Introduction

Vibrio parahaemolyticus is a gram-negative, rod-shaped, and halophilic bacteria that is the leading cause of seafood-borne bacterial infections. The gastrointestinal tract is mainly impacted by vibriosis disease. This occurs through the ingestion of infected and contaminated seafood. The pathogens are found in the pond, river, and ocean water. Vibrio parahaemolyticus is consumed with raw oysters and various other forms of shellfish. This organism is particularly prevalent in seafood that is not properly cooked. The symptoms of this disease include watery diarrhea along with abdominal cramps, vomiting, and fever. Vibrio cholerae causes the same gastrointestinal, but can also infect the bloodstream of immunocompromised people causing fever, chills, hypotension, and skin lesions. Fifty percent of people infected with Vibrio vulnificus succumb to the infection.

Currently, the oysters are harvested and put through a process called depuration to clean them of local bacteria (coliforms). In this process, oysters and other shellfish are put in a tank with filtered and sterilized seawater under conditions that better facilitate their depuration. This causes the expulsion of the contents from the oyster. It also reduces the separation of these contaminants from the shellfish and prevents recontamination. The depuration cleans the systems of harmful bacteria like Salmonella, but is less effective at removing other pathogens and viruses in its current design. It is also inconsistent or inefficient at removing bacteria, and water native bacteria like Vibrio. A study by the Aquaculture Research Station at Louisiana State University Agricultural Center, while inconclusive, showed evidence that cooling oysters in dry ice has an effect on Vibrio populations, however, it is not a common practice.

Connecticut has seen an increase in Vibrio infections over the last two-oyster harvesting seasons. As water temperatures increase Vibrio bacteria populations expand but the time and rate of expansion is not known. We started the study in late May because we got the best data. In addition to this, it is also the time when oyster reproduction is at its highest. This means that there could be a rise in foodborne Vibrio infections during the summer (as seen in the past two years). It is estimated that there are about 4300 Vibrio parahaemolyticus and many of those reported. It is also reported that there are 95 cases of Vibrio vulnificus per year only half of which are culture confirmed. In addition to this, evidence shows that these numbers are rising.

Oyster Collection

From May to October, Vibrio infection reported to CDSS and FOSS was recorded. According to CDSS, the number of infections annually per 100,000 people increased from 0.09 to 0.28. The number of annual infections per 100,000 people according to FOSS increased from 0.15 to 0.42.

Materials and Methods

Oyster Collection

18-35 oysters were collected weekly from June 6th to July 31st at oyster beds at the Sound School in New Haven, Connecticut. These oysters were scrubbed with a scrub brush to prevent internal contamination with shell organisms. Samples were collected and processed within an hour of collection.

Oyster Processing

A 9-lb commercial blender (Model: 3-1/2) was placed on a balance and its mass was taken. The oysters were then shocked and their tissue was placed in the blender to create a pooled sample. The blender was mixed more with the tissue inside and the difference between the two measurements was used to determine the mass of the oysters. 100 mL of 0.85% artificial seawater was added into the blender and the tissue was blended at full speed for 1 minute. The total volume of the pooled sample was taken by pouring it from the blender into a graduated cylinder. DNA Extraction

Using Montell™-isolated colonies of Vibrio parahaemolyticus and Vibrio vulnificus were taken from the plates and placed in separate 1.5 mL centrifuge tubes containing 40uL of sterile water. The tubes were then placed in a centrifuge and spun at 13,200 rpm for 5 minutes in order to pellet the bacteria. The supernatant was discarded and 200uL of sterile water was added to each tube. The tubes were then placed in a 100°C water bath for 10 minutes. The tubes were then removed and centrifuged at 13,200 rpm for 2 minutes. The supernatant was used for PCR. Chelex Montell™-isolated colonies of Vibrio parahaemolyticus and Vibrio vulnificus were taken from the plates and added to separate 1.5 mL centrifuge tubes containing 200uL of 0.5M sodium cholate. These tubes were boiled for 10 minutes. The tubes were then centrifuged at 13,200 rpm for 5 minutes. The tubes were removed and the supernatant was transferred to separate 1.5 mL centrifuge tubes.

PCR Confirmation

The 100 bp and 500 bp primers were used to confirm presence of Vv and Vp and 1500 bp primers were used to confirm presence of Vp. PCR reactions were loaded into Ready-To-Go PCR tubes with 3uL of DNA. 1uL of each primer (forward and reverse), and 2uL of distilled water. The PCR tubes were loaded with each DNA sample being loaded as according the above table. The tubes were then briefly centrifuged. They were then placed in the thermocycler with the following program:

Initial incubation: 94°C for 5 minutes
15 cycles: 
94°C for 1 minute 
55°C for 1 minute 
72°C for 1 minute
Final extension: 72°C for 1 minute.

The PCR products were held at 4°C. 5uL of loading dye was mixed with 1uL of each PCR product. 1:5 agarose gel was prepared and each mixture was loaded into a well. Electrophoresis was run at 200V for 40-45 minutes.

Results and Discussion

Oyster Collection:

Our results show an overall increase in Vibrio CFUs over the course of the summer. Our first four collections show a constant increase in number of Vv CFUs until our fifth collection. The fifth collection was performed after Hurricane Arthur passed through the Connecticut area around the 4th of July. The hurricane contributed to the drop in Vv numbers by decreasing the salinity of the water and increasing the amount of sediment and runoff from the mainland. A second collection of eight oysters (Collection 5.5) was performed during the same week. The number of CFUs from the second collection dropped slightly from the previous (Collection 5) showing that the large drop in Vv CFUs from week 4 to week 5. The numbers continued to drop for the sixth collection, but they shot up again to a much higher CFU counts for collection 7. The week after collection 7, heavy rain came through and area and lowered the Vibrio number for collection 8.

The trend displayed by the Vp CFUs mimicked the trend set by the Vv, except for the fifth collection which was performed after Hurricane Arthur. The Vp numbers continued to climb while the Vv numbers dropped dramatically. The confirmatory collection showed Vp numbers that were more on par with the trend set by the Vv numbers. In addition, icing the oysters has a high chance of killing them depending their age and size.

The regression line on the chart of Colony Forming Units (cfu) vs. Weeks has a positive slope indicating an increasing trend. This trend shows that the number of Vibrio parahaemolyticus in Eastern Oyster (Crassostrea Virginica), has increased over the course of the summer months. This means that the risk of infection with Vv is higher when eating uncooked oysters. The regression line on the chart of Colony Forming Units (cfu) vs. Weeks shows the trend. It displays the same increased risk of infection during the summer months.

The standard deviation of collections from 5 and onward were higher than previous weeks. This is due to the weather conditions that occurred around these times. Week 5 CFU count was lowered due to the passing of Hurricane Arthur. In addition, there was heavy rainfall towards the end of the study.