A SURVEY TO DETERMINE THE PREVALENCE OF RAW OYSTER CONSUMPTION IN HIGH RISK INDIVIDUALS FOR VIBRIO VULNIFICUS INFECTION

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INTRODUCTION

*Vibrio vulnificus* is a toxin-forming, lactose fermenting, halophilic bacteria found in coastal and brackish waters of the United States. *Vibrio vulnificus* infection can lead to morbidity, amputation, and even mortality in high-risk patient groups. This includes individuals with cancer, hepatic disease, renal disease, Diabetes Mellitus, HIV-infection, and the elderly (1,2,3,4,5). The elderly are included in this list because of compromised health and weakened immunity from the aging process (6).

*Vibrio vulnificus* produces extracellular proteins that have proteolytic or elastase and hemolytic activities, toxic to tissue culture cells (2). These products work together causing severe disease that is observed in infected people (2). Symptoms reported with *V. vulnificus* primary septicemia include: fever, chills, skin lesions, nausea, vomiting, diarrhea, hypotension, and shock. Warm water greater than 20 degrees celsius and salinity between 0.7 and 1.6 percent is optimal for *Vibrio vulnificus* proliferation (2). During the summer months when ocean water temperatures increase, the numbers and incidence of infection dramatically rise.

The death rate from *Vibrio vulnificus* primary septicemia has been reported as 45-60% in several studies (4). Berg et al (1) reports average mortality to be 46-75 percent. Mortality can approach 100 percent if the individual has hypotension (7). There is a strong relationship between eating raw oysters and primary septicemia among infected individuals (4). Most patients with septicemia report eating raw oysters 24 to 48 hours before the onset of symptoms (4) and raw oysters are now thought to be the main vehicle for entrance of *Vibrio vulnificus* via the gastrointestinal tract (8). Johnston et al (9) found that 89 percent of patients who developed primary septicemia had eaten raw oysters within two weeks before the onset of illness.

Klontz et al (10) reported 66 percent of persons with *Vibrio vulnificus* primary septicemia had liver disease, and 95 percent had both liver and chronic disease. Eight-eight percent of persons with wound infections due to *Vibrio vulnificus* had exposed that wound to seawater (10).

In 1988, the State of Florida reported 57 *Vibrio* infections. Of these, fourteen were *Vibrio vulnificus* infections. The median age of patients was 37 years, and the majority of cases were reported in July. Fifty-three percent were associated with raw oyster consumption and 23 percent with wound exposure.

Because organisms that cause food poisoning and infection occur naturally, they are difficult to control. In 1988, FDA funded research to develop a gene probe to detect *Vibrio vulnificus* in seafood (6). Unfortunately, there are no required sanitation or other public health controls to test for *Vibrio vulnificus*.

For people in high-risk categories, knowledge of the dangers of raw seafood ingestion due to *Vibrio vulnificus* can mean the difference between life and death (11). Educational efforts
have targeted the medical profession for recognition and treatment of the disease. Less effort has been given to prevention of infection. Educational workshops revealing good manufacturing principles for seafood processors is one educational effort. Depuration using ultraviolet light and an added bactericide helps decrease bacterial count and increase the shelf life of oysters that are contaminated (12). However, the area of prevention that has been neglected is the need for increased "at risk" consumer awareness of the dangers of raw oyster consumption.

Because high mortality rates have been associated with Vibrio vulnificus infections, patients must be warned about the life threatening infections and complications associated with consumption of raw oysters and other seafoods. The purpose of this study was to survey a subgroup of high-risk individuals and determine the percentage of individuals advised against raw oyster consumption, the source of information, and prevalence of raw oyster consumption. A comparison to a study in 1988 by Johnson et al (13) was used to determine whether awareness has increased in two years. This study was developed to help determine a need for patient education and the appropriate sources of education.

