Outbreak of *Vibrio parahaemolyticus* Gastroenteritis Associated with Alaskan Oysters


**BACKGROUND**

*Vibrio parahaemolyticus*, the leading cause of seafood-associated gastroenteritis in the United States, typically is associated with the consumption of raw oysters gathered from warm-water estuaries. We describe a recognized outbreak of *V. parahaemolyticus* infection associated with the consumption of seafood from Alaska.

**METHODS**

After we received reports of the occurrence of gastroenteritis on a cruise ship, we conducted a retrospective cohort study among passengers, as well as active surveillance throughout Alaska to identify additional cases, and an environmental study to identify sources of *V. parahaemolyticus* and contributors to the outbreak.

**RESULTS**

Of 189 passengers, 132 (70 percent) were interviewed; 22 of the interviewees (17 percent) met our case definition of gastroenteritis. In our multiple logistic-regression analysis, consumption of raw oysters was the only significant predictor of illness; the attack rate among people who consumed oysters was 29 percent. Active surveillance identified a total of 62 patients with gastroenteritis. *V. parahaemolyticus* serotype O6:K18 was isolated from the majority of patients tested and from environmental samples of oysters. Patterns on pulsed-field gel electrophoresis were highly related across clinical and oyster isolates. All oysters associated with the outbreak were harvested when mean daily water temperatures exceeded 15.0°C (the theorized threshold for the risk of *V. parahaemolyticus* illness from the consumption of raw oysters). Since 1997, mean water temperatures in July and August at the implicated oyster farm increased 0.21°C per year (P<0.001 by linear regression); 2004 was the only year during which mean daily temperatures in July and August at the shellfish farm did not drop below 15.0°C.

**CONCLUSIONS**

This investigation extends by 1000 km the northernmost documented source of oysters that caused illness due to *V. parahaemolyticus*. Rising temperatures of ocean water seem to have contributed to one of the largest known outbreaks of *V. parahaemolyticus* in the United States.
**VIBRIO PARAHAEOMOLYTICUS**, A GRAM-
negative, halophilic bacterium that inhab-
its warm estuarine waters worldwide, is
the most common cause of seafood-associated bac-
terial gastroenteritis in the United States. The
most common vehicle for this infection in the United
States is the consumption of raw or improperly
cooked oysters. Before the summer of 2004, Alas-
kan waters were thought to be too cold to support
levels of *V. parahaemolyticus* high enough to cause
disease.

Thermostable direct hemolysin (encoded by *tdh*)
is a virulence factor that occurs in more than 90
percent of clinical strains of *V. parahaemolyticus* but
usually in less than 1 percent of environmental iso-
lates. A single nonpathogenic strain of *V. para-
haemolyticus* was reported from environmental cul-
ture in Alaska only once. From 1995 to 2003, the
Alaska Department of Environmental Conservation
(DEC) tested approximately 400 Alaskan oysters
and other marine environmental samples for vibrio
species; none yielded *V. parahaemolyticus* (DEC: un-
published data).

**METHODS**

**THE OUTBREAK**

On July 16, 2004, the DEC notified the Alaska Sec-
tion of Epidemiology of several cases of gastroen-
teritis among passengers on a cruise ship (78-pas-
enger capacity) that was sailing in Prince William
Sound. On July 19, a health official from the Nevada
Office of Epidemiology notified staff at the Section of
Epidemiology of the diagnosis of laboratory-
confirmed *V. parahaemolyticus* gastroenteritis in a
Nevada resident (Patient A). The illness had started
on July 4, while the patient was a passenger on the
ship. The patient reported having eaten raw oysters
served on board the ship the day before the onset of
the illness. In collaboration with various state and
federal agencies, we began an investigation.

**EPIDEMIOLOGIC INVESTIGATION**

We performed a retrospective cohort study on pas-
sengers from four July 2004 cruises to determine
the burden of gastrointestinal illness among pas-
sengers and risk factors for illness. The case defini-
tion for this part of the study was a person with
acute onset of three or more episodes of watery di-
arrhea in a 24-hour period while on board the ship
during the summer of 2004. We administered a
questionnaire by telephone or in person to all the
passengers that we contacted, recording demo-
graphic information, characteristics of the illness,
and information about the food consumed on
board the ship, and we calculated attack rates and
risk factors for illness.

