighteen hours ⁴³. Use of overnight incubation in alkaline peptone water enrichment at 42°C has been demonstrated to produce significantly higher rates of isolation of V. cholerae ⁴⁴.

Several primary isolation media are available for pathogenic vibrios, most utilizing the resistance of V. cholerae to bile and the differential characteristics of sucrose fermentation or tellurite reduction ⁴⁵. Thiosulfate-Citrate-Bile salts-Sucrose agar (TCBS) has been the historical medium of choice, although there have been reports of unacceptable lot-to-lot variation ⁴⁶. Use of this medium requires diligent quality control procedures.

The biochemical identification of environmental isolates of V. cholerae begins with the use of a small number of screening tests. Selection of screening tests must be appropriate for the resources of a particular laboratory and

the nature of the study ⁴⁷. Most screening protocols use Gram stain morphology, the oxidase reaction (N,N-Dimethylphenelynediamine oxalate), sodium chloride as a growth requirement, and a limited number of fermentation reactions.

Common additional elements of many definitive identification schemes include the results of ornithine and lysine decarboxylation tests; arginine dihydrolase tests; and fermentation reactions of glucose, arabinose, mannitol, sucrose, salicin and inositol. O1 and non-O1 *V. cholerae* are susceptible to the vibriostatic agent O/129 (2,4-diamino-6,7-diisopropylpteridine phosphate) and produce a β -galactosidase ⁴⁸. *V. cholerae* does not have a lactose permease and appears as a non-lactose fermenter on ordinary enteric media.

MATERIALS AND METHODS.

Sites.

Figure 1 displays the San Lorenzo River watershed and the location of the sampling sites. One hundred fourteen samples were taken from eleven sites between 12/4/88 and 12/4/89. Six sites (003,202,022,060,180, and 245) were chosen for continuous monitoring. Once sampling began at one of these six sites, a minimum of one sample per month was taken from that site. To assess the distribution of V. cholerae in the San Lorenzo River, samples were taken at five other sites.

The six sampling points chosen for continuous monitoring during this survey are accessible to the general public and are known as "natural bathing sites." They are monitored on a weekly basis for fecal coliform and fecal streptococcal levels by the Environmental Health Branch of the County of Santa Cruz Health Services Agency. These sites were chosen because of their history of observation, public importance, and likelihood of continued monitoring.

Site 003 is located at the railroad trestle as it bridges the San Lorenzo River, approximately 100 meters from the outflow point of the river into Monterey Bay. It is adjacent to an amusement park and a large beach, both serving to attract large crowds during the warm, summer months. Although frequently posted as unsafe for recreational use due to high fecal coliform levels, many people, especially young children, wade and swim in the warm, slow-moving water. Nineteen samples were taken from this site between 12/4/88 and 12/4/89.

Site 202 is 2.7 kilometers (km) from site 003. This section of the San Lorenzo River flows through the approximate center of the City of Santa Cruz. A city-owned park occupies the eastern bank of the river, including the sampling site. This site probably represents the margin of usual tidal effects, i.e., points up-river are not subject to saltwater intrusion during normal tides [S. Peters, Santa Cruz County Environmental Health Department, personal communication of unpublished data]. Fourteen samples were taken at this site between 1/2/89 and 12/4/89.

Site 022 is located 4.6km from site 003 and is the site least accessible to the public. Reaching it requires a steep descent down a rocky ledge. This site is inaccessible during wet weather, which was the situation through March, 1989. Samples were taken on 1/2/89 and 1/29/89 slightly upstream, and are included in the overall data analyses. Monitor sampling did not commence at this site until 4/18/89. Sixteen samples in all were taken between 1/2/89 and 12/4/89.

Site 060 is located at the entrance to Henry Cowell Redwood State Park. It is 12.3km from site 003. Eighteen samples were collected at this site between 12/4/88 and 12/4/89.

Site 180 is near the center of the town of Ben Lomond, approximately 200m downstream from a town park. The site is 17.8km from site 003. Eighteen samples were taken here between 12/4/88 and 12/4/89.

Site 245, 22.6km from site 003, is approximately 150m downstream from a municipal park in the town of Boulder Creek. Eighteen samples were taken at this site between 12/4/88 and 12/4/89.

Samples were taken at five additional sites to assess the distribution of V. cholerae. Information on these additional sites is summarized below.

Site 001 was the entry point of the San Lorenzo River to Monterey Bay. Two samples were taken to verify the presence of V. cholerae in Monterey Bay waters, as reported by Kenyon et al ⁵. This site was discontinued because of dramatic changes in its location, mostly influenced by sand accumulation.

Site 005 was located 1.5 km from site 003. This site was not readily accessible to the public. Three samples were taken between 1/2/89 and 4/18/89.

Site 020 was 2km from site 003. Samples were taken from this site on 1/29/89 and 4/18/89. Because this was a difficult site to reach and was also the entry point of a tributary creek, sampling was discontinued.

Site 349 was 30.7km from site 003. This site was discontinued due to low water flow in January, 1989, after three samples had been taken.

Site 762 represented a single sample taken on a tributary creek to the San Lorenzo River after a reported sewage leak.

Figure 1 The San Lorenzo River Watershed

Water Quality:

A Horiba model U-7 Water Quality Checker [Horiba instruments, Irvine CA.] was utilized to provide water quality information. Measurements were taken of pH, dissolved oxygen (parts per million, ppm), temperature (°C), conductivity (µmhos/cm), and turbidity (ppm). Additional turbidity measurements were made on a majority of the samples using a Hach Turbidometer, with data reported in Nephelometer Turbidity Units (NTU).

Direct colony counts of fecal coliforms and fecal streptococci were made using standard membrane filtration ⁴⁹ (0.45µm pore size; Millipore Corp) and mFC broth [Difco] and KF streptococcus agar [Difco], respectively.

Collection and Transport:

At each site a sample for V. cholerae isolation and a sample to determine fecal coliform and fecal streptococci counts were taken. A sample for V. cholerae consisted of ten liters taken as a succession of one liter portions. Each portion of river water was removed with a one liter stainless steel bucket. The portions were filtered serially through a double thickness of 15cm X 17cm sterile cotton gauze [Kendall Kerlix® Super Sponges. Kendall Co. Boston, Mass.]. The gauze was held in a non-sterile, pre-rinsed polypropylene funnel [Nalgene # 4252-0150. Nalge Co., Rochester, N.Y.]. The

filtrate was allowed to run back into the river, downstream of the sampling point. After filtration of ten liters, the gauze pads held approximately 100ml of retained water. The pads and their contents were placed in 100ml of triple-strength alkaline peptone water (pH 8.5). An additional 100ml of sample water was added to the enrichment broth to provide coverage of the gauze pads. This dilution resulted in a single-strength enrichment broth.

