Vibrio parahaemolyticus GASTROENTERITIS ASSOCIATED WITH CONSUMPTION OF ALASKAN OYSTERS


Alaska Division of Public Health, DHSS
Section of Epidemiology
3601 C Street, Suite 540
Anchorage, AK 99503
E-mail: joe_mclaughlin@health.state.ak.us

BACKGROUND: Vibrio parahaemolyticus (Vp), the leading cause of seafood-associated gastroenteritis in the United States, is typically associated with consumption of raw oysters gathered from warm estuarine waters. We describe a Vp outbreak that occurred in Alaska during the summer of 2004.

METHODS: We conducted a retrospective cohort study among cruise ship passengers to determine illness attack rates and risk factors, active surveillance to identify additional case-patients not associated with the cruise ship, and an extensive environmental study to identify sources of Vp and factors that might have contributed to the outbreak.

RESULTS: Of 189 passengers, 132 (70 percent) were interviewed; 22 (17 percent) met our gastroenteritis case definition. Raw oysters were the only significant predictor of illness after multiple logistic regression analysis. Cruise ship case-patients consumed a median of one oyster. Active surveillance identified numerous additional case-patients. Pulsed-field gel electrophoresis patterns were highly conserved across clinical and oyster isolates. Seventy-four percent of environmental isolates were positive for the presence of the thermostable direct hemolysin gene (a known virulence factor).

CONCLUSIONS: This large Vp outbreak associated with consumption of raw Alaskan oysters demonstrated a low infectious dose and extraordinarily high proportions of pathogenic environmental isolates.
Vibrio parahaemolyticus OUTBREAKS IN SPAIN: IMPLICATIONS FOR THE EU

Jaime Martinez-Urtaza
Instituto de Acuicultura
Universidad de Santiago de Compostela
Spain
ucmjmur@usc.es

V. parahaemolyticus infections have increased globally during the last decade. From 1996, outbreaks of illnesses due to this organism have been detected in several Asian countries, Chile and the USA. By contrast, the risk of infections caused by V. parahaemolyticus in Europe has been considered low according to recent reviews of epidemiological information. Data obtained after an exhaustive survey of unreported cases at Spanish hospitals have shown that V. parahaemolyticus infections in Spain are more common than previously assumed. This organism was isolated from patients with gastroenteritis in Barcelona (1986, 1987 and 1999), Zaragoza (1993) and Madrid (1998 and 2000). In Galicia (NW Spain), 84 cases of V. parahaemolyticus infections were identified retrospectively from hospital records from 1997 to 2000. A single outbreak of 64 cases in 1999 was associated with oyster consumption. Most Spanish clinical isolates were serotype O4:K11 and pulsed-field gel electrophoresis (PFGE) analysis demonstrated these to be a unique clone distinct from Asian and American clinical strains. In July 2004, a second large V. parahaemolyticus outbreak of 80 illnesses occurred in A Coruña. The outbreak isolates belonged to the serotype O3:K6 and O3:K untypeable, had the toxR, tilh, and tdh genes, lacked the thr gene, and were positive for the group specific-PCR assay. Results from PFGE and arbitrarily primed PCR analyses unequivocally linked the outbreak isolates to the pandemic clone of V. parahaemolyticus. The epidemiological investigation associated with this outbreak identified boiled crab as the most probable vehicle of infection.

Investigations of the environmental circumstances existing during the outbreak episodes have revealed that the sudden increment of infections detected in 1999 and 2000 were coincident with the presence of exceptional oceanographic conditions (dominant downwelling periods and warm seawater temperatures), which suggests that changing environmental conditions could drive the increment of V. parahaemolyticus levels in the marine environment of Galicia. By contrast, no unusual oceanographic conditions were observed in the nearby dates of the 2004 outbreak. This observation, together with the pandemic nature of the isolates, directs the most probable origin of the contamination to the ballast water from ships.

The emergence of V. parahaemolyticus infections in the European continent is an important public health concern and stresses the urgent need for the revision of the current status of this organism in the European microbiological surveillance system for infectious gastroenteritis and its possible incorporation in the control programs for shellfish harvesting areas and ready-to-eat seafood in Europe.
THE ROLE OF HUMAN VOLUNTEER STUDIES IN RISK MANAGEMENT: EVIDENCE FROM VIRUS INFECTIVITY DATA SUBJECTED TO QUANTITATIVE RISK ASSESSMENTS

Mark D. Sobsey

University of North Carolina
CB# 7431, McGavran-Greenberg Hall, room 4114a
Chapel Hill, NC 27599-7431 USA
E-mail: Sobsey@email.unc.edu

A limited number of human volunteer studies have been done to predict the risks of viral infection and illness from ingestion of raw mollusks. Two main types of studies have been done: those using safety-tested inocula of specific human enteric viruses and those using actual shellfish harvested from natural waters. In this presentation will briefly review the available data from both types of studies, the strengths and weaknesses of these data and the challenges faced in trying to use such data for microbial risks assessments and risk management decisions. Also to be discussed are the future potentials for such studies and the challenges presented by new regulations governing microbial inocula quality and safety, health protection and ethics in human volunteer studies.