METHODS

A. SAMPLE:

Subjects in this survey (n = 164) were volunteers recruited from seven different Florida locations encompassing the cities of Gainesville, Dunedin, West Palm Beach, Palatka, and Naples. Data were collected on scheduled dates from August - November, 1990, and all patients entering the clinics on these dates were asked to participate in the survey. No attempts were made to randomize the sample. Six groups at risk for Vibrio vulnificus infection were identified. These included hepatic disease, renal disease, Diabetes Mellitus, oncology, HIV infection, and the elderly. Elderly volunteers (n = 29) were recruited from the Geriatric Research Center in Dunedin, Florida. These subjects were included because of increased susceptibility to Vibrio vulnificus infection. Subjects from Dunedin were surveyed at their places of residence. None were currently being treated for an illness which made them susceptible to Vibrio vulnificus infection. Subjects with hepatic disease (n = 30) and renal disease (n = 18) were recruited from outpatient clinics at Shands Hospital in Gainesville, Florida. Oncology patients (n = 21) were surveyed from North Florida Regional Medical Center in Gainesville, Florida. Finally, HIV positive subjects (n = 62) were recruited from outpatient clinics in Alachua County, Palm Beach County, and Collier County. Four patients with diabetes were surveyed at Putnam Community Hospital in Palatka, Florida.

B. QUESTIONNAIRE DEVELOPMENT:

Validity and reliability of the questionnaire used in this survey were determined by Johnson et al (13) in 1988 during an initial study of the awareness of hazards related to raw seafood consumption. The original questionnaire was revised and illustrated to make it more esthetically pleasing, but the questions were not altered. Therefore, the results from Johnson et al (13) can be statistically compared to this current study. Survey information was gathered from medical records and individual interviews. The subjects were asked to provide information about current food habits and nutrition knowledge.

C. DATA COLLECTION PROCEDURES:

This study was reviewed and approved by the Health Center Institutional Review Board at the University of Florida. Individuals meeting the criteria for one of the high-risk groups were asked to participate in this study. All received and signed an Informed Consent. The questionnaire took from three to seven minutes to complete and were mostly self-completed. After being surveyed, participants received a pamphlet on Vibrio vulnificus and basic principles of avoiding infection were discussed. Any questions or problems that participants had were also addressed.
Ages, diagnoses, and medications were validated through medical records, except for the elderly population, where medical records were not available. Data were collected for 164 individuals from August until November, 1990.

RESULTS AND DISCUSSION

Participants in this study were categorized into six high-risk groups for *Vibrio vulnificus* infection. Table 1 summarizes each high-risk group, the mean age, and standard deviation of participants.

<table>
<thead>
<tr>
<th>HIGH RISK GROUP</th>
<th>N</th>
<th>MEAN AGE</th>
<th>STD. DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic Disease</td>
<td>30</td>
<td>40</td>
<td>15.6</td>
</tr>
<tr>
<td>Renal Disease</td>
<td>18</td>
<td>39</td>
<td>18.8</td>
</tr>
<tr>
<td>HIV positive</td>
<td>62</td>
<td>36</td>
<td>9.4</td>
</tr>
<tr>
<td>Oncology</td>
<td>21</td>
<td>56</td>
<td>14.4</td>
</tr>
<tr>
<td>Elderly</td>
<td>29</td>
<td>84</td>
<td>3.5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>04</td>
<td>55</td>
<td>20.0</td>
</tr>
</tbody>
</table>

There is a statistical difference (p < .05) when comparing high risk groups for the ratio of males to females, which can mostly be attributed to the HIV positive population. Table 2 summarizes the frequency and percentage of males and females in the high risk groups for *Vibrio vulnificus* infection.

<table>
<thead>
<tr>
<th>HIGH RISK GROUP</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
</tr>
<tr>
<td>Hepatic Disease</td>
<td>17</td>
<td>10.4</td>
</tr>
<tr>
<td>Renal Disease</td>
<td>6</td>
<td>3.7</td>
</tr>
<tr>
<td>HIV positive</td>
<td>53</td>
<td>32.3</td>
</tr>
<tr>
<td>Oncology</td>
<td>4.3</td>
<td>14</td>
</tr>
<tr>
<td>Elderly</td>
<td>5.5</td>
<td>20</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total Male</strong></td>
<td>57.3</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

In this study, it was determined that 29 participants were taking medication that would make them more susceptible to *Vibrio vulnificus* infection. Twelve of the 21 participants in the Oncology group were receiving chemotherapy and 17 of the 18 renal participants were on corticosteroid therapy. Both medications are known to increase susceptibility to infection due to their immunosuppressive effects. Sixty-three percent of the participants in this study (n=104) were not instructed in any "special diet". Forty-one percent were females (n=43) and 59 percent...
(n=61) were males. Thirty-seven percent (n=50) of the participants were instructed on a special diet. Forty-five percent were female (n=27) and 55 percent (n=33) were males. There was no statistical difference between males and females at p < .10.