Samples were transported as collected. Samples were transported without refrigeration. Collections were timed so that samples would arrive at the laboratory within two hours.

Enrichment:

Enrichment began immediately upon sampling when the collected material was placed into the enrichment broth (1.0% peptone and 1.0% sodium chloride with the pH adjusted to 8.5 ± 0.2). Samples were placed into a 42°C incubator upon arrival in the laboratory.

Isolation:

After overnight incubation at 42°C, a loopful of surface growth was streaked upon the surface of a Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar plate [Difco]. Plates were incubated in a normal air incubator at 37°C. The

following day, typical colonies were picked to T₁N₁ (1.0% tryptone, 1.0% NaCl, 2.0% agar, pH 7.2±0.2) agar slants⁵⁰.

Identification:

Growth present on T₁N₁ slants was subjected to a battery of biochemical tests. Oxidase testing by the Kovac's method⁵¹ using the reagent N,N-Dimethylphenelynediamine oxalate, coupled with a typical Gram stain appearance of curved, negative rods comprised the first level of screening. Cultures chosen for follow-up were tested for ability to grow in 1.0% tryptone broth without added salt; gas production from glucose; fermentation reactions of glucose, lactose, mannitol, and inositol; decarboxylation of lysine and ornithine; and dihydrolation of arginine.

Serology:

All isolates biochemically identified as V. cholerae by the above scheme were tested by slide agglutination for the presence of the O group 1 antigen with commercial polyvalent antiserum [Difco].

Statistical Analysis:

Data analysis was performed using StatView 512+ (Brainpower, Inc., Calabasas, CA) run on a Macintosh Plus (Apple Computers, Cupertino, CA). The point biserial

correlation coefficient was used for correlation analysis of dichotomous and continuous variables ⁵². Significant water quality factors were compared using one-way analysis-of-variance (ANOVA) and Fisher's protected t-test. To lend statistical validity, only data from continuously monitored sites were used in statistical tests of significance.

Quality Control:

All media, antisera, and reagents used for this study were tested for proper performance with organisms of known reactivity, per the guidelines of the Laboratory Field Services Branch of the California State Department of Health Services ⁵³.

RESULTS

Samples and Isolates:

Tables A-F (see Appendix) summarize the measurements of physical parameters and culture results of all samples taken during this study.

Fifty (43.9%) of the 114 samples taken during the course of this study yielded isolates of non-O1 Vibrio cholerae. All isolates demonstrated the same morphological and biochemical profile described in Table 1.

None of the isolates produced an agglutination reaction with polyvalent V. cholerae O1 antiserum.

Prevalence of V. cholerae in the San Lorenzo River on a given sampling day ranged from 0.0% to 100%. Point prevalence data are given in Table 2. Figure 2 indicates the fluctuation observed in the number of sites yielding V. cholerae isolates for each sampling day.

Isolation rates for V. cholerae at all sites ranged from 100% to 0.0%, as indicated in Table 3. This table also demonstrates that for sites with more than three samples taken, isolation rates ranged from 84.2% to 11.2% . As shown in Figure 3, isolation rates declined with increasing distance from the ocean waters of Monterey Bay.

TABLE 1: San Lorenzo River Vibrio cholerae Survey
 Santa Cruz, CA-12/88 - 12/89
 Morphology and biochemical reactions of V. cholerae
 isolates

Morphology	Curved cells, 0.5µm X 2µm
Gram Stain	Negative
Oxidase	+
Growth on TCBS agar	Smooth, yellow colony
Growth on T ₁ N ₁ agar	+
Growth in Tryptone Broth without added sodium chloride	+
Fermentation of: Sucrose	+ (TCBS agar)
Lactose	- (KIA agar)
Glucose	+
Mannitol	+
Inositol	-
Gas from Glucose	-
Hydrogen sulfide produced	- (KIA)
Lysine decarboxylation (Moeller)	+
Ornithine decarboxylation	+
Arginine dihydrolation	-

TABLE 2: San Lorenzo River Vibrio cholerae Survey
Santa Cruz, CA-12/88 - 12/89
Point Prevalence Data

Sampling Date	ALL SITES		MONITORING SITES	
	# positive/ # of sites	Prevalence rate (%)	# positive/ # of sites	Prevalence rate (%)
12/4/88	1/6	17	0/4	0
12/11/88	1/6	17	1/4	25
1/2/89	2/3	67	NA	NA
1/15/89	0/6	00	0/5	0
1/29/89	1/3	67	NA	NA
2/26/89	0/5	00	0/5	0
3/8/89	1/5	20	1/5	20
3/22/89	1/6	17	1/5	20
4/18/89	7/8	88	5/6	83
5/8/89	3/6	50	3/6	50
5/22/89	2/6	33	2/6	33
6/7/89	2/6	33	2/6	33
6/27/89	3/6	50	3/6	50
7/11/89	3/6	50	3/6	50
8/11/89	5/6	83	5/6	83
8/29/89	3/6	50	3/6	50
9/19/89	2/6	33	2/6	33
10/25/89	6/6	100	6/6	100
11/14/89	4/6	67	4/6	67
12/4/89	3/6	50	3/6	50
	50/114	43.9%	44/100	44.0%

NA = NOT APPLICABLE

FIGURE 2

San Lorenzo River Vibrio cholerae Survey

Site point prevalence

TABLE 3: San Lorenzo River Vibrio cholerae Survey
 Santa Cruz, CA-12/88 - 12/89
 Sample Site Isolation Rates

Sample Site #	Samples Taken	Samples Positive	%+
001	2	1	50.0
003	19	16	84.2
005	3	3	100.0
020	2	1	50.0
202	14	10	71.4
022	16	7	43.8
060	18	5	27.8
180	18	5	27.8
245	18	2	11.1
349	3	0	00.0
762	1	0	00.0

Water Quality:

Water quality data for each sample are summarized in Appendix 1 (A-E). Mean values in each category of measurement were determined for date of sample (Table 4) and for sampling site (Table 5).

