Relationship between *V. parahaemolyticus* levels in market bivalve molluscs and reported illnesses in Los Lagos, Chile

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Introduction

Following emergence of the pandemic clone of *Vibrio parahaemolyticus* in 1996 (Okuda *et al.* 1997), there has been a sharp increase in *V. parahaemolyticus* cases worldwide. Large outbreaks attributable to the pandemic clone have occurred with high attack rates in Chile, and especially in the Los Lagos region in southern Chile (Gonzalez-Escalona *et al.* 2005, Martinez-Urtaza *et al.* 2008). The size of these outbreaks in comparison to the burden of *V. parahaemolyticus* illness in the US suggests that the virulence of pandemic strains may be substantially greater than strains outside the pandemic clone. To investigate this hypothesis a survey of *V. parahaemolyticus* levels in market bivalve mollusks commonly consumed and associated with illnesses in the Puerto Montt area of Los Lagos was undertaken for comparison to the rate of reported illness.

Materials & Methods

Market samples of shellfish (mussels, clams) were collected at approximately 2-week intervals and analyzed in duplicate from December 29, 2008 until March 27, 2009. The same markets were also sampled on May 5 and June 2, 2009, when the number of *V. parahaemolyticus* cases was expected to be zero. A total of 39 samples (12 bivalves/sample) were collected and analyzed in duplicate (78 sub-samples). The abundance of *V. parahaemolyticus* in shellfish was quantified using a most probable number format followed by real-time PCR detection of the thermolabile hemolysin (*tlh*) for total *V. parahaemolyticus* and the thermostable direct hemolysin (*tdh*) and the *tdh*-related hemolysin (*trh*) for pathogenic *V. parahaemolyticus*. The number of *V. parahaemolyticus* cases reported each week in the Puerto Montt area was obtained from the Department of Epidemiology, Ministry of Health Chile. For each bi-weekly sampling period, mean levels of vibrio (*tlh, tdh*) were estimated by fit of a generalized linear mixed model (GLMM) to the MPN-PCR observations. The correspondence between resulting estimates of abundance of vibrio in shellfish and the number of reported cases of infection in Puerto Montt occurring immediately following each market sampling date was determined by Poisson regression analysis.

Results and Discussion

In samples collected from late December through March, total *V. parahaemolyticus* (*tlh*) was found in 93% (65/70) of the samples at levels between <0.03 and 1100 MPN/g. During the same period, pathogenic *V. parahaemolyticus* (*tdh+*) was detected in 26% of the samples with levels ranging between 0.03 and 120 MPN/g. Only 22% of the samples collected in May and June had detectable *V. parahaemolyticus* (0.036-20 MPN/g) levels and pathogenic *V. parahaemolyticus* was not detected in any of these samples. None of the samples during the entire study were positive for *trh*.

Bi-weekly estimates of mean levels of total (*tlh*) and pathogenic (*tdh*) *V. parahaemolyticus* are shown in Figure 1 in comparison to the number of cases reported over the two week period following each sampling date. The correspondence between incidence of illness and levels of *V. parahaemolyticus* was found to be stronger for *tlh* than *tdh*, which may be a consequence of the uncertainty of estimates for *tdh*. The correspondence between reported illness and mean *tlh* levels is shown in Figure 2. Based the fit of the regression, there was greater than a 1 log increase in reported cases corresponding to a 2 log increase in total *V. parahaemolyticus*.
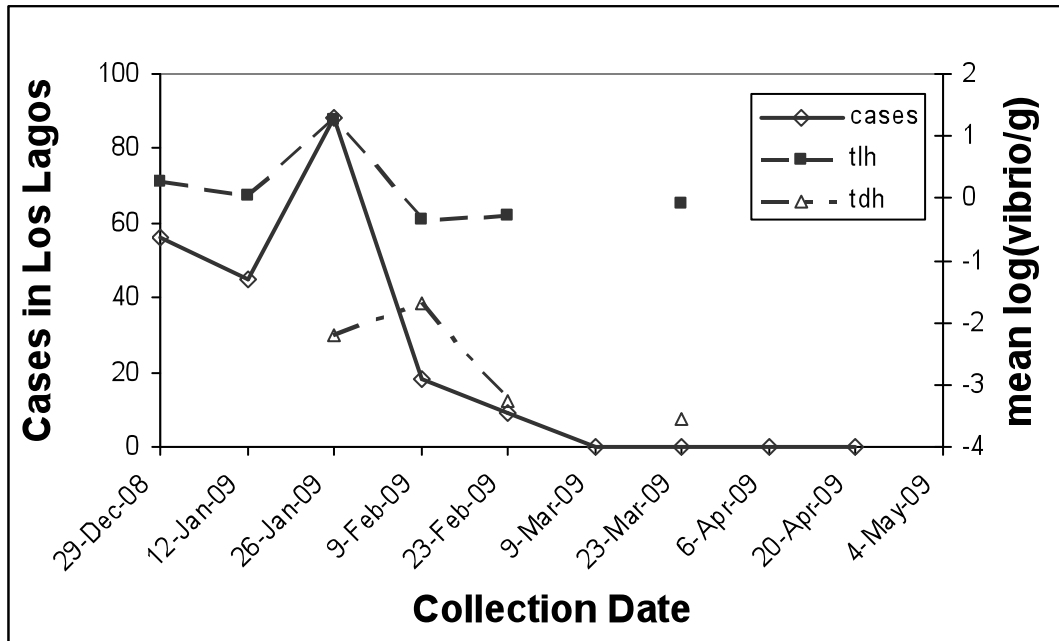

