

ORIGINAL ARTICLE

# Outbreak of *Vibrio parahaemolyticus* Gastroenteritis Associated with Alaskan Oysters

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## ABSTRACT

### 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.

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**V**IBRIO PARAHAEMOLYTICUS, A GRAM-negative, halophilic bacterium that inhabits warm estuarine waters worldwide,<sup>1</sup> is the most common cause of seafood-associated bacterial gastroenteritis in the United States.<sup>2</sup> The most common vehicle for this infection in the United States is the consumption of raw or improperly cooked oysters.<sup>3</sup> Before the summer of 2004, Alaskan 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 isolates.<sup>4-7</sup> A single nonpathogenic strain of *V. parahaemolyticus* was reported from environmental culture in Alaska only once.<sup>8</sup> 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: unpublished data).

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## METHODS

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### THE OUTBREAK

On July 16, 2004, the DEC notified the Alaska Section of Epidemiology of several cases of gastroenteritis among passengers on a cruise ship (78-passenger 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 passengers from four July 2004 cruises to determine the burden of gastrointestinal illness among passengers and risk factors for illness. The case definition for this part of the study was a person with acute onset of three or more episodes of watery diarrhea 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 demographic 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 Municipality of Anchorage Department of Health and Human Services, we performed active surveillance by communicating with the public and with health care providers through the media in an effort to identify additional persons in whom gastroenteritis had developed as a result of consuming Alaskan oysters. The case definition for this portion of the investigation 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 during 2004.

### ENVIRONMENTAL INVESTIGATION

Sanitarians from the Food and Drug Administration (FDA) performed a detailed investigation of the ship and obtained food and water samples for laboratory 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 collect 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 Molluscan Broodstock Program, funded by the Department of Agriculture, were used to analyze temperature trends and associations. National Oceanic and Atmospheric Association data from buoys were used to analyze long-term trends in water temperature in the Gulf of Alaska.<sup>9</sup>

### LABORATORY INVESTIGATION

Stool samples collected from patients were forwarded 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. parahaemolyticus* were estimated with the use of a most-probable-number (MPN) procedure<sup>10</sup> and MPN

tables as previously described.<sup>11</sup> Isolates identified as *V. parahaemolyticus* were tested for *tdh* by polymerase-chain-reaction (PCR) analysis at the Washington Department of Health Laboratory. All *tdh*-positive *V. parahaemolyticus* isolates were forwarded to the Centers for Disease Control and Prevention (CDC) for serotyping. Selected isolates were subtyped with the use of pulsed-field gel electrophoresis (PFGE) with *NotI*, *SfiI*, and *ApaI* enzymes. The gels were normalized and DNA fingerprints were compared with the use of BioNumerics software.<sup>12</sup>

#### 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.

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## RESULTS

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#### 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.

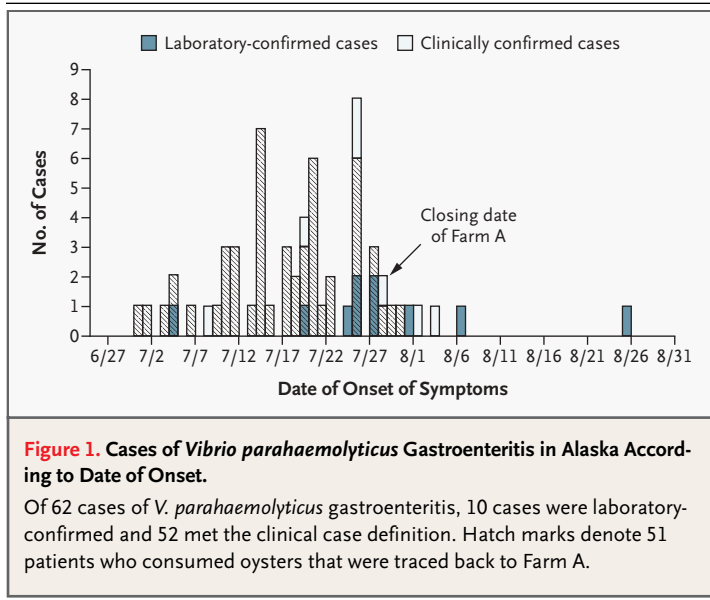
Attack rates for persons who ate oysters were as follows: 29 percent for Cruises 1, 2, and 3 combined (of 48 persons who consumed oysters, 14 became ill); 21 percent for Cruise 1 (3 persons became ill); 42 percent for Cruise 2 (5 persons became ill); and 27 percent for Cruise 3 (6 persons became ill).