Statistical analysis:

Correlation coefficients were determined for all measures of water quality versus isolation of V. cholerae. Table 6 is a compilation of the data for all samples taken during the study. Table 7 is a compilation of correlation data comparing water quality factors to the number of sites yielding isolates of V. cholerae on a given sampling day, i.e., point prevalence. Also included in these tables are F-test values from one-way ANOVA for each factor and probability estimates.

On two occasions, multiple tube enumeration of V. cholerae from site 003 yielded most-probable-number 95% confidence interval estimates of one to seventeen organisms per 100 milliliters of sample. Samples taken at the same time using the gauze-filtration technique detected V. cholerae at these low densities.

TABLE 4: San Lorenzo River Vibrio cholerae Survey
Santa Cruz, CA 12/88 - 12/89
Mean water quality values by date of sample

Date of Sample	D.O. a	pH	Temp. °C	Cond. ^b	Turb. 1c	Turb. 2 d	FC e / 100ml	FS f / 100ml	FC / FS g
12/4/88	7.9	7.3	10.1	955	34.8	ND	376.1	2257.7	0.6
12/11/88	12.7	7.3	10	993.3	10.8	ND	254.2	2263.7	1.7
1/2/89	10.5	8.1	10	273.3	13.3	ND	273.3	ND	ND
1/15/89	12.2	7.6	6.5	323.3	19.3	ND	72.2	ND	ND
1/29/89	10.1	8.1	11.2	333.3	25.7	ND	90	ND	ND
2/26/89	11.1	7.9	11.6	420	20.8	ND	125.8	ND	ND
3/8/89	10.5	7.9	13.3	358	13.2	4.8	1068.4	2642	0.6
3/22/89	11	8	15.4	650	24	2.1	253.3	170	1.5
4/18/89	11.3	8.2	19.5	525	37.3	1.4	187.5	70.6	2.8
5/8/89	8.8	8	17.7	2966.7	15.2	1.9	524	248.3	2.1
5/22/89	10	8	15.4	1050	26.8	1.5	412.2	348.3	1.2
6/7/89	8.8	8.1	18	2566.7	11.7	1.5	526.3	290.2	2.1
6/27/89	10.7	8.2	19.7	2816.7	27	1.1	878.3	420.7	1.9
7/11/89	8.7	7.8	18.2	2133.3	8.9	1.1	967	420.5	1.6
8/01/89	8.9	8.1	19.7	1700	11	1	2395.3	814	2.2
8/29/89	8.7	8.2	18.8	1216.7	ND	0.8	333.3	539.3	0.7
9/19/89	8.7	7.6	15.1	1100	34.7	1.5	459.3	616.7	0.7
10/25/89	8.9	7.8	14.1	383.3	ND	55.3	1586.7	4450	0.4
11/14/89	10.2	8.1	11.9	666.7	27.5	1.3	330.8	2136.7	0.8
12/4/89	10.9	8.1	7.9	433.3	12.3	1.5	157.7	400	0.4

^a Dissolved oxygen

^e Fecal coliform count.

^b Conductivity micromhos/cm

^f Fecal Streptococcus count

^c Turbidity (ppm) by ratio turbidometer

^g Ratio of fecal coliform to fecal streptococci

^d Turbidity (NTU) by nephelometer at 25°C

^h ND= not determined

TABLE 5: San Lorenzo River Vibrio cholerae Survey
Santa Cruz, CA 12/88 - 12/89
Mean Water Quality Values by Site

Sample Site	D.O. a	pH	Temp. °C	Cond.b	Turb. 1C	Turb. 2 d	FC e / 100ml	FS f / 100ml	FC / FS g
001	9.7	6.7	13.6	28400.0	8.0	ND	420.0	345.0	1.2
003	9.5	7.3	16.1	5678.9	30.4	4.5	884.5	643.4	2.9
005	11.7	7.9	14.8	533.3	16.3	1.4	310.0	ND	ND
020	10.8	8.5	16.0	450.0	43.0	1.2	330.0	160.0	3.8
202	9.0	8.0	16.0	442.9	23.8	5.0	1879.3	2095.9	2.1
022	10.6	8.3	15.4	406.3	16.9	5.8	229.7	767.4	0.6
060	9.7	7.9	14.0	411.1	18.1	10.1	304.7	1528.3	0.5
180	10.4	8.1	14.2	411.1	17.9	5.7	326.3	1390.1	0.9
245	10.1	8.0	13.0	427.8	21.8	2.0	279.4	735.7	1.3
349	11.5	7.9	6.7	366.7	15.3	ND	1.4	ND	0.1
762	11.1	7.9	14.0	500.0	19.0	ND	108.0	400.0	0.3

a Dissolved oxygen

e Fecal coliform count.

b Conductivity micromhos/cm

f Fecal Streptococcus count

c Turbidity (ppm) by ratio turbidometer

g Ratio of fecal coliform to fecal streptococci

d Turbidity (NTU) by nephelometer at 25°C

h ND= not determined

TABLE 6: San Lorenzo River Vibrio cholerae Survey
Santa Cruz, CA-12/88 - 12/89
Water Quality Parameters vs V. cholerae Isolation

Measurement	Count	Correlation	Alpha = 0.05	r ²	ANOVA F-test	Probability
Dissolved oxygen	114	-0.114	0.195	0.013	2.272	0.0534
pH	114	-0.094	0.195	0.009	13.346	0.0001
Temperature	114	0.492	0.195	0.242	1.269	0.2835
Conductivity	114	0.387	0.195	0.15	9.452	0.0001
Turbidity (ppm)	102	0.025	0.195	0.001	1.73	0.1367
Turbidity (NTU)	83	-0.167	0.217	0.028	0.339	0.8878
Fecal coliform(FC) per 100ml	114	0.255	0.195	0.065	2.983	0.0151
Fecal strep. (FS) per 100ml	97	-0.170	0.205	0.032	1.086	0.374
Ratio FC/FS	97	0.495	0.205	0.245	5.62	0.0001

TABLE 7: San Lorenzo River Vibrio cholerae Survey
Santa Cruz, CA-12/88 - 12/89
Correlation of water quality and V. cholerae point
prevalence

Measurement	Count	Correlation	Alpha =0.05	R ²	ANOVA F-test	Probability
Dissolved oxygen	20	0.316	0.444	0.1	7.938	0.0001
pH	20	-0.481	0.444	0.231	1.688	0.052
Temperature	20	-0.451	0.444	0.204	40.577	0.0001
Conductivity	20	-0.103	0.444	0.011	0.597	0.8999
Turbidity (ppm)	18	-0.019	0.468	<0.01	1.226	0.2545
Turbidity (NTU)	14	-0.526	0.532	0.276	14.036	0.0001
Fecal coliform(FC) per 100ml	20	-0.522	0.444	0.272	0.966	0.5072
Fecal strep. (FS) per 100ml	16	-0.163	0.497	0.027	3.052	0.0002
Ratio FC/FS	16	-0.192	0.497	0.037	1.234	0.2481

DISCUSSION:

Method:

The use of a gauze filtration method for collecting samples for Vibrio cholerae in conjunction with overnight enrichment at 42°C demonstrated the presence of non-O1 V. cholerae in waters taken from the San Lorenzo River in Santa Cruz County. Using other techniques, Rhodes et al. ³² demonstrated V. cholerae in surface waters in Colorado, while Blake et al. ³³ isolated the organism from standing water in Louisiana. Other published environmental assessments of this organism known to this researcher have focused on marine or estuarine locations.