In collaboration with the staff of the Municipal-
ity of Anchorage Department of Health and Human
Services, we performed active surveillance by com-
municating with the public and with health care
providers through the media in an effort to identify
additional persons in whom gastroenteritis had de-
veloped as a result of consuming Alaskan oysters.
The case definition for this portion of the investiga-
tion was a person with acute onset of three or more
episodes of watery diarrhea in a 24-hour period
that started within two days after the consumption
of raw oysters collected from Alaskan waters dur-
ing 2004.

**ENVIRONMENTAL INVESTIGATION**

Sanitarians from the Food and Drug Administra-
tion (FDA) performed a detailed investigation of the
ship and obtained food and water samples for lab-
oratory testing. On July 21, sanitarians from the
DEC traveled to the ship’s oyster source (Farm A) in
Prince William Sound to inspect the facility; to col-
lect water, sediment, and oyster samples; and to
record water temperatures. On August 2, the DEC
initiated a program to monitor *V. parahaemolyticus*
in all other active oyster farms in Alaska. Starting
on August 3, the DEC contacted retail outlets and
wholesale shippers in Alaska to identify the sources
of oysters associated with patients identified by the
Alaska Section of Epidemiology. Recordings of the
water temperature at Farm A, documented every
two hours since 1997 by researchers at the Mollus-
can Broodstock Program, funded by the Depart-
ment of Agriculture, were used to analyze temper-
ature trends and associations. National Oceanic
and Atmospheric Association data from buoys were
used to analyze long-term trends in water tempera-
ture in the Gulf of Alaska.

**LABORATORY INVESTIGATION**

Stool samples collected from patients were forward-
ed to the Alaska State Public Health Laboratory and
tested for *V. parahaemolyticus*, Shiga toxin–producing
*Escherichia coli*, shigella, salmonella, campylobacter,
and norovirus. Environmental samples were sent to
the DEC laboratory for analysis. Counts of *V. para-
haemolyticus* were estimated with the use of a most-
probable-number (MPN) procedure and MPN
Oysters remained a significant predictor of illness on board the cruise ship. Of the 132 passengers interviewed, 22 (17 percent) met our case definition for seafoods that were significant predictors of illness on bivariate analysis with the use of the Pearson chi-square test. The Pearson chi-square test was used to calculate a prevalence ratio and 95 percent confidence intervals were calculated with the use of backward stepwise multiple logistic-regression analysis. Adjusted odds ratios and their 95 percent confidence intervals for the association of illness on bivariate analysis with the use of the Pearson chi-square test were compared with the use of BioNumerics software.

Statistical analysis

Adjusted odds ratios and their 95 percent confidence intervals were calculated with the use of backward stepwise multiple logistic-regression analysis for seafoods that were significant predictors of illness on bivariate analysis with the use of the Pearson chi-square test. The Pearson chi-square test was used to calculate a prevalence ratio and 95 percent confidence interval for the association of tdh positivity of environmental isolates from Farm A with the month of specimen collection. Simple linear regression analyses were used to determine trends in water temperature for Farm A and the Gulf of Alaska in July and August. A one-way analysis of variance with Tukey’s post hoc multiple-comparison test was used to analyze differences in mean yearly water temperatures at Farm A in July and August. A t-test was used to compare mean water temperatures at Farm A in July 2004 with those in August 2004. Statistical analyses were performed with the use of SPSS software (version 11.5.0). Statistical significance was defined as P<0.05; all P-values are two-sided.

Results

Epidemiologic investigation

Of 189 passengers in the cohort, 132 (70 percent) were interviewed: 36 of 52 passengers on Cruise 1 (sailing from July 2 to 5), 21 of 27 passengers on Cruise 2 (July 9 to 12), 42 of 62 passengers on Cruise 3 (July 12 to 16), and 33 of 48 passengers on Cruise 4 (July 21 to 23). Of the 132 passengers interviewed, 22 (17 percent) met our case definition of the illness. Consumption of any of three seafood items served on board the ship, including raw oysters, was a significant predictor of illness on bivariate analysis. Only the consumption of raw oysters remained a significant predictor of illness after multiple logistic-regression analysis (adjusted odds ratio, 5.2; 95 percent confidence interval, 1.47 to 18.54). The median number of oysters consumed by persons who became ill was one (range, one to six); the median incubation period was 24 hours (range, 12 to 36). There was no correlation between the incubation period and the number of oysters consumed.