The presentation will include information and data analysis from human volunteer dose-response infectivity studies that have been done with safety-tested inocula for hepatitis A virus, Norwalk Virus, Rotavirus, Polioviruses, Echoviruses, Coxsackieviruses and Adenoviruses. The need to “mine” these previous data for human infectivity dose-response relationships and the development of risk estimates relevant to ingestion of raw shellfish will be discussed and examples will be presented. Specifically, historical data for Norwalk Virus and Hepatitis A Virus will be presented with new dose-response analyses. Examples of the dose-response analyses of these data are shown in the Figures below. Also to be presented are the results of human volunteer studies on risks of gastroenteritis from ingestion of raw oysters and clams that were done in the 1980s. The extent to which these data can be used for risk assessments and risk management decisions will be considered.

Dose-Response Modeling of Virus Illness from Historical Human Volunteer Data:
Panel A (left): Norwalk Virus
Panel B (right): Hepatitis A Virus
*Vibrio parahaemolyticus* IN CLINICAL AND SHELLFISH SAMPLES DURING THE DIARRHEA OUTBREAKS OF 2004 AND 2005 IN SOUTHERN CHILE

Romilio T. Espejo*, Jessica Toro, Cristina Hernández, Loreto Fuenzalida, and Jaime Romero

Instituto de Nutrición y Tecnología de los Alimentos
Universidad de Chile, El Llano 5524, Macul, Santiago, Chile
E-mail respejo@inta.cl

Large diarrhea epidemic outbreaks associated to seafood consumption have taken place the austral summers of 2004 and 2005 in the environs of Puerto Montt Chile (41°S 72°W, approximately). The 2004 epidemic reached approximately 1.500 clinical cases and the epidemic of 2005 about 3.600. Samples of clinical cases and shellfishes were analyzed to understand the origin and spread of these epidemics. Our observations indicate that the epidemics were directly related to the presence of the O3:K6 serovar pandemic clone, in spite this clone was a minor component of a small *V. parahaemolyticus* population in shellfishes.

The epidemic in 2005 was not exclusive of the Puerto Montt area. About 11.000 clinical cases were reported in the whole country. Nevertheless, it is likely that seafood from the Puerto Montt region caused most of the cases because this region produces about 80% of the seafood consumed in the large cities. Analysis of the stools from clinical patients indicated that both epidemic outbreaks were caused by the O3:K6 pandemic clone of *V. parahaemolyticus* that emerged in Southeast Asia in 1996. Before 2004, *V. parahaemolyticus* infection had not been reported in this region and their absence was thought to be due to the low seawater temperature that seldom reaches more than 16 °C.

Analysis of the shellfish samples obtained at the midst of the epidemics (January-March) showed that 53% contained *V. parahaemolyticus*. However the total load was low, ranging from 3 to 93 g⁻¹, and only 4 of the 108 positive samples showed O3:K6 pandemic isolates. *V. parahaemolyticus* population in shellfish was mainly composed by non-pandemic strains, which could be differentiated into 14 clonal groups. None was *tdh* or *trh* positive. Only four of the 14 clonal groups were found both summers, suggesting either temporal changes in population composition or a much larger diversity than that observed by us in these two years.

| Total and pandemic *V. parahaemolyticus* in clinical and shellfish samples |
|-----------------------------|-------|----------|
| Samples        | Total | *V. parah* | O3:K6 pandemic |
| Clinical       | 64    | 63       | 58 (90%) |
| Shellfish      | 204   | 108      | 3 (3%)   |

Clonal groups were differentiated by a new method developed to allow rapid and simple comparison of the bacterial origin. This method permits direct genome restriction enzyme analysis (DGREA) with equal discriminatory index than RFLP-PFGE. It is based on digestion of bacterial DNA with a six base-restriction endonuclease, separation of the fragments with sizes ranging from 500 to 2.500 bp by polyacrylamide gel electrophoresis, and comparison of the fragments patterns after visualization by silver nitrate staining.