Spira and Ahmed ³⁶ have proved the effectiveness of the gauze filtration method and DePaolo ⁵⁰ has reported significantly higher yields of V. cholerae from oysters using 42°C enrichment. By combining these two techniques, this research demonstrates that V. cholerae can be isolated using simple equipment and routine laboratory media.

The approved qualitative methods listed in the 15th edition of Standard Methods for the Examination of Water and Wastewater for isolating Vibrio spp. are the Moore swab, membrane filtration, filtration through diatomaceous earth, and glass microfibre filtration. With the exception of the Moore swab, all the filtration techniques may be inappropriate for turbid waters. Turbid samples require

clarification by centrifugation before processing. The equipment required for these techniques is not easily adapted for field use, especially when multiple samples are desired. The latter three methods suggest sample volumes of one to ten liters per sample. The usual practice when using the membrane, diatomaceous earth, or glass microfibre filtration techniques is to collect the sample and process it remotely, i.e., in the laboratory. The gauze filtration technique developed for this study uses equipment that is easily acquired and easily transported. It allows the concentration of large volumes of sample (e.g., ten liters), into a small enrichment volume, 300ml in this instance. This method can be used with turbid water samples.

The Moore swab technique is practical for field use and is suitable for turbid waters. Spira ⁵⁴ has pointed out the disadvantages of this technique:

- 1) It is ineffective in still waters.
- 2) A precise sample time is not available.
- 3) Swabs may be lost or taken away before the investigator returns.

This researcher would state another disadvantage:

- 4) No precise correlation to a measurement of water quality is possible.

The gauze filtration technique does not have these disadvantages. It allows a point sample to be taken with

attendant water quality information. It can be used in non-flowing waters as well as in vigorous streams. The disadvantage of this technique is that it does not allow for enumeration of V. cholerae from a water source. Although preliminary tests indicate this method may be sensitive to low numbers of V. cholerae, further research is needed. Comparative studies of this method and a quantitative technique such as the most-probable-number multiple tube enumeration method would clarify the sensitivity and effectiveness of the gauze filtration technique.

Prevalence:

Non-O1 V. cholerae was isolated from nine of eleven sampling sites. The overall prevalence rate was 44%. As indicated in Table 3, isolation rates varied between sites. Higher rates were observed (Figure 3) at sites closer to the waters of Monterey Bay. In order to exclude the effects of the assessment samples that were taken, prevalence rates were graphed that excluded the sites not continuously monitored. This information is presented in Figure 4. A clear pattern of elevated isolation rates is evident in the spring, summer and fall months. Kaper et al.²⁶ and others have demonstrated a seasonal variation in V. cholerae populations. This study suggests a similar trend, with higher isolation rates occurring between April and December, 1989.

FIGURE 4: San Lorenzo River Vibrio cholerae Survey
 Santa Cruz, CA-12/88 - 12/89
 Point prevalence rate at monitor sites

FIGURE 5: San Lorenzo River *Vibrio cholerae* Survey
 Santa Cruz, CA-12/88 - 12/89
 Mean water temperature vs point prevalence

Table 7 summarizes the analysis of the data grouped by date of sample collection and compares mean water quality factors to prevalence of V. cholerae on a given day (point prevalence). This analysis demonstrates significant ($p < 0.05$), positive correlations with V. cholerae isolation and pH, temperature, and the fecal coliform count. One-way ANOVA indicates that the mean values for pH and the fecal coliform count do not vary significantly from values expected by chance variation. The mean water temperature exhibits a moderate (0.451), significant correlation with isolation of the bacterium on a given day. The variation seen in this parameter was highly significant ($p = 0.0001$). Figure 5 displays a graphic representation of the relationship of mean water temperature to prevalence of V. cholerae in the San Lorenzo River. The prevalence rate clearly rises and falls with the mean water temperature. This finding agrees with other research 19,23,25.

A major study of four coastal areas by Seidler and Evans ⁵⁵ demonstrated that V. cholerae was isolated from waters ranging from 8°C to 36°C. The highest numbers of the organism were found in the 21°C to 28°C range. Figure 6 shows that V. cholerae isolations in this study were found in water temperatures ranging from 7.1°C to 23.0°C. Twenty percent of the isolates were found in the 18°-20°C range, 50% at

16°-22°C, and 70% in the 14° to 24°C range. The results of this study show a greater likelihood of isolating V. cholerae when water temperatures are between 14°C and 24°C, although the bacterium was isolated from samples with lower temperatures. West and Lee ⁵⁶ were unable to isolate V. cholerae from brackish waters below 9°C in Kent, England. This study slightly extends the lower limit observed by these researchers and by Seidler and Evans.

An anomalous rise in the prevalence of V. cholerae in the San Lorenzo River was noted on 10/25/89. All samples yielded isolates of V. cholerae. These samples were taken one week after the October 17, 1989, Loma Prieta earthquake. The earthquake measured 7.2 on the Richter scale. Minutes before and for weeks after the quake, an extraordinary amount of groundwater was inexplicably released to the surface. As can be seen in Table 4, the mean turbidity value for the samples taken on 10/25/89 shows a >10-fold increase (nephelometer turbidity units) over the next highest value. Turbidity measured as parts-per-million was beyond the capacity of the device used.

Kaysner et al.²⁴ demonstrated V. cholerae in sediment samples taken in bays and estuaries of California, Oregon, and Washington. The high prevalence of V. cholerae observed in the San Lorenzo River after the earthquake suggests that sediment deposits may yield V. cholerae isolates. Disruption

of the riverbed by natural forces such as storm run-off and earth movement and man-made disruptions such as levee work or bridge repair may transiently alter the V. cholerae population.

