# Correlation between Environmental Factors and Prevalence of *Vibrio parahaemolyticus* in Oysters Harvested in the Southern Coastal Area of Sao Paulo State, Brazil[▽]

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The presence of *Vibrio parahaemolyticus* in 123 oyster samples collected from an estuary on the southern coast of Sao Paulo state, Brazil, was investigated. Of the 123 samples, 99.2% were positive with densities ranging from <3 to $10^5$ most probable number (MPN)/g. Densities correlated significantly with water temperature ($r = 0.48$; $P < 0.001$) but not with salinity ($r = -0.09$; $P = 0.34$). The effect of harvest site on counts was not significant ($P > 0.05$). These data provide information for the assessment of exposure of *V. parahaemolyticus* in oysters at harvest.

Infections caused by *Vibrio parahaemolyticus* have been reported in several countries (1, 3–5, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26). Among other pathogenic features, *V. parahaemolyticus* strains produce a thermostable hemolysin, known as thermostable direct hemolysin (TDH), as well as TRH (a TDH-related hemolysin) (25, 29). However, not all strains are pathogenic, as less than 1% of food or environmental strains produce TDH or TRH (2, 7, 9, 10, 11).

The most important vehicle for this microorganism is raw or partially cooked shellfish (8, 13, 25, 29). In this study, the densities of *V. parahaemolyticus* in oysters collected in six oyster bed sites in the estuary of Cananeia (25°S; 48°W) in the southern coastal area of Sao Paulo state, Brazil (Fig. 1) between May 2004 and June 2005 were determined using the most probable number (MPN) technique by the method of De Paola and Kaysner (12). Each sample consisted of 15 oysters, pooled in a plastic bag, and transported in a cold box to the laboratory located in the city of Sao Paulo, Brazil. The temperature during transportation did not exceed 13°C, and the travel time was around 5 h. In the laboratory, the oysters were kept under refrigeration (4 to 8°C) and analyzed within 24 h of collection. Oysters were cleaned and shucked by the method of Cook et al. (6). Identification of *V. parahaemolyticus* was based on traditional and API 20E strip biochemical tests (bioMérieux, France), using *V. parahaemolyticus* ATCC 17802 as the reference strain. The observed prevalence of this bacterium was high, as the microorganism was detected in 99.2% (122/123) of the samples and the densities varied between 0.78 and 5.04 log MPN/g.

Strategies for the control of *V. parahaemolyticus* in oysters depend on understanding the seasonal and geographical distribution and the effects of environmental parameters on the growth of this pathogen. To verify the influence of salinity and temperature of seawater on the density of *V. parahaemolyticus*, samples of water ($n = 123$) were collected from the same depth of oyster beds using 250-ml plastic flasks. Salinity was determined using a salinometer (model RS10; Rosemount Analytical, Cedar Grove, NJ), and the temperature was determined at the time of collection using a digital thermometer (Hanna Instruments). The results are shown in Fig. 2.

Total *V. parahaemolyticus* densities did not correlate significantly with water salinity, as determined by Pearson coefficient ($r = -0.09$; $P = 0.34$). However, the mean salinity varied significantly according to the sampling site and season ($P < 0.05$) (Table 1). The highest mean salinity (24.2 ppt) was detected at site 5 and was 1.4 times higher than at site 2 (17.3 ppt), the lowest mean salinity detected in this study.

The weak correlation between water salinity and *V. parahaemolyticus* densities in oysters suggests that salinity *per se* is a secondary factor for growth of this bacterium, as are turbidity and chlorophyll content in water (27, 30). These results agree with those obtained by Deepanjali et al. (9) and Martinez-Urtiga et al. (21), who did not find correlation between these two parameters. However, they are in contrast with the results reported by DePaola et al. (11), who observed correlation ($P < 0.05$) between salinity and total density of *V. parahaemolyticus*.