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. parahaemolyticus*, 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,<sup>13</sup> 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).

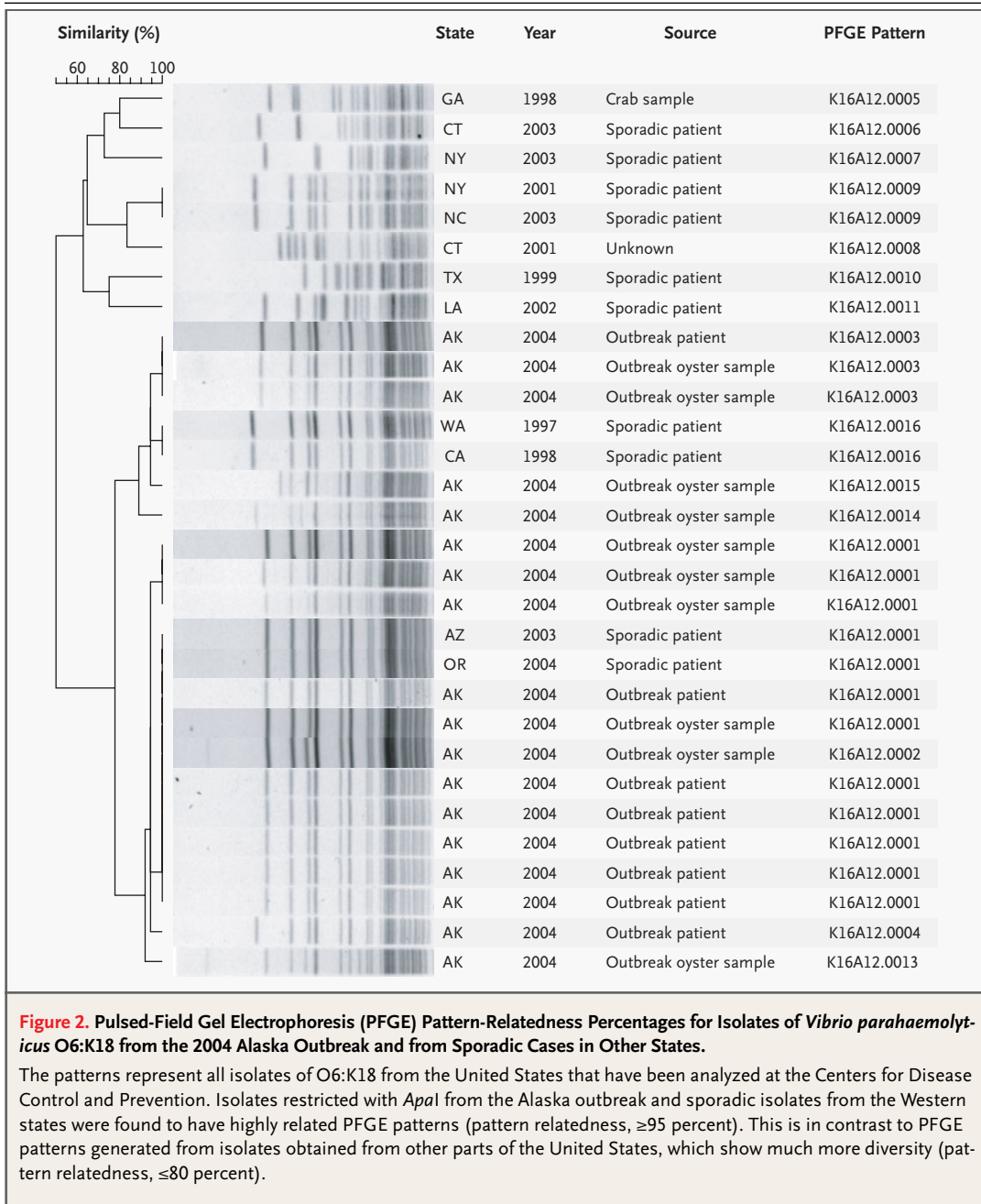
#### LABORATORY RESULTS

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 daily marine water temperatures at Farm A in July and August from 1997 to 2004 showed a 0.21°C increase per year ( $r^2 = 0.14$ ,  $P < 0.001$ ); water temperatures in July and August were significantly warmer in 2004 than in each of the six previous