FIGURE 6: San Lorenzo River *Vibrio cholerae* Survey
 Santa Cruz, CA-12/88 - 12/89
V. cholerae isolates vs temperature

Site Analysis:

Recovery of V. cholerae was successful at all sites where greater than three samples were taken. The two sites from which V. cholerae isolates were not obtained were sites 349 and 762. As stated before, site 762 is a tributary creek and not part of the San Lorenzo River. It will be excluded from further discussion.

Site 349 is the site most distant from the ocean. Three negative samples were taken between December, 1988 and January, 1989. Sampling was discontinued because the volume of water flowing at this point was insufficient to collect a ten liter sample in a reasonable amount of time. It was decided that the possible exposure to the public was not significant and this site did not warrant continued monitoring.

Success at isolating the organism at all sites after repeated sampling suggests that other sites on the San Lorenzo River may yield V. cholerae as well. This suggestion may be important if other sites are identified that have potential human exposure.

Table 6 indicates that water temperature, water conductivity, the fecal coliform count, and the ratio of the fecal coliform count to the fecal streptococcus count were the environmental factors having a significant correlation with isolation of V. cholerae. However, sample temperature

did not vary significantly ($p=0.28$) between sites, leaving three factors with significant ($p < 0.05$) variation having a positive, significant ($p < 0.05$) correlation with V. cholerae isolation. The strength of these correlations ranged from low (fecal coliform 0.25) to moderate (conductivity 0.387; FC/FS ratio 0.495). These three factors account for 46% of the observed variation.

A positive, significant correlation was observed between isolation of V. cholerae and water conductivity. The variation observed between sites was significantly different ($p=0.0001$). The conductivity measured at site 003 differed from all other sites using two tests of variability, the Fisher protected least-square-difference test and Scheffe's F-test. No significant variation was observed between the other monitoring sites.

Measurement of electrical conductivity yields information on the amount of free ions present in a sample. Comparisons to other studies can be made using the conversions that one milliequivalent (meq) per liter of a monovalent ion is approximately equal to 0.01 times the conductivity at 25°C. One part per thousand ($^{0}/_{00}$) sodium chloride (NaCl) equals 17meq/l. Comparison of this study to other studies assumes that sodium chloride comprises the majority of free ions measured by conductivity. This assumption is probably valid

for the lower sites of the San Lorenzo River, but may not be valid for the upper river sites.

In the course of this study the salinity range of all samples was 0.1⁰/₀₀ to 18.5⁰/₀₀ NaCl. V. cholerae was isolated from waters ranging from 0.1⁰/₀₀ to 14.9⁰/₀₀ NaCl. The range of samples for the monitoring sites was the same as the range for the monitoring sites yielding V. cholerae isolates: 0.2⁰/₀₀ to 9.3⁰/₀₀ NaCl.

Figure 7 shows the relationship between mean salinity and isolation rates at the monitoring sites. Isolation of V. cholerae clearly declines as the mean salinity declines.

Colwell et al. ²⁷ demonstrated that increased salinity enhanced V. cholerae survival in Chesapeake Bay. Hood et al. ⁵⁷ found that in Florida estuaries, the greatest numbers of V. cholerae were isolated from waters of 10-25⁰/₀₀ NaCl. Lee et al. ³¹ found that few V. cholerae were isolated near Kent, England, in canal waters containing <1⁰/₀₀ NaCl, while the largest number of isolates were recovered from samples measuring 1-7⁰/₀₀ NaCl. The results of the current study indicate that a similar relationship exists in the San Lorenzo River between salinity (as measured by conductivity) and isolation of V. cholerae.

FIGURE 7: San Lorenzo River Vibrio cholerae Survey
Santa Cruz, CA-12/88 - 12/89

Comparison of isolation rates vs salinity

Fecal coliform counts were significantly ($p < 0.05$) correlated with isolation of V. cholerae at a given site. The correlation was low (0.255) and represents 6.5% of the statistical variation. Analysis by protected t-test (Fisher PLSD) indicates significant variation between sites at the 95% confidence level. This statistic is skewed by a single, very high fecal coliform count at site 202 on 8/1/89. When the ANOVA is recalculated, excluding this datum point, significant differences are indicated in the fecal coliform counts between site 003 and sites 022, 060, 180, and 245, and between site 202 and sites 022, 060, 180, and 245. Sites 003 and 202 thus had fecal coliform counts that differed significantly from the other monitoring sites, but did not differ significantly from each other.

Sites 003 and 202 also had the highest isolation rates of V. cholerae. Bradford ⁵⁸ concluded that fecal coliform counts fluctuated independently from V. cholerae counts. Colwell ²⁷ found no relationship between fecal coliform counts and V. cholerae isolation in Chesapeake Bay. Kenyon et al. ²⁸ isolated greater numbers of V. cholerae in coastal areas of Santa Cruz County that had elevated fecal coliform levels. A statistical association exists in the Santa Cruz County locale among fecal coliform counts and V. cholerae isolation. This relationship may reflect unknown environmental factors

that differ from those in the studies of Colwell and Bradford.

The ratio of the fecal coliform count to the fecal streptococcus count (FC/FS) is used in concert with other measures of water pollution. It is recommended by the Environmental Protection Agency of the United States government as an indicator of recent water contamination by human waste ⁵⁹.

Van Orden ⁶⁰ has pointed out that fecal streptococcus counts become unreliable at increasing distance from the point of contamination, and that tidal influences will distort FC/FS ratios.

This study found a significant ($p < 0.05$), positive correlation with FC/FS ratios and isolation of V. cholerae at a given site. The correlation was moderate (0.495) and represents 24.5% of the statistical variation. One-factor ANOVA for this parameter shows significant variation between sites ($p = 0.0001$) with significant differences observed at the 95% confidence level between site 003 and all other monitoring sites, and between site 202 and sites 022 and 060. No other significant differences between sites were noted.

This finding adds additional weight to the possibility that there is a definite relationship in Santa Cruz County waters to isolation of V. cholerae and indicators of fecal pollution. Dufour and Ballentine ⁵⁹ assert that fecal

streptococci persist longer in marine waters than in fresh waters. This study has demonstrated increased salinity and an elevated FC/FS ratio at site 003. If the number representing the fecal streptococcus count could be adjusted to account for the greater survival brought about by the elevated salinity, the number would only decrease. This would raise the FC/FS ratio and strengthen the observed relationship. No such "correction factor" is known to this researcher.

