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A Research Note

Campylobacter jejuni in a Washington State Shellfish Growing Bed Associated With Illness

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ABSTRACT

Consumption of raw Pacific oysters (*Crassotea gigas*) harvested from a Washington State recreational shellfish bed were associated with illness. Illness occurred within 2 d of ingestion of a half-dozen shellstock oysters. Each oyster consist of approximately 20 g of meat. The duration of illness lasted 2 d. Routinely, *Campylobacter* species have been found in several shellfish beds in the Puget Sound Bay. Its presence in the marine environment appears to be incidental and primarily, comes from wild birds, farm runoff, and sewage bypasses. This paper describes the first reported case of *Campylobacter* gastroenteritis associated with raw oyster consumption in the State of Washington.

Consumption of raw shellfish has notably been implicated in illness throughout the world. The causative etiologic agents belonging to the group gram-negative bacteria: the genera *Vibrios*, *Salmonella*, *Shigella*, and *Aeromonas* have been responsible for shellfish-associated illness (*12*). Recently, *Campylobacter* has been added to this list of agents. A reported outbreak in 1980 associated with shellfish transmission of *Campylobacter jejuni* occurred among participants of a firemen's banquet (*8*). Sixteen of 28 participants became ill with Campylobacterosis after ingesting raw clams. With continuing evidence for *C. jejuni* as the etiologic agent in shellfish-associated outbreaks, this organism should be included in the general screening.

Case history

The first report of a shellfish-borne human illness for 1991 was received by the Shellfish Program of the Washington State Department of Health in February.

The patient, a healthy 45-year-old white male, became ill after consuming a half-dozen raw oysters harvested (1/20/92) from recreational state park (Penrose State Park) beach site in South Puget Sound during a weekend outing with three other individuals. The oysters were refrigerated until eaten raw for lunch the next day.

The patient experienced onset of illness 48 h later (1/23/91). He experienced fever, diarrhea, abdominal pain, and nausea. A stool culture obtained by the hospital was positive for *C. jejuni*.

Meals consumed prior to onset of illness consisted of omelets, pasteurized milk, a barbecue beef sandwich, and french fries. No poultry (also associated with Campylobacteriosis) was consumed prior to the incubation period. The three other people who were with the patient during the weekend did not consume any raw or cooked oysters and did not become ill. The barbecue beef sandwiches was the only food shared by members of the party of four.

MATERIALS AND METHODS

Sample collection

On February 29, 1991, samples for laboratory analysis were collected from Penrose State Park consisting of oyster shellstock, seawater, and bird (seagulls and ducks) excreta. Weight and volume measurements for these samples included the following: three plastic bags each containing 1 dozen oysters, with a total weight of 2,459 g of meat for all three bags; six sterile plastic bottles each containing 100 ml of seawater; and bird excreta swabs in 2/13 X 100 mm Cairy-Blair transport medium. All samples were transported in ice in an ice-chest container. Upon arrival in the laboratory, samples were immediately refrigerated at 5°C for analysis the following day. Samples were analyzed within 12 h of collection.

Sample preparation and analysis

Shellstock. Oysters were analyzed as described by the American Public Health Association (*3*). Each oyster composite was homogenized with a Stomacher 400 (Tekmar, Cincinnati, OH) without adding diluent. Twenty-five gram portions of the blended sample were weighed into sterile 400-ml Tekmar bags containing 225 ml of enrichment broth.

Bird excreta. Excreta were analyzed by adding each swab to a sterile 400-ml Tekmar bag containing 100 ml of enrichment broth.

Seawater. Approximately 600 ml of seawater were filtered through 0.45-μM pore size, 47-mm membrane (Zetabind, Cuno Laboratory Products, Meriden, CT). Membrane was placed in 400-ml Tekmar sterile plastic bag containing 100 ml of enrichment broth and fragmented with a sterile pipette.

Enrichment broth . Enrichment broth consisted per liter of the following: nutrient broth #2 (Oxoid) with 0.6% yeast extract (Difco); 5% lysed horse blood (Oxoid); and 0.025% each of ferrous sulfate (Baker), sodium metabisulfite (Sigma), and sodium pyruvate (Sigma). Sample homogenates in enrichment broth were preincubated at 35°C in ambient atmosphere for 2 h. After incubation, antibiotics consisting per milliliter of sodium cefoperazone (15 µg) (Pfizer); amphotericin B (2 µg) (Sigma); colistin sodium sulphomethate (4 µg) (Fluka); vancomycin hydrochloride (10 µg) (Sigma); and trimethoprim lactate (10 µg) (Burroughs Wellcome) were added to the enriched sample homogenate, and then incubated at 42°C for 48 h in modified (microaerophilic) atmosphere consisting of 5% O_2, 10% CO_2, and 85% N_2. Gas mixture was bubbled throughout the enrichment broth at approximately 10-15 ml per minute. Following incubation, 5 ml of each sample were filtered (Autovial sypringeless sterile filters 0.65 µm, Genex Corp., Gaithersburg, MD). Filtered and nonfiltered samples were plated onto *Campylobacter* blood-free selective charcoal agar base (CBFSCA) with the following antibiotics added per liter: sodium cefoperazone (30 mg), rifampicin (10 mg) (Sigma), and amphotericin B (2 mg) and then incubated at 37°C for 48 h.