Of 164 participants only 25.6 percent (n=42) remember being told to avoid eating raw oysters. Table 3 summarizes the frequency and percentage of participants told to avoid raw oysters. Of the 42 participants who remember being told to avoid raw oysters, 28.6 percent (n=12) were females and 71.4 percent (n=30) were males, which was statistically significant at p < .05. Only 19.5 percent (n=50) participants remember being told to cook raw oysters before eating them. Of the 32 participants who knew to cook raw oysters before eating them, 21.9 percent (n=7) were females and 78 percent (n=25) were males. This, again was statistically significant at p < .05. Overall, men were more aware of the advisability of avoiding raw oysters or of cooking before eating them than women. This significance again may be attributed to the HIV positive population. The HIV positive group is mostly males and 34 percent (n=21) of the patients were aware of the advisability of avoiding raw oyster consumption, the highest percentage out of all the high risk groups. The other percentages are summarized in Table 3 and are as follows: Hepatic Disease (30%), Diabetes (25%), Renal Disease (22%), Oncology (19%), and Elderly (10%). Accurate statistical significance using Chi-Square could not be determined, since the number of subjects was < 5 in four of the six high risk groups.

The overall treatment of an HIV positive individual in South Florida commonly involves counseling on food safety practices. This may explain the higher level of knowledge of the dangers of raw oyster consumption. Elderly in this study are not under the supervision of health professionals for an illness that makes them more susceptible to *Vibrio vulnificus*. Therefore, they have less opportunity to learn of the dangers of raw oyster consumption. This level of awareness is similar to the healthy general public. The results of this study show that only 10 percent of the elderly surveyed are aware of the hazards of raw seafood consumption, the smallest percentage of all groups. The source of education for this group is principally mass media.

Sources of nutrition information about the dangers of raw oyster consumption varied from health professionals to mass media. Table 4 summarizes where participants received their information.

Table 3: Frequency and Percentage of Participants Told to Avoid Raw Oysters in Each High Risk Group.

<table>
<thead>
<tr>
<th>HIGH RISK GROUP</th>
<th>N</th>
<th>FREQUENCY</th>
<th>% IN GROUP</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic Disease</td>
<td>30</td>
<td>9</td>
<td>30</td>
<td>5.5</td>
</tr>
<tr>
<td>Renal Disease</td>
<td>18</td>
<td>4</td>
<td>22</td>
<td>2.4</td>
</tr>
<tr>
<td>HIV positive</td>
<td>62</td>
<td>21</td>
<td>34</td>
<td>12.8</td>
</tr>
<tr>
<td>Oncology</td>
<td>21</td>
<td>4</td>
<td>19</td>
<td>2.4</td>
</tr>
<tr>
<td>Elderly</td>
<td>29</td>
<td>4</td>
<td>10</td>
<td>1.8</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4</td>
<td>1</td>
<td>*25</td>
<td>* .6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>25.6%</td>
<td></td>
</tr>
</tbody>
</table>

*Because of small population size numbers might not be indicative of sub-group of Diabetics at risk for *Vibrio vulnificus*.
Most learned to avoid raw oysters through more than one source, 37.8 percent (n=17). Television was a source of information alone for eight people. This was attributed to a "20/20" special on the hazards of raw oysters.