An interesting picture of the ecology of V. cholerae has emerged from this study. V. cholerae was isolated significantly more frequently at sites with elevated conductivity, fecal coliform counts and fecal coliform/ fecal streptococcus ratios. On sampling days when the water temperature was elevated, significantly more sites yielded V. cholerae. These findings support the conclusions of other researchers that salinity and water temperature are significant factors in the prevalence of V. cholerae in an aquatic system. This survey does not support the conclusions of other researchers that a relationship between the prevalence of V. cholerae and indicators of fecal pollution does not exist.

V. cholerae was isolated more frequently (>50%) from the lower San Lorenzo River sites, i.e. between the entry point of the river to Monterey Bay and the site 2.7km upstream.

Three factors may account for greater prevalence at these sites:

1] Environmental conditions at these sites are more supportive of growth and survival of V. cholerae, leading to a greater opportunity to detect the bacterium. This concept is supported by the higher temperatures and salinity (by conductivity) observed at these sites.

2] V. cholerae is being introduced from an outside source, such as leaking sewers, animal excrement, or ocean water intrusion. The correlation of V. cholerae isolation to elevated counts of fecal coliforms supports the conjecture that sewage may contribute to V. cholerae prevalence. The absence of endemic foci of V. cholerae gastroenteritis in Santa Cruz County negates it. A more likely explanation is that both types of organisms are supported by the same environmental conditions.

Animal reservoirs of V. cholerae have been proposed, but a model has yet to be established. Presence of V. cholerae in marine shellfish and crustaceans is well established.

Lee et al. ³¹ reported complete failure to isolate V. cholerae from a large number of cattle, sheep, pigs, and poultry. Their results show the presence of the bacterium in 6% of healthy gulls sampled, but this may be a transitory outcome of the birds having ingested infected shellfish. Kelly et al. ⁶¹ reported non-O1 V. cholerae isolated from a

single fish and three crayfish from a drainage dish associated with a human case of cholera.

Introduction of V. cholerae into the San Lorenzo River by waterfowl cannot be ruled out, but the correlation of high fecal coliform/ fecal streptococcus ratios with V. cholerae isolation negates this concept. Pollution by waterfowl, which are known to harbor high numbers of fecal streptococci, would be expected to lower the observed ratios.

The potential for fish to contribute organisms to the river environment exists, but seems unlikely given the consistent prevalence observed in the upper river. Steelhead trout (Oncorhynchus mykiss) spawn in the upper river, but only during late December and April.

Research by Kenyon et al.⁵ demonstrated non-O1 V. cholerae in the marine waters of Monterey Bay at the outflow point of the San Lorenzo River and at other points along the Santa Cruz County coastline. Mixing of ocean water with the San Lorenzo River is evident by the elevated conductivity observed in samples taken from the lower river. Introduction of V. cholerae during tidal changes is possible, and mixing of sea water that harbors the bacterium with river water may contribute to the greater prevalence of V. cholerae observed at sites 003 and 202.

Additional sampling from both the lower river and the waters of Monterey Bay could clarify this issue. Biotyping of

isolates with a large battery of biochemical reactions and serotyping of similar biotypes would resolve whether the organisms found in the San Lorenzo River are the same type as those found in the Bay.

Tidal effects could not reasonably explain the presence of V. cholerae in the upper sites of the river. Salt water intrusion does not appear to occur beyond site 202. Biotyping of the isolates taken during this study and serotyping of similar biotypes would clarify whether different stains exist in the upper and lower river.

3] V. cholerae from the upper river is accumulating in the waters of the lower river.

This study detected V. cholerae from non-stagnant, flowing water. Given the movement of the water, it is possible that these organisms could become concentrated in the lower river sites as the flow of the river becomes restricted upon entry to the Monterey Bay. Bohn and Buckhouse ⁶² assert that coliform numbers tend to increase at downstream points in streams. It is reasonable to assume that vibrios also would increase in numbers. The same techniques as in [2] above could be used to resolve this question. Additional sampling with complete biochemical characterization and serotyping of V. cholerae strains isolated from all environments would clarify this issue.

The V. cholerae isolates from this study have not been tested for virulence or pathogenic factors. Their potential as a public health threat is unknown. Given the documented evidence of non-toxigenic, non-O1 V. cholerae gastroenteritis and extra-intestinal infections, the presence of the organism in recreational waters must be acknowledged. Additional testing as discussed in conjunction with further in-depth investigation will ensure that environmental protection of public waters is promoted.

SUMMARY

Non-O1 Vibrio cholerae was isolated from the San Lorenzo River in Santa Cruz County, California. The use of gauze filtration of water samples in conjunction with enrichment at 42°C successfully demonstrated the presence of the bacterium.

V. cholerae was isolated at all sites when more than three samples were taken. A seasonal variation was observed. V. cholerae was more prevalent during the spring, summer, and fall.

Certain sites demonstrated a higher prevalence of V. cholerae. Prevalence at these sites was positively correlated with elevated salinity, elevated fecal coliform counts, and elevated ratios of fecal coliforms to fecal streptococcal counts.

The source of the organism was not determined. Additional research is warranted to clarify this question. The findings of this research support the hypothesis proposed by Colwell et al. that V. cholerae is an autochthonous member of brackish aquatic systems. This research suggests that the organism may be a naturally occurring member of fresh water systems, as well.

The gauze filtration method of sample collection coupled with 42°C enrichment incubation may provide an effective, sensitive protocol to monitor the presence of V. cholerae in

public waters. Further study to determine the true sensitivity of this technique is warranted.

Use of this technique with the water quality monitoring protocols for fecal coliforms currently in use, would yield a more complete picture of the true prevalence of V. cholerae in Santa Cruz County aquatic environments. It is recommended that the Environmental Health Department and the Health Services Agency of the County of Santa Cruz support these investigations.

Non-O1 Vibrio cholerae is a clinically recognized agent capable of producing extensive morbidity and has been associated with three known hospitalized cases from Santa Cruz County. It is recommended that the County of Santa Cruz Health Services Agency issue a Physician's Advisory, so that medical practitioners in the area can be made aware of the potential for vibrio infection.