Identification procedures

Plates were observed for typical colonies. Wet mounts were prepared and examined under oil with a phase-contrast microscope (Zeiss). Typical cells of *Campylobacters* were curved shape (gull) rods joined forming zigzags and migrated across the microscopic field with a corkscrew-like motion. Colonies directly from CBFSCA were tested with the Meritec™ Campy (jcl) (Meridian Diagnostics, Inc., Cincinnati, OH) a rapid, sensitive latex agglutination test for the identification to the genus level of *C. jejuni*, *Campylobacter coli*, and *Campylobacter laridis*. Colonies exhibiting agglutination were further tested biochemically. Confirmation of suspected *Campylobacters* were tested by the following biochemicals and growth requirements: growth at 25°C (-), 35-37°C (+), 42°C (+), 3.5% NaCl (-), and 1% glycine (+); nitrate reduction (+), H_2S production in 0.02% cysteine-HCl (+), oxidase, and catalase. For differentiation between species, isolates were tested for ability to hydrolyze hippurate and antibiotic resistance to nalidixic acid and cephalothin.

RESULTS AND DISCUSSION

Campylobacter species have been found routinely in West Coast shellfish beds in the States of California, Oregon, and Washington (*1*). Sample types include oysters, cockles, sediment, seawater, freshwater (streams and tributaries), bird excreta, and cattle manure. *C. jejuni* is found in many animals (*4,6,14*). Its transient existence in the marine environment primarily comes from wildbirds (*11,13*), farm runoffs (*2*), surface water (*7*), and sewage bypasses (*9,10*). Subsequently, shellfish are subject to contamination by this bacterium. Recent reports have appeared in the literature concerning the presence of *Campylobacter* in shellfish-associated illness (*12*). All of these reported cases occurred from the State of Florida, with the exception of one report from the State of New Jersey.

The first report of shellfish-borne illness caused by *Campylobacter* in the State of Washington was received by the Shellfish Program of the Washington State Department of Health. Oysters harvested from Penrose State Park located in the South Puget Sound area were associated with illness. Penrose State Park is located in a remote area, only open to the public on weekends. The park is not impacted by a high density residential area. *C. jejuni* were found in all sample types (oysters, seawater, and bird excreta) collected from Penrose State Park. One observation that was noted at the park, was the presence of a "duck pond" caused by the high tide on the surf. This pond drains directly on the shellfish and, thus, could provide a consistent source of *Campylobacters*. Additional water samples, approximately 75 d after the outbreak, were collected for laboratory analysis. Low levels of *Campylobacter* (0.23 MPN/L) were found. This area continues to be a source of *Campylobacter* affecting shellfish. *Campylobacters* survive well in shellfish held at cold temperatures. In survival experiments in shucked oyster meats, it has been shown that *C. jejuni* (inoculum level of 1 x 10^5 CFU/g) survives for 22 d at 4°C in ambient atmosphere; however, at 10, 15, and 26°C, survival was 5, 5, and 2 d, respectively (*1*). This suggests that *C. jejuni* survives much better in a colder environment. This observation has been noted by other investigators (*5*). Water temperatures in the Northwest particularly in Puget Sound range from 1 to 15°C throughout the year. The presence of *Campylobacter* in the marine environment together with cooler prevailing temperatures makes oysters or other shellfish in these environments a possible source of *Campylobacter* infection. For example, at a nearby commercial shellfish bed located 10 miles north from Penrose State Park, a 16-month sampling of Allyn (North Bay), Washington, was conducted. The temperature range in that period of time from 1 to 10°C. *Campylobacters* were isolated at 22, 19, and 35% in samples of oysters, sediment, and freshwater, respectively (*1*). The shellfish beds are directly impacted by the community of Allyn. The shellfish beds are located approximately 180 m from the nearest residence. One major contributor of *Campylobacters* affecting the shellfish beds is a man-made duck pond located in back of a residential dwelling. The pond is continually draining directly into the shellfish beds. Recently, the State of Washington, Department of Health, and local public health officials surveyed the shoreline at Allyn (North Bay) and found a significant number of defective leaking septic systems which were contributing fecal pollution to the immediate area where commercial shellfish were harvested (*15*). These findings led to the closure of commercial and recreational harvest of shellfish in North Bay. The North Bay area produces annually about 35,000 gal (ca. 9,247 L) of oysters. The oysters sell on the average for $20.00 per gal. This has had an economic impact to the oyster industry. This action has led to improvements and/or upgrading of septic systems.

More evidence is supporting *Campylobacter*'s role in shellfish-associated gastroenteritis illnesses. This report describes a single case of oyster-associated gastroenteritis with strong evidence for *C. jejuni* as the etiologic agent. Shellfish beds located near potential pollution sources such as sewage effluents, farm runoffs, and waterfowl reservoirs can present a health risk in the consumption of raw oysters.

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The concentration of PR imine decreased over time reaching 9-11% of the initial PR imine content after 2 d in a manner similar to that obtained in the experiment carried out with bovine serum. However, the decrease in the amount of PR imine was associated with a simultaneous increase in the amount of PRT as a result of the conversion. After 2 d the PRT level accounted for about 54 and 78% of the 62 and 125 µg PR imine added to serum, respectively. The mechanism of the conversion is not clear and needs to be elucidated.

In conclusion, our results indicate that the presence of PR imine naturally occurring in blue-veined cheese is not of great toxicological significance. The level of PR imine was minimal (equivalent to a maximum of 42 ppb in the cheese). Moreover, more toxic PRT, which may result from the conversion in animal serum, is likely to be sequestered by the serum albumins. Nevertheless, additional analyses of different blue-veined cheeses and more in depth in vivo investigations of the behavior of PR imine need to be conducted in order to clarify the actual risk involved.

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