Overall, 36 percent of participants (n=59) eat raw oysters. The largest percentage of participants eating raw oysters in a particular group is Oncology, with 61.9 percent surveyed. Fifty-eight percent of the elderly admit to raw oyster consumption; both of these groups rated lowest in knowledge of the hazards of raw oyster consumption. The results for the remaining groups are as follows: HIV positive (29%, n=18), Diabetes Mellitus (25%, n=1), Hepatic Disease (23, n=7), and Renal Disease (17%, n=3). HIV positive individuals have been known to increase raw oyster consumption because of the high zinc content in oysters. Zinc is an important part of immune function. However, the belief that increased zinc intake above the RDA improves immune function is inaccurate and may lead to dangerous food practices.

The largest reason for not eating raw oysters was not liking them, (63%, n=67). The second largest reason was being told to avoid them, (20.8%, n=22). Two reported under "comments" that they no longer like raw oysters because they became ill after eating them.

Only 9.1 percent (n=15) of total participants reported eating raw oysters in the past six months. This might be attributed to the time of the survey. Oyster season occurs during the winter months, and the previous six months before the survey included spring and summer months, when oyster consumption is lowest.

Knowledge and information does not necessarily ensure compliance. Of the 42 participants that were told to avoid raw oysters, 21 percent (n=9) still eat them. Knowledge must be present for compliance to occur, and compliance is seen in 33 out of the 42 participants who were told to avoid them.

A chi-square test for probability was used to help determine if there is a significant difference between the knowledge of participants surveyed in 1988 by Johnson et al (13), and this current study. It was reported in 1988, that out of 57 participants, eight (14%) knew to avoid eating raw oysters. This current study shows that out of 164 participants, 42 (25.6%) know to avoid raw oysters. This is significant at p < .10. In other words, there has been a significant increase in knowledge of the dangers of raw oyster consumption. The two factors most likely to account for the improvement, is the inclusion of the HIV positive group and the recent "20/20" television special on hazards of raw oysters.

Table 4: Where Knowledge of the Hazards of Raw Oysters was Learned

<table>
<thead>
<tr>
<th>SOURCE OF INFORMATION</th>
<th>FREQUENCY</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctor</td>
<td>3</td>
<td>6.7</td>
</tr>
<tr>
<td>Dietitian</td>
<td>4</td>
<td>8.9</td>
</tr>
<tr>
<td>Nurse</td>
<td>2</td>
<td>4.4</td>
</tr>
<tr>
<td>Friend</td>
<td>2</td>
<td>4.4</td>
</tr>
<tr>
<td>Relative</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>Television</td>
<td>8</td>
<td>17.8</td>
</tr>
<tr>
<td>Magazine</td>
<td>3</td>
<td>6.7</td>
</tr>
<tr>
<td>Newspaper</td>
<td>3</td>
<td>6.7</td>
</tr>
<tr>
<td>Bible</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>Personal Beliefs</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>More Than One Answer</td>
<td>17</td>
<td>37.8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>
CONCLUSIONS

The subjects surveyed in this study were not randomly selected and in some groups, the numbers surveyed were small. Therefore, they are not representative of the entire population of individuals who fall into one of the high-risk groups for *Vibrio vulnificus* infection. These results, however, do indicate that among clinics surveyed, there appears to be a significant increase in awareness about the microbiological hazards associated with raw oyster consumption.

Because *Vibrio vulnificus* is such a virulent organism, and mortality rates for infection range from 45-60% (4), education efforts must target prevention. In the past ten years, *Vibrio vulnificus* infections continue to occur despite attempts to educate the medical profession and oyster processors. Suggested ways to further knowledge of hazards of raw oyster and seafood consumption may be through: 1. PSAs (Public Service Announcements) during the summer months especially, 2. Increased distribution of educational materials, such as pamphlets through the Department of Natural Resources, 3. Issuing of warning labels in restaurant menus or marquis about hazards of raw seafood ingestion for high-risk groups. Louisiana, as of August 1, 1990 will require warning labels on all raw shellfish produced or sold in the state as a form of prevention (12).

In conclusion, these data can show that there is still a lack of awareness of the dangers of raw seafood consumption in high risk individuals. Knowledge, however, appears to have increased in the past two years due to health professionals and mass media. Future efforts should be aimed at the consumer and targeted towards prevention since available treatments for *Vibrio vulnificus* infections are ineffective in a majority of the cases.