Attack rates for persons who ate oysters were as follows: 29 percent for Cruises 1, 2, and 3 combined (of 48 persons who consumed oysters, 14 became ill); 21 percent for Cruise 1 (3 persons became ill); 42 percent for Cruise 2 (5 persons became ill); and 27 percent for Cruise 3 (6 persons became ill).

We identified 62 people (14 patients from the retrospective cohort study who reported having consumed oysters before the onset of illness and 48 other persons) who met our case definition for active surveillance (Fig. 1). Forty-six patients (74 percent) were male. The median age was 47 years (range, 7 to 73). In addition to diarrhea, symptoms included abdominal cramping (in 82 percent), chills (44 percent), myalgias (36 percent), self-reported fever (34 percent), headache (32 percent), vomiting (29 percent), diarrhea with mucus (21 percent), and bloody diarrhea (7 percent). The median duration of illness was 5 days (range, 1 to 13). Twelve patients (19 percent) were evaluated by a health care provider; none were hospitalized.

The V. parahaemolyticus isolate from the stool of Patient A was serotype O6:K18 and tdh-positive. Stool samples were collected from nine other persons who met the expanded surveillance case definition. All samples yielded V. parahaemolyticus; no additional pathogens were identified. Isolates from eight of the nine samples were sent to the CDC for serotype analysis; seven were serotype O6:K18 and one was serotype O1:K56. PCR results were positive for tdh in all eight isolates.

Environmental investigation

According to a detailed 2004 logbook and interviews by the DEC with the two owners of Farm A, six dozen to seven dozen oysters were harvested from float lines twice a week ( Tuesdays and Saturdays) from mid-May through mid-July, placed into a clean bucket with ice usually within one hour (possibly, on rare occasions, within four hours) after harvesting, and delivered to the cruise ship by means of a skiff. The oysters were then shucked by cooks on the ship and promptly consumed by the passengers. The usual time from harvest to con-
sumption was less than three hours. According to DEC recommendations, the last delivery of oysters to the ship was on July 20. Instead of being served to passengers, these oysters were refrigerated overnight and submitted to the DEC laboratory on July 21. One sample of 5 oysters and one sample of 12 oysters were tested; both samples contained V. para-haemolyticus, yielding 2.1 and 3.5 MPN per gram, respectively. The following four serotypes were identified from 10 isolates grown: O6:K18 (5 isolates), O1:K9 (3), O5:K17 (1), and O10:K68 (1). All 10 isolates were positive for tdh. The ship’s water samples were negative for fecal coliform bacteria.

From July to October 2004, 96 environmental samples (e.g., oysters, water, and sediment) were collected from 17 Alaskan oyster farms; 31 samples (32 percent) were positive for V. parahaemolyticus, yielding 88 isolates. Samples positive for V. parahaemolyticus came from eight oyster farms located in Prince William Sound and southeast Alaska. Of 77 isolates, 57 (74 percent) were positive for tdh, yielding 11 strains; O1:K9, O1:K25, O1:K54, O1:Kunk, O3:K20, O3:K56, O4:K12, O4:K63, O5:K17, O6:K18, and O10:K68. The most frequently occurring serotypes were O1:K9 (17 isolates), O4:K63 (14 isolates), and O6:K18 (10 isolates). All O6:K18 isolates were from oysters from Farm A. According to the recommendation of the National Shellfish Sanitation Program, the DEC closed Farm A on July 28. Three other farms with tdh-positive environmental samples that were associated with at least one patient also were closed.

Eighty-two percent of the 62 patients identified by active surveillance ate oysters that could be traced back to Farm A (Fig. 1), and the remaining 18 percent consumed oysters from three other farms. Forty-two of the 51 persons (82 percent) who consumed oysters associated with Farm A received the oysters directly from the grower.