The results of this study corroborate existing evidence (10, 11, 27, 30) indicating that the temperature of seawater has a significant correlation ($r = 0.48$; $P < 0.001$) on the densities of *V. parahaemolyticus* in oysters, but they are at odds with results reported by Deepanjali et al. (9), who observed no statistically significant correlation with seawater temperature. The temperature variations observed in the present study (15°C) were lower than those observed by DePaola et al. (22°C) (11) but higher than those reported by Deepanjali et al. (10°C) (9).

The relationship between *V. parahaemolyticus* density and water temperature and salinity were analyzed by multiple linear regression. Results showed that salinity was not significant

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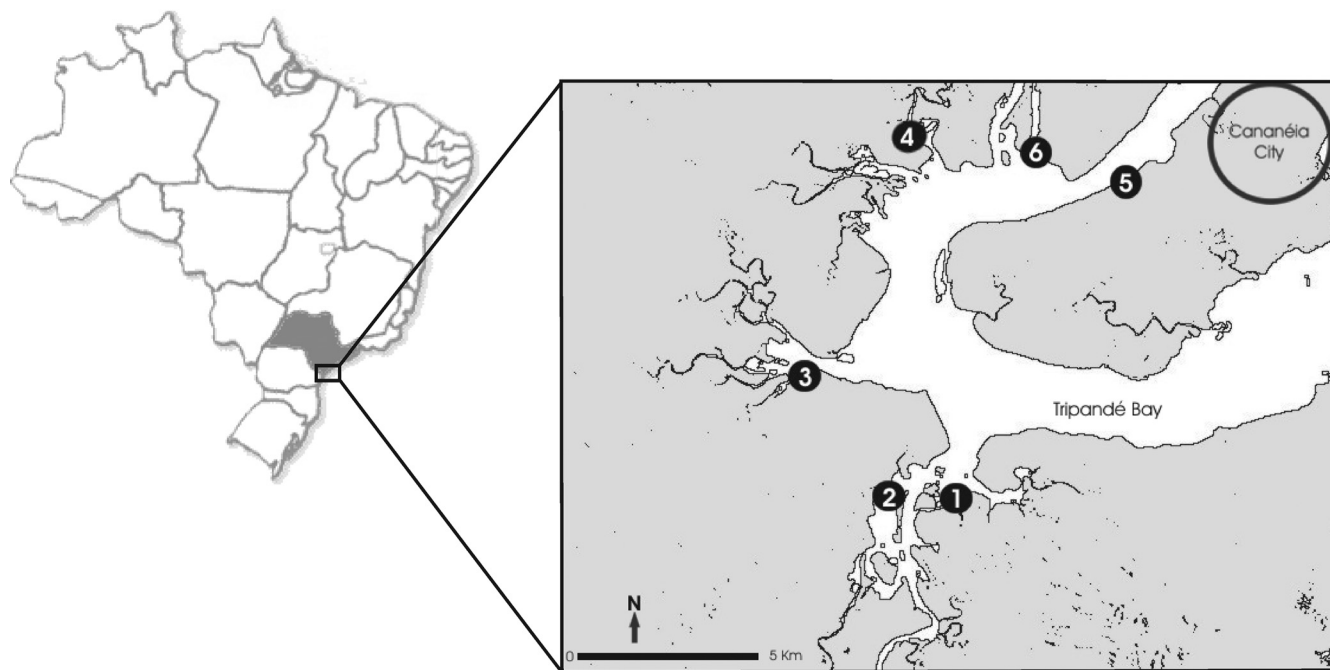▽ Published ahead of print on 18 December 2009.