REFERENCES


RELATIVE PREVALENCE OF ECONOMIC FRAUD
IN THE SEAFOOD INDUSTRY

Martha Hudak-Roos, E. Spencer Garrett,
G. Malcolm Meaburn, Ph.D., Lloyd Regier, Ph.D., and John Tennyson, Ph.D.
National Marine Fisheries Service
Pascagoula, MS

With the recent focus on improved seafood inspection much has come to
light on public health considerations in consuming fish and fishery
products. However, the occurrence of fraudulent practices in the trade
of seafood has been largely overlooked as a hazard to the consumer. Economic
fraud data was scrutinized as part of the Model Seafood Surveillance Project
(MSSP) to design a new mandatory seafood inspection system based upon the
Hazard Analysis Critical Control Point (HACCP) concept. The prevalence of
fraud, along with the cost to the consumer, was estimated and placed in
proper perspective with other hazards to be controlled in the new system.

INTRODUCTION

Fraudulent practices in the seafood industry represent a specific set
of hazards to the consumer. These economic fraud hazards are overshadowed
by product safety concerns. In this era of tightened resources, regulatory
effort is focused toward what makes people sick. Databases such as those
maintained at the Centers for Disease Control (CDC) help us understand the
prevalence and significance of public health problems in consuming seafood,
but there is little corresponding data to aid in understanding the magnitude
and impact of economic fraud.

Not all economic fraud is intentional. It can be perpetuated because
of a lack of information or misunderstanding. No matter what the reason,
however, industry's desire for a "level playing field" to combat fraud is
strong and consumers want full value.

One of the first documented indications of this industry concern was a
1985 survey conducted by the National Fisheries Institute (NFI) (7). The
survey concluded that economic fraud issues demanded attention. Quotes such
as, "I know a packer that right out and says, I've got some beautiful
turbot; we'll relabel it any way you want" and "There's no problem more
serious than non-enforcement of labeling laws", were dominant throughout
this report. There was general agreement among the industries (processing,
distributing, and importing firms) as well as retailers and restauranteurs
that there is widespread abuse of overglazing and overbreading of fishery
products, inaccurate net weights, and species substitution. However, the
same survey participants were in general agreement that fishery products
were labeled according to regulations (Tables 1 and 2).

A more recent survey conducted by the Southeastern Fisheries
Association (SFA) within their "Industry and Regulatory Interface Project"
(5) also revealed concern over economic fraud issues. A scale of one to
seven was used to weight problem areas where seafood product quality is
violated or lost. Included in these areas were economic fraud issues such
as species substitution, use of glaze, and use of phosphates, along with
non-economic fraud issues such as on-board vessel handling practices,
processing, frozen storage, and use of sulfites. Only one issue, related to
vessels, received a greater overall weight than the three economic fraud issues (Figure 1).

Table 1. 1985 NFT MEMBERSHIP SURVEY RESULTS
Processing/Distributing/Importing Firms

<table>
<thead>
<tr>
<th></th>
<th>AGREE %</th>
<th>PART. AGREE %</th>
<th>TOTAL %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Overglazing of fishery products is a widespread abuse.</td>
<td>37</td>
<td>35</td>
<td>72</td>
</tr>
<tr>
<td>B. Overbreading of fishery products is a widespread abuse.</td>
<td>34</td>
<td>22</td>
<td>56</td>
</tr>
<tr>
<td>C. Inaccurate net weights are a widespread abuse.</td>
<td>30</td>
<td>28</td>
<td>60</td>
</tr>
<tr>
<td>D. Improper substitution of fishery products is a widespread abuse.</td>
<td>30</td>
<td>39</td>
<td>69</td>
</tr>
<tr>
<td>E. Fishery products are labeled according to regulations.</td>
<td>23</td>
<td>45</td>
<td>68</td>
</tr>
</tbody>
</table>