Figure 1. Bi-weekly number of reported cases of *V. parahaemolyticus* in the Puerto Montt area versus *tlh* and *tdh* levels in market shellfish during the market survey period.
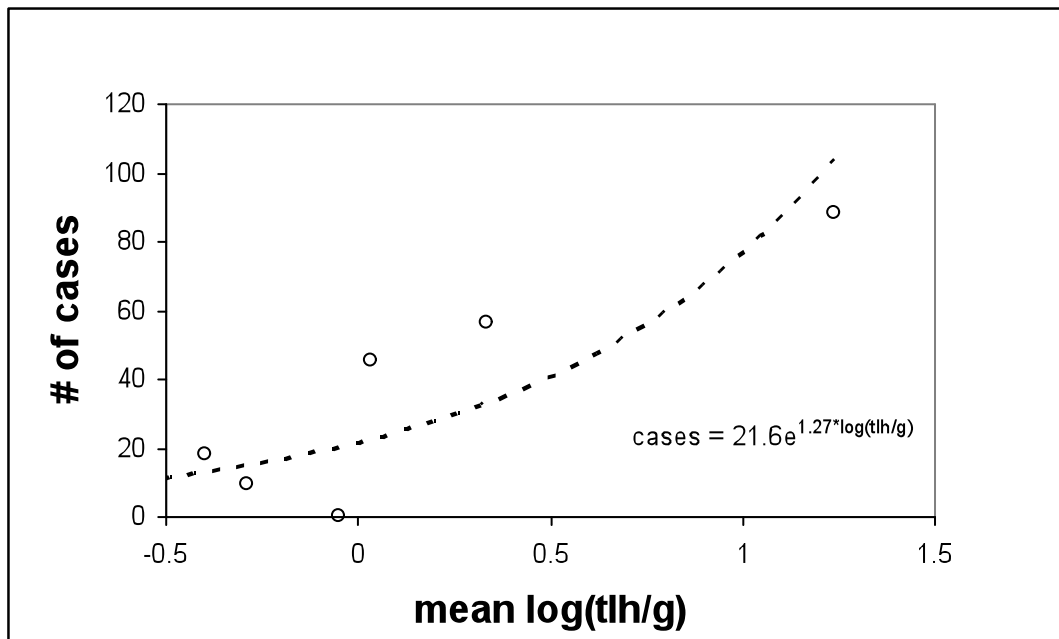

Figure 2. Relationship between bi-weekly reported cases of infection and mean levels of total *V. parahaemolyticus* in market shellfish in Puerto Montt.

Conclusions

As an assessment of probable dose-response, the bi-weekly frequency of illnesses reported in Los Lagos correlated with the estimated abundance of total *V. parahaemolyticus* in market samples. Levels of pathogenic (*tdh*) *V. parahaemolyticus* were frequently below the limit of detection and estimates of bi-weekly mean exposure levels too uncertain to identify a direct correlation with occurrence of illness. Total *V. parahaemolyticus* levels observed during the present study were found to be considerably lower than that observed in market oysters from the US during comparable warm weather months (Cook *et al.* 2002, DePaola *et al.* 2010) and the proportion of the total population that are *tdh+* appear comparable (~0.5%). In comparison to per capita burdens of illness in each country, these differences suggest that pandemic strains may be more virulent than non-pandemic strains. A total of 1616 illnesses were reported in Chile from June 2008 to April 2009 and ~19% of these were culture-confirmed. The corresponding per capita rate of culture-confirmed illness is ~2 per 100,000 which is over 10-fold greater than that observed in the US where less than 200 infections per year are culture-confirmed in a population of over 300 million. These findings support a growing body of evidence that even among the *V. parahaemolyticus* strains possessing the virulence determinant, *tdh*, there is wide variation from one region to the next with regard to dose response and that neither levels of total nor pathogenic *V. parahaemolyticus* is a universal indicator of risk.

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