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APPENDIX: Table (A) Water Quality Measurements
 San Lorenzo River *Vibrio cholerae* Survey-Santa Cruz, CA - December 1988- December, 1989

Site	Date Sampled	D.O. a	pH	Temp. °C	Cond. b	Turb. 1c	Turb. 2 d	FC e / 100ml	FS f / 100ml	FC / FS g	<i>V. cholerae</i> isolated
001	12/04/88	7.5	6.7	13.2	25300	15	ND	660	350	1.9	X
001	12/11/88	11.8	6.7	13.9	31500	1	ND	180	340	0.5	
003	12/04/88	8.6	6.8	12.5	5300	15	ND	740	760	1.0	
003	12/11/88	11.7	6.4	13.8	12100	3	ND	520	540	1.0	X
003	1/02/89	8.6	7.8	9.8	2200	15	ND	40	ND	ND	X
003	1/15/89	11.2	6.9	10.2	3400	21	ND	40	ND	ND	
003	2/26/89	8.5	7.2	12.2	1000	2	ND	484	ND	ND	
003	3/08/89	9.5	7.1	14.9	2900	12	ND	2260	2550	0.9	X
003	3/22/89	10.1	7.4	18.6	1500	34	2.4	1070	220	4.9	X
003	4/18/89	11.6	8.4	21.0	300	43	1.2	30	40	0.8	X
003	5/08/89	7.0	6.5	18.1	15800	13	1.6	2035	300	6.8	X
003	5/22/89	10.6	7.0	16.2	4300	48	1.0	200	130	1.5	X
003	6/07/89	6.9	6.9	17.9	13300	7	1.4	1740	240	7.3	X
003	6/27/89	12.4	7.3	22.2	14400	3	1.2	630	120	5.3	X
003	7/11/89	9.9	7.4	20.5	10800	107	1.0	336	80	4.2	X
003	8/01/89	10.5	7.5	23.0	7600	4	1.4	604	88	6.9	X
003	8/29/89	9.2	7.4	21.2	5400	ND	0.8	16	16	1.0	X
003	9/19/89	7.3	6.9	15.0	4600	169	3.6	1400	1030	1.4	X
003	10/25/89	8.0	7.8	15.5	400	ND	40	4160	3600	1.2	X
003	11/14/89	9.0	7.7	13.9	2000	102	1.8	280	170	1.6	X
003	12/04/89	10.2	7.7	8.6	600	16	1.6	220	410	0.5	X

a Dissolved oxygen
 b Conductivity micromhos/cm
 c Turbidity (ppm) by ratio turbidometer
 d Turbidity (NTU) by nephelometer at 25°C.
 e Fecal coliform count.
 f Fecal *Streptococcus* count
 g Ratio of fecal coliform to fecal streptococci
 ND= not determined
 X= isolate recovered

TABLE (B)
San Lorenzo River Vibrio cholerae Survey-Santa Cruz, CA - December 1988- December, 1989

Site	Date Sampled	D.O.	pH	Temp. °C	Cond.	Turb. 1	Turb. 2	FC / 100ml	FS / 100ml	FC / FS	V. cholerae isolated
005	1/02/89	13.0	8.2	10.7	300	11	ND	580	ND	ND	X
005	1/29/89	10.2	7.5	11.7	200	16	ND	200	ND	ND	X
005	4/18/89	11.9	8.0	22.0	1100	22	1.4	150	40	3.8	X
020	1/29/89	9.2	8.4	11.0	400	18	ND	60	ND	ND	
020	4/18/89	12.3	8.6	21.0	500	68	1.2	600	160	3.8	X
202	1/02/89	9.9	8.2	9.4	300	14	ND	200	ND	ND	
202	1/29/89	10.8	8.3	11.0	400	43	ND	10	ND	ND	
202	4/18/89	11.4	8.3	19.6	500	20	1.0	140	20	7.0	X
202	5/08/89	8.8	8.7	18.2	400	18	1.0	140	320	0.4	X
202	5/22/89	9.6	7.9	15.6	400	53	1.4	415	290	1.4	X
202	6/07/89	9.5	7.8	18.9	400	9	1.0	925	341	2.7	X
202	6/27/89	8.0	8.4	21.1	700	66	1.2	4200	1150	3.7	X
202	7/11/89	6.4	7.9	18.8	400	9	1.0	4960	1540	3.2	X
202	8/01/89	7.5	8.1	20.4	1000	35	0.8	13200	3540	3.7	X
202	8/29/89	6.9	8.2	20.3	100	ND	1.2	1640	900	1.8	X
202	9/19/89	7.5	7.4	16.0	400	1	1.4	380	500	0.8	
202	10/25/89	8.8	7.6	14.8	400	ND	47	0	7250	0.0	X
202	11/14/89	10.7	7.9	12.3	400	5	1.5	100	8900	0.0	X
202	12/04/89	10.8	7.7	8.1	400	12	1.5	0	400	0.0	

TABLE (C)
San Lorenzo River *Vibrio cholerae* Survey-Santa Cruz, CA - December 1988- December, 1989

Site	Date Sampled	D.O.	pH	Temp. °C	Cond.	Turb 1	Turb 2	FC / 100ml	FS / 100ml	FC / FS	V. <i>cholerae</i> isolated
22	1/15/89	13.2	7.7	7.0	300	22	ND	30	ND	ND	
22	2/26/89	12.1	8.2	12.3	500	21	ND	3	ND	ND	
22	3/08/89	12.8	8.3	13.7	400	20	2.0	704	540	1.3	
22	3/22/89	11.8	8.4	16.1	500	36	2.2	30	30	1.0	
22	4/18/89	11.6	8.4	21.0	300	43	1.2	30	40	0.8	
22	5/08/89	9.3	8.5	17.4	400	17	1.2	66	120	0.6	
22	5/22/89	10.5	8.4	15.4	400	15	1.4	62	536	0.1	
22	6/07/89	8.9	8.5	18.2	400	8	1.4	68	600	0.1	
22	6/27/89	11.6	8.8	19.5	500	4	1.2	64	524	0.1	X
22	7/11/89	9.7	9.0	17.8	400	10	1.0	54	80	0.7	X
22	8/01/89	8.9	8.6	19.0	400	5	1.0	94	664	0.1	X
22	8/29/89	9.6	8.6	18.4	400	ND	0.6	50	1660	0.0	X
22	9/19/89	9.4	7.9	15.4	400	2	1	172	700	0.2	X
22	10/25/89	9.1	7.8	14.4	400	ND	64	1700	4650	0.4	X
22	11/14/89	10.5	8.2	12.1	400	17	1.2	362	180	2.0	
22	12/04/89	11.3	8.2	8.6	400	16	1.6	186	420	0.4	X