Table 2. 1985 NFT MEMBERSHIP SURVEY RESULTS
Retailers and Restauranteurs

<table>
<thead>
<tr>
<th></th>
<th>AGREE %</th>
<th>PART. AGREE %</th>
<th>TOTAL %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Overglazing of fishery products is a widespread abuse.</td>
<td>27</td>
<td>19</td>
<td>46</td>
</tr>
<tr>
<td>B. Overbreading of fishery products is a widespread abuse.</td>
<td>37</td>
<td>26</td>
<td>63</td>
</tr>
<tr>
<td>C. Inaccurate net weights are a widespread abuse.</td>
<td>33</td>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>D. Improper substitution of fishery products is a widespread abuse.</td>
<td>33</td>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>E. Fishery products are labeled according to regulations.</td>
<td>8</td>
<td>42</td>
<td>50</td>
</tr>
</tbody>
</table>
Except for these documented industry concerns, economic fraud information or data is most difficult to obtain. We have classified the available information into three categories: databases (specific and incidental), published reports, and observations and interviews.

**DATABASES**

There are few databases designed specifically to collect economic fraud information. In most cases, when fraud data are reported, they are incidental discoveries.

Specific economic fraud databases include data from the National Seafood Inspection Laboratory (NSIL), state directed surveys, and weights and measures programs.

The NSIL analyzes samples upon specific request (i.e., for suspect products). The most comprehensive data in this database relate to species substitution. Over a three year period (fiscal years 88-90) 59 percent of samples labeled cod, 57 percent of the product labeled haddock, 56 percent of the product labeled flounder or sole, and 51 percent of the product labeled red snapper were found not to be cod, haddock, flounder or sole, and red snapper, respectively. Figures like these are confirmed by some states. For example, Florida conducted a survey in Fiscal Year (FY) 88-89 which indicated that 38 percent of the fish fillets sampled for species identification were misbranded.

Incorrect weights and measures also can be indicators of economic fraud and may be identified from specific databases. For example, states have bureaus that deal with the collection of weights and measures data. Further, the U.S. Department of Commerce (USDC), in its voluntary inspection
program, analyzes product lots for specific criteria, including weights and measures.

We contacted six states' Weights and Measures Divisions. Only California and Michigan had product specific data. Alabama, Florida, and New York could not break out seafood related data. Washington's data was not computerized; they could not supply data to us without a labor intensive activity.

In 1989 seafood labeled by retail markets in California was surveyed. Twenty-eight percent of the packages sampled for net weight contained less than labeled; however, the average error for all of the retailers surveyed was +3 percent. In other words, the retailers, on the whole, were overpacking. Similarly, packages of seafood labeled by the processor were sampled. In this survey, 12.5 percent of the packages were less than labeled. Again, however, the overall tendency was to overpack. For processors' labels the average error was +2 percent.

The sampling of seafood packages for weights in Michigan offered a different insight. While general sampling of seafood products again gave a two percent overpack, specific shortweight problems were evident for Individually Quick Frozen (IQF) shrimp (Table 3). State officials in Michigan as well as Florida confirmed that inaccurate net weight problems occur more frequently with IQF products.

An examination of USDA lot inspection certificates for an average three month period confirms our findings from state data (Figure 2). When "proper net weight" is defined by specification, i.e., what the buyer will accept, the percent average error is +1.9. If "proper net weight" is defined in exact units of measure (0.01), then the average error is +1.5 percent.

The economic impact of underpacking IQF shrimp was examined. An analysis using the Michigan data's average error of 8.3 percent underpacking of IQF shrimp is exemplified in the following: Let a processor produce 917,000 pounds of IQF shrimp (any product type). He sells this lot as 1,000,000 pounds. Assuming a wholesale price of $4.00 per pound, the processor receives $3,322,000 in fraudulent profit. The consumer, on average, purchasing one pound of IQF shrimp receives 0.92 pound. At a retail selling price of $6.36 (1) per pound, the consumer overpaid by $0.51. Frozen Food Age (1) reports that retailers sold 9.8 million pounds of frozen peeled shrimp, cooked and raw, from October, 1989 to October, 1990. A large grocery chain has estimated that 80 percent of their frozen shrimp is IQF. Thus in this example, U.S. consumers were losing $4 million on underpacking of IQF shrimp.