years examined ( $P \leq 0.001$ ) (Fig. 4). Mean yearly surface-water temperatures from 1976 to 2004 in

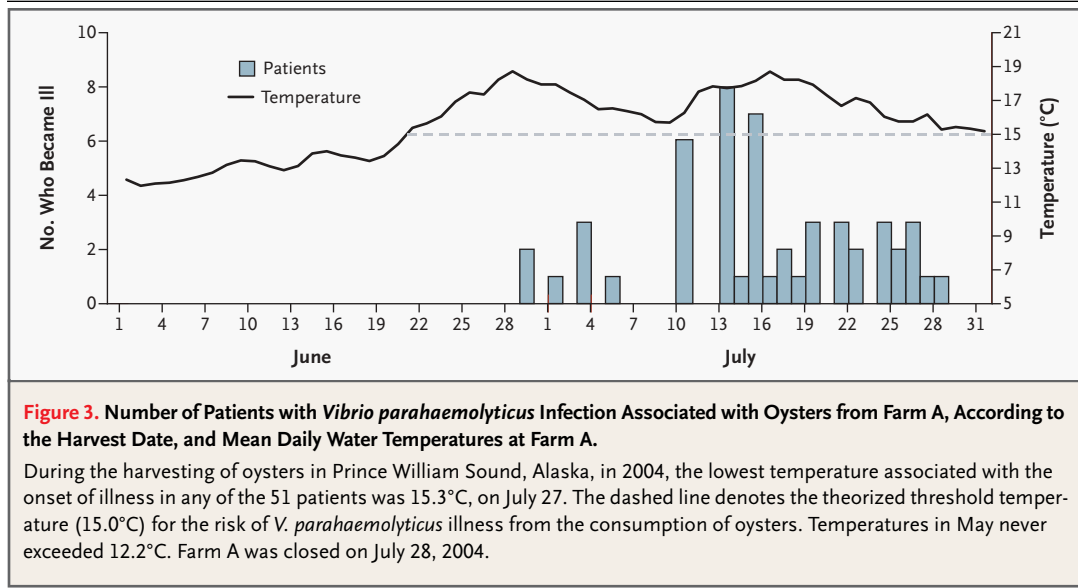


the Gulf of Alaska also showed a trend of increasing water temperatures (Fig. 5).

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 high-

er than 60 degrees — more than 1000 km north of British Columbia, previously the northernmost area reported to have locally acquired illness.<sup>14</sup> 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.<sup>15-17</sup> Although water temperatures have reached 15.0°C