Twenty-nine environmental samples from Farm A were collected from July 21 through September 15; the median count of V. parahaemolyticus from 13 oysters was 3.5 MPN per gram (range, 0.3 to 430.0). Of 55 isolates from environmental samples of V. parahaemolyticus, 47 (85 percent) were tested for the presence of tdh. Of 34 isolates obtained in July, 31 (91 percent) were positive for tdh; of 10 isolates obtained in August, 2 (20 percent) were positive for tdh. Three isolates were obtained in September; all were negative for tdh. The prevalence ratio for tdh positivity in July as compared with that in August was 4.6 (95 percent confidence interval, 1.2 to 14.0).

LABORATORY RESULTS

Eighteen isolates (8 human and 10 oyster) confirmed as V. parahaemolyticus O6:K18 were subtyped with the use of PFGE. ApaI showed the most discrimination among the 18 isolates, yielding seven unique PFGE profiles. The predominant ApaI pattern, K16A12.0001, was found in both oyster and human isolates (Fig. 2). PFGE patterns generated from O6:K18 isolates submitted to the CDC during this outbreak and previously from sporadic cases in other states were highly conserved in Western states but not in Eastern states.

TEMPERATURE DATA

From May 1 (the beginning of the distribution season) until the closing of Farm A, on July 28, Farm A sold approximately 3000 oysters per week, and mean daily water temperatures exceeded 15.0°C on 38 days. All oysters from Farm A that were implicated in the outbreak were harvested at temperatures above 15.0°C (Fig. 3). The mean monthly water temperatures in 2004 at Farm A were cooler in July than in August (16.6°C and 17.4°C, respectively; P<0.001). Mean yearly surface-water temperatures from 1976 to 2004 in
the Gulf of Alaska also showed a trend of increasing water temperatures (Fig. 5).

**DISCUSSION**

This report documents a large outbreak of *V. parahaemolyticus* serotype O6:K18 in the United States, and it expands the range of epidemiologically confirmed *V. parahaemolyticus* illness to a latitude higher than 60 degrees — more than 1000 km north of British Columbia, previously the northernmost area reported to have locally acquired illness. Furthermore, our findings provide evidence to support the hypothesis that a water temperature above 15.0°C at the time of oyster harvest is an appropriate threshold indicator of increased risk of *V. parahaemolyticus* infection from the consumption of raw oysters. Although water temperatures have reached 15.0°C at the time of oyster harvest, the reported outbreaks occurred at temperatures below this threshold. It is possible that other factors, such as the distance between the oyster harvest and the consumer, contribute to the risk of infection. Additional studies are needed to further understand the relationship between water temperature and the risk of *V. parahaemolyticus* infection from the consumption of raw oysters.
at Farm A each year since 1997, 2004 was unusual because mean temperatures were above 15.0°C for a much longer period and were almost 2°C warmer than during any of the previous years.

The data presented here demonstrate a trend of rising temperatures at Farm A and in the Gulf of Alaska. International studies have also documented increasing ocean-water temperatures, most notably in the higher latitudes of the Northern Hemisphere. Additional reports have documented the emergence of *V. parahaemolyticus* outbreaks associated with oysters harvested from areas with water temperatures similar to those found near southern Alaska. Warming ocean waters may well have contributed to this outbreak, but changing patterns of animal migration and discharged ballast water may also have played a role. Furthermore, unrecognized outbreaks in past years may also have occurred.

The high prevalence of *tdh*-positive environmental isolates of *V. parahaemolyticus* (74 percent) in this investigation contrasts with findings in all other reported environmental investigations. The highest proportion of *tdh*-positive environmental isolates previously reported (approximately 3 percent) was associated with Puget Sound oysters. Some studies with limited *tdh*-positive environmental data have implied an increasing prevalence of pathogenic *V. parahaemolyticus* with cooler water temperatures. In this outbreak, *V. parahaemolyticus* was more likely to be *tdh*-positive during the colder month of July than during August,

![Figure 3. Number of Patients with *Vibrio parahaemolyticus* Infection Associated with Oysters from Farm A, According to the Harvest Date, and Mean Daily Water Temperatures at Farm A.](image)

During the harvesting of oysters in Prince William Sound, Alaska, in 2004, the lowest temperature associated with the onset of illness in any of the 51 patients was 15.3°C, on July 27. The dashed line denotes the theorized threshold temperature (15.0°C) for the risk of *V. parahaemolyticus* illness from the consumption of oysters. Temperatures in May never exceeded 12.2°C. Farm A was closed on July 28, 2004.