FIG. 1. Locations of oyster bed sites in the Cananeia estuary on the southern coast of Sao Paulo state, Brazil. (Courtesy of E. E. de Miranda and A. C. Coutinho [Embrapa Monitoramento por Satélite] [http://www.cdbrasil.cnpm.embrapa.br].)

either for linear effects or for squared effects ($P > 0.05$). For temperature, while the parameter of linear effect was significant ($P < 0.05$), the squared effect was not ($P > 0.05$). Considering the goodness of fit of the model, the following linear regression described the density in oysters the best (Fig. 3): $\log_{10}$ MPN *V. parahaemolyticus*/g $= -0.944 + (0.175 \times$ temperature). The lack of model fitness test was not significant and was considered adequate to express the relationship between *V. parahaemolyticus* density and seawater temperature, in spite of the low $R^2$ (0.23).

The effect of temperature was further summarized by rank correlation and the use of a smoothing technique (moving average) in which densities corresponding to temperatures within a range of 1°C were pooled to estimate an arithmetic mean of densities in successive intervals. The moving average was calculated using a length of three values. Although seawater temperature and *V. parahaemolyticus* densities were correlated in the present study, the mean densities reached a plateau at temperatures above 24°C and below 20°C (Fig. 4) where the density was not significantly influenced by temperature, consistent with observations reported also by DePaola et al. (11). Our findings could explain the lack of correlation
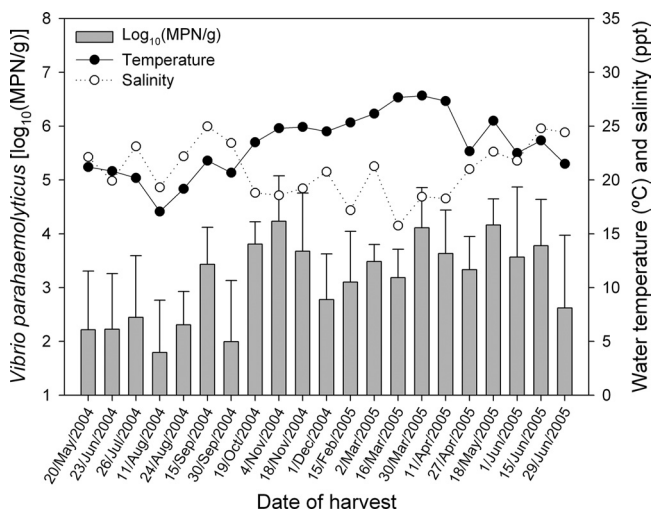


FIG. 2. Total densities of *Vibrio parahaemolyticus* in oysters from the southern coast of Sao Paulo state, Brazil. Each bar or point represents the arithmetic mean of six sites, and each error bar represents the standard deviation.

TABLE 1. Seasonal distribution of the total density of *Vibrio parahaemolyticus* in oysters, water temperature, and salinity in the southern coastal area of Sao Paulo state, Brazil

| Variable | Season | No. of samples | Mean[a] | SD | Range |
|---|---|---|---|---|---|
| *Vibrio parahaemolyticus* density ($\log_{10}$ MPN/g) | Winter | 35 | 2.44 A | 1.06 | <0.48–4.38 |
| | Spring | 29 | 3.26 B | 1.17 | 1.04–5.04 |
| | Summer | 24 | 3.47 B | 0.75 | 1.54–5.04 |
| | Fall | 35 | 3.48 B | 1.01 | 1.04–5.04 |
| Temp (°C) | Winter | 35 | 20.1 A | 1.9 | 14.4–24.0 |
| | Spring | 29 | 23.6 B | 1.8 | 20.0–26.0 |
| | Summer | 24 | 26.7 C | 1.4 | 24.1–29.2 |
| | Fall | 35 | 23.9 B | 2.2 | 20.6–28.3 |
| Salinity (ppt) | Winter | 35 | 22.3 A | 4.5 | 12.2–29.8 |
| | Spring | 29 | 20.2 AB | 4.4 | 11.2–29.4 |
| | Summer | 24 | 18.2 B | 4.3 | 5.3–25.2 |
| | Fall | 35 | 21.8 A | 3.9 | 8.7–28.2 |