TABLE (D)
San Lorenzo River *Vibrio cholerae* Survey-Santa Cruz, CA - December 1988- December, 1989

Site	Date Sampled	D.O.	pH	Temp. °C	Cond.	Turb. 1	Turb. 2	FC / 100ml	FS / 100ml	FC / FS	V. cholerae isolated
60	12/4/88	3.2	7.4	9.9	400	11	ND	630	4200	0.2	
60	12/11/88	12.9	7.5	9.3	400	19	ND	100	9050	0.0	
60	1/15/89	12.0	7.6	6.7	400	10	ND	130	ND	ND	
60	2/26/89	10.5	8.0	12.2	500	23	ND	96	ND	ND	
60	3/8/89	9.9	8.0	13.0	400	12	5.5	760	2680	0.3	
60	3/22/89	10.5	8.1	14.7	500	18	2.2	70	120	0.6	
60	4/18/89	9.8	8.1	17.3	500	36	2.2	125	60	2.1	X
60	5/8/89	8.6	8.1	17.5	400	13	4.2	252	465	0.5	X
60	5/22/89	10	8.3	15.6	400	31	3.2	112	370	0.3	
60	6/7/89	8.7	8.6	17.9	400	4	3.4	180	260	0.7	
60	6/27/89	10.6	8.6	18.4	400	79	1.5	188	370	0.5	
60	7/11/89	8.9	5.7	16.4	400	9	1.6	260	365	0.7	
60	8/01/89	9.8	8.4	17.8	400	11	1.5	144	208	0.7	X
60	8/29/89	9.3	8.4	17.2	400	ND	0.8	104	184	0.6	
60	9/19/89	9.2	7.9	14.3	400	2	0.8	204	630	0.3	
60	10/25/89	8.9	7.8	13.7	300	ND	112	1440	3300	0.4	X
60	11/14/89	10.6	8.2	11.4	400	5	1.0	550	1860	0.3	X
60	12/4/89	11.1	8.2	8.2	400	7	1.4	140	330	0.4	

TABLE (E)

San Lorenzo River Vibrio cholerae Survey-Santa Cruz, CA - December 1988- December, 1989

Site	Date Sampled	D.O.	pH	Temp. °C	Cond.	Turb. 1	Turb. 2	FC / 100ml	FS / 100ml	FC / FS	V. <u>cholerae</u> isolated
180	12/04/88	9.0	7.6	8.6	500	27	ND	65	7660	0.0	
180	12/11/88	13.5	7.7	7.6	400	34	ND	40	3500	0.0	
180	1/15/89	12.3	7.8	5.6	300	17	ND	70	ND	ND	
180	2/26/89	12.5	8.0	10.9	500	36	ND	10	ND	ND	
180	3/08/89	10.4	8.1	12.6	300	10	6.0	768	2160	0.4	
180	3/22/89	11.7	8.0	14.4	400	18	2.0	130	90	1.4	
180	4/18/89	11.1	8.1	18.0	500	45	1.2	155	80	1.9	X
180	5/08/89	10.0	8.1	18.2	400	18	1.4	236	115	2.1	
180	5/22/89	9.7	8.1	15.9	400	6	0.7	1408	544	2.6	
180	6/07/89	9.4	8.4	18.6	400	1	0.8	130	160	0.8	
180	6/27/89	10.6	8.2	19.5	400	7	0.8	104	280	0.4	
180	7/11/89	8.7	8.4	18.8	400	13	1.5	80	128	0.6	
180	8/01/89	8.7	8.1	20.5	400	1	0.8	60	76	0.8	X
180	8/29/89	9.1	8.4	19.1	500	ND	0.5	130	248	0.5	
180	9/19/89	10.2	7.8	15.6	400	33	1.0	300	380	0.8	
180	10/25/89	9.7	7.9	13.6	400	ND	61	1400	5320	0.3	X
180	11/14/89	10.1	8.2	10.8	400	7	1.0	528	1060	0.5	X
180	12/04/89	11.0	8.3	7.1	400	14	1.5	260	440	0.6	X

TABLE (F)
San Lorenzo River Vibrio cholerae Survey-Santa Cruz, CA - December 1988- December, 1989

Site	Date Sampled	D.O.	pH	Temp. °C	Cond.	Turb. 1	Turb. 2	FC / 100ml	FS / 100ml	FC FS	V. cholerae isolated
245	12/04/88	9.4	7.6	8.3	400	131	ND	160	560	0.3	
245	12/11/88	13.4	7.7	7.6	400	6	ND	685	80	8.6	
245	1/15/89	12.3	7.8	5.1	300	22	ND	160	ND	ND	
245	2/26/89	11.9	8.0	10.3	500	22	ND	36	ND	ND	
245	3/08/89	9.9	8.1	12.2	400	12	5.8	850	5280	0.2	
245	3/22/89	11.0	8.1	14.6	500	19	1.6	112	160	0.7	
245	4/18/89	10.5	7.8	16.4	500	21	1.5	270	125	2.2	X
245	5/08/89	9.1	7.9	16.5	400	12	2.0	415	170	2.4	
245	5/22/89	9.7	8.0	13.8	400	8	1.4	276	220	1.3	
245	6/07/89	9.6	8.2	16.4	500	41	1.0	115	140	0.8	
245	6/27/89	10.7	8.1	17.3	500	3	0.6	84	80	1.1	
245	7/11/89	8.5	8.3	16.7	400	2	0.8	112	330	0.3	
245	8/01/89	7.9	8.1	17.7	400	10	0.6	270	308	0.9	
245	8/29/89	7.9	8.3	16.8	500	ND	0.6	60	228	0.3	
245	9/19/89	8.8	7.8	14.2	400	1	1.2	300	460	0.7	
245	10/25/89	9.0	8.0	12.7	400	ND	8	820	2580	0.3	X
245	11/14/89	10.2	8.3	10.7	400	29	1.0	165	650	0.3	
245	12/04/89	11.2	8.3	6.9	400	9	1.5	140	400	0.3	
349	12/04/88	9.6	7.9	7.9	400	20	ND	1	16	0.1	A. hydrophila
349	12/11/88	13.1	7.8	7.9	400	2	ND	0	72	0.0	
349	1/15/89	11.9	8.0	4.4	300	24	ND	3	ND	ND	
762	3/22/89	11.1	7.9	14.0	500	19	ND	108	400	0.3	