![Figure 4. Mean Daily Water Temperatures in July and August at Farm A, Prince William Sound, Alaska.](image)

The temperature logger at Farm A was located at a depth of 5 ft on one of the oyster trays. Water temperatures were not available for 1999 because the temperature logger was not functioning that summer. The mean water temperatures in July and August for each of the years shown, starting with 1997, were 15.1, 14.6, 14.3, 14.7, 14.9, 15.5, and 16.9°C, respectively. The dashed red line denotes the theorized threshold temperature (15.0°C) for the risk of *V. parahaemolyticus* illness from the consumption of oysters; 2004 was the only year during which temperatures logged in July and August at Farm A did not fall below this threshold.
...ingly high, considering the low levels of *V. parahaemolyticus* found in oysters from Farm A and the low quantity of oysters consumed by passengers (median, one oyster), but *V. parahaemolyticus* outbreaks associated with MPN estimates that were substantially lower than the current standards have previously been reported.14,29 However, MPN estimates are inherently imprecise and tend to underestimate levels of pathogens.22 Nevertheless, the MPN estimates reported here are 1500 times lower than the current National Shellfish Sanitation Program guidelines for harvested oysters (5000 MPN per gram) and 3000 times lower than the FDA’s level of concern (10,000 MPN per gram) for ready-to-eat seafood, including raw oysters.13

It could be argued that the oysters tested during this investigation might have contained different concentrations of pathogenic *V. parahaemolyticus* than the oysters consumed by the passengers on the cruise ship; however, high attack rates continued for three cruises during a two-week period, the same *V. parahaemolyticus* serotype was isolated from a majority of patients tested and from samples from Farm A collected during the outbreak, oysters delivered to the cruise ship were always under the control of the harvester until being shucked by cooks on the ship, and environmental sanitation experts reported no evidence of tampering with the records of time or temperature.30

It is uncertain why oysters from Farm A were disproportionately associated with the outbreak. One possible explanation is that the principal strain involved with the outbreak, which was identified only at Farm A, was more pathogenic than other strains. Other potential explanations, such as differences in temperature, salinity, and the content of the sediment, are still being investigated.

Many of the serotypes reported in this study have also been found in Puget Sound.28 In addition, the O6:K18 isolates associated with the outbreak described in the current study had PFGE profiles similar to those of O6:K18 isolates found in sporadic cases in other Western states. These findings suggest an exchange of *V. parahaemolyticus* between Puget Sound and Alaska, possibly through migrating sea birds or marine mammals or the discharge of ballast water.31

Finally, this investigation underscores the importance of disease reporting by health care providers. Had the clinician who diagnosed the index patient not notified health officials, it is possible that the cause and extent of this outbreak might not have been elucidated.

In conclusion, we recommend that when water temperatures at oyster farms exceed 15.0°C, control measures should be considered, including the establishment of a monitoring program for *tdh*-positive *V. parahaemolyticus*, the moving of oyster nets into cooler waters, the implementation of postharvest processing of oysters, and the issuance of public advisories to cook oysters. Furthermore, because the present outbreak occurred at MPN estimates substantially below the current standards of the National Shellfish Sanitation Program and the FDA, we recommend that the guidelines be reconsidered and that region-specific risk assessments be performed to account for potential ecologic variation of *V. parahaemolyticus*. Health care providers evaluating patients with acute gastroenteritis should ask specifically about recent consumption of oysters and report patients with *V. parahaemolyticus* to public health officials. Finally, more research is needed to identify environmental factors that contribute to the growth of pathogenic *V. parahaemolyticus* and to subsequent outbreaks of *V. parahaemolyticus* infection, as well as to assess differences in virulence among various *tdh*-positive strains of *V. parahaemolyticus*.

Supported by grants from the U.S. Department of Agriculture and the National Ocean Service (to Dr. DePaola).

We are indebted to the following persons and organizations for their substantial contributions to this investigation: Rick Sawaski, Nevada Office of Epidemiology; Mike Ostasz and Manny Soarez, Alaska Department of Environmental Conservation; Bruce Chandler and staff, Municipality of Anchorage; Department of Health and Human Services; Bernard Jilly and staff, Alaska Section of Laboratories; staff at the Molluscan Broodstock Program, funded by the Depart-