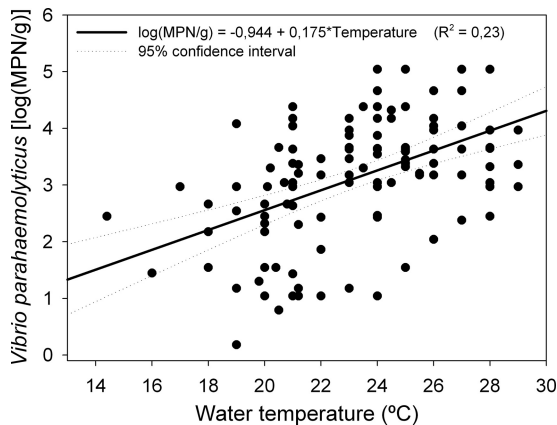[a] Values with different letters are significantly different ($P < 0.05$).

FIG. 3. Goodness of fit regression model of *V. parahaemolyticus* density in oysters and water temperature.

among those parameters detected in tropical oysters by Deepanjali et al. (9) when the temperature varied from 25 to 35°C.

The influence of the season of the year and site of collection on the mean densities was assessed by analysis of variance and Tukey's test, when necessary. As shown in Table 1, the *V. parahaemolyticus* densities were similar in the samples collected during spring, summer, and autumn but differed significantly ($P < 0.05$) in those collected during winter. Densities among samples collected during summer varied less compared to other seasons. Densities above $10^5$ MPN/g were detected in six (4.9%) oyster samples (three samples during spring, two samples during summer, and one sample during autumn) collected when the temperature was higher than 24°C and the salinity was higher than 15 ppt. The effect of harvest site on densities was not significant ($P > 0.05$).

Previous studies performed with oysters collected in the same region in Brazil have shown a low incidence of pathogenic *V. parahaemolyticus* (23, 28). Similar results were observed in the present study, as only one oyster sample (0.8%) and only one isolate among 2,243 isolates tested (0.044%) were
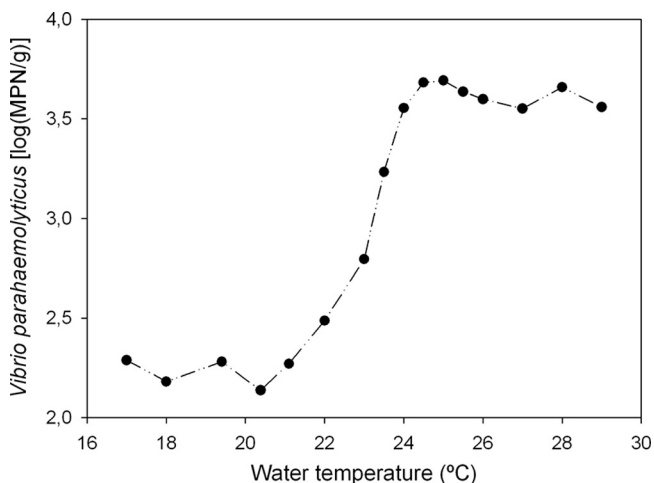
Kanagawa and *tdh* positive. Besides the Kanagawa reaction (24), all strains have been tested for *tlh*, *tdh*, and *trh* genes using PCR (12). The pathogen-positive sample presented with a low density of *V. parahaemolyticus* (3 MPN/g) and was collected during winter, when the temperature was 21°C. Due to the low incidence of pathogenic strains in the samples, correlation between pathogenicity and water temperature or salinity could not be determined.

This study indicates that the presence of *V. parahaemolyticus* in oysters cultivated in the southern coast of Sao Paulo state, Brazil, is high, but pathogenic strains are seldom detected. These results on the ecology and characteristics of *V. parahaemolyticus* are valuable for future risk assessments related to this pathogen in oysters at harvest.

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FIG. 4. Relationship between the mean density of *V. parahaemolyticus* in oysters and seawater temperature in Cananeia estuary, Sao Paulo state, Brazil.

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