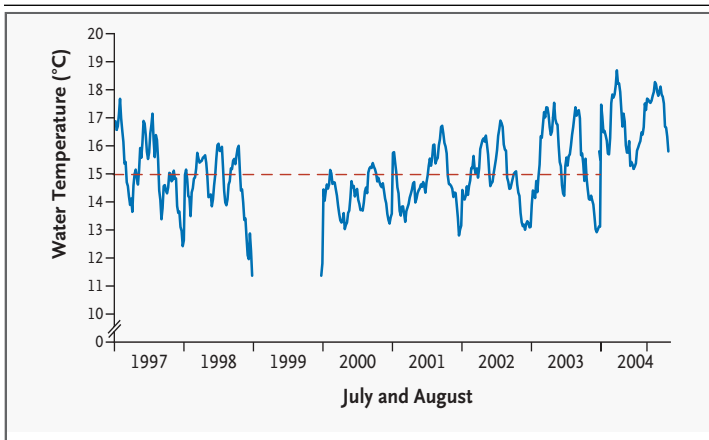
at Farm A each year since 1997, 2004 was unusual because mean temperatures were above 15.0°C for a much longer period and were almost 2°C warmer than during any of the previous years.

The data presented here demonstrate a trend of rising temperatures at Farm A and in the Gulf of Alaska. International studies have also document-

ed increasing ocean-water temperatures, most notably in the higher latitudes of the Northern Hemisphere.<sup>18,19</sup> 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.<sup>14,20</sup> 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.<sup>8,21-24</sup> The highest proportion of *tdh*-positive environmental isolates previously reported (approximately 3 percent) was associated with Puget Sound oysters.<sup>23,25-28</sup> Some studies with limited *tdh*-positive environmental data have implied an increasing prevalence of pathogenic *V. parahaemolyticus* with cooler water temperatures.<sup>22,23,24</sup> In this outbreak, *V. parahaemolyticus* was more likely to be *tdh*-positive during the colder month of July than during August, further indicating that *tdh*-positive strains might have a selective advantage in surviving colder conditions (or possibly that they are the first strains introduced into the environment and are gradually replaced by nonpathogenic strains as the water warms).

The attack rate (29 percent) among passengers who ate oysters aboard the cruise ship is surpris-



**Figure 4. Mean Daily Water Temperatures in July and August at Farm A, Prince William Sound, Alaska.**

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.

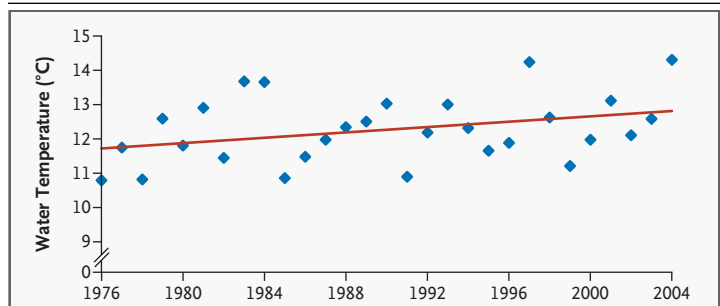
ingly high, considering the low levels of *V. parahaemolyticus* found in oysters from Farm A and the low quantity of oysters consumed by passengers (median, one oyster), but *V. parahaemolyticus* outbreaks associated with MPN estimates that were substantially lower than the current standards have previously been reported.<sup>14,29</sup> However, MPN estimates are inherently imprecise and tend to underestimate levels of pathogens.<sup>22</sup> 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.<sup>13</sup>

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.<sup>30</sup>

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.<sup>28</sup> 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.<sup>31</sup>

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.



**Figure 5.** Mean Surface-Water Temperatures in July and August in the Gulf of Alaska, 1976 to 2004, According to National Oceanic and Atmospheric Administration (NOAA) Buoy Station 46001.

Shown is a 0.04°C yearly increase in surface-water temperatures approximately 500 km south of Farm A, the nearest point for which extensive NOAA buoy temperature data are available. The corresponding P values for the mean yearly, monthly, and daily surface-water temperatures in July and August from 1976 through 2004, according to data from NOAA Buoy Station 46001, are  $P=0.07$ ,  $P<0.001$ , and  $P<0.001$ , respectively. The equation for the linear trend is  $y=0.04x-72.80$  ( $r^2=0.13$ ).

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 ecological 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*.

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