Non-specific (incidental) databases are more numerous than specific databases. Examples of these databases include Food and Drug Administration (FDA) consumer complaints, FDA adverse samples and import detentions, and state adverse samples.

In FY 90, FDA's consumer complaint database indicated that 16 percent of complaints in which no illnesses were reported were related to economic fraud. When all complaints including those related to illness were considered a ten percent economic fraud complaint ratio was obtained.
In regard to FDA's adverse samples, a 1988 Government Accounting Office (GAO) study (2) on seafood safety reported that eight FDA district offices had 1,514 adverse samples in 1986. Of these, 220 were misrepresented. This is a 14.5 percent violative rate. Again, these FDA data were not specific to economic fraud. In most cases, economic fraud data were discovered incidentally. Hence, in actuality, the data probably are underestimated.

Similarly, import detentions indicate economic fraud violations in seafood products. In 1989 FDA's detentions of seafood products for economic violations related to 0.2 percent of the weight, but 13 percent of the total number of detentions.

In 1989-90 the state of Florida analyzed over 800 samples of seafood products and found that, of those checked for economic fraud, 14 percent were misbranded. Additionally, 12 percent were adulterated and 21 percent in non-compliance, both of which may include some economic fraud violations. Again, it must be cautioned that the 800 samples were not all examined for economic fraud.

Thus while these data represent scanty information not arrived in a systematic way, the numbers are such that it is clear that substantial economic fraud exists and is a consumer hazard. It also appears that economic fraud is underreported since the datasets were not designed specifically to obtain economic fraud information (Table 4).

In order to calculate the potential costs of economic fraud to consumers, for illustrative purposes assume ten percent economic fraud in the U.S. seafood supply. In 1989 there were 12.3 billion pounds of round weight fishery products (8). If one assumes a 50 percent loss in processing these products, the supply becomes 6.15 billion pounds. If there is ten percent economic fraud in the industry, then 615 million pounds of fishery products on the market in 1989 would have been fraudulent. Since consumers spent $28.1 billion in 1989 (8), then the average price paid, assuming 6.15 billion pounds of after processing supply, was $4.57 per pound. Making the assumption that minimally ten percent of the price of the product equates to an economic fraud, this price differential relates to $0.46 per pound due to economic loss and total economic fraud of $283 million (based upon 615 million pounds of fraudulent product).

Even if we assume that 60, 40, or 30 percent is lost in processing, the result is still $283 million per year because of the principle of mathematical equivalency. Consider further that short weights or substitutions are more likely found on high value products rather than the average $4.57 per pound estimate. This implies that the ten percent fraud value and final national fraud level may be larger.
Table 3. MICHIGAN TEST RESULTS FROM SEAFOOD SAMPLING FOR WEIGHTS (9/87-11/90)

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>RESULTS</th>
<th>PERCENT RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-20.9 to -5.9</td>
</tr>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-7.4 to +5.2</td>
</tr>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-13.8 to -12.8</td>
</tr>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-5.3 to -2.7</td>
</tr>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-15.3 to -1.8</td>
</tr>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-18.6 to -11.3</td>
</tr>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-4.2 to +3.0</td>
</tr>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-12.8 *</td>
</tr>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-7.5 to -5.2</td>
</tr>
<tr>
<td>IQF SHRIMP</td>
<td>SHORT</td>
<td>-18.2 to -1.8</td>
</tr>
</tbody>
</table>

*RANGE NOT PROVIDED FOR THIS SAMPLE

Figure 2. USDC LOT INSPECTION CERTIFICATES
Table 4. SUMMARY
NON-SPECIFIC ECONOMIC FRAUD DATABASES

<table>
<thead>
<tr>
<th>PERCENT</th>
<th>DATABASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>of 1989 IMPORT DETENTIONS</td>
</tr>
<tr>
<td>14</td>
<td>of FY 89-90 SEAFOOD SAMPLES ANALYZED IN FLORIDA</td>
</tr>
<tr>
<td>14.5</td>
<td>of 8 FDA DISTRICT OFFICES' (1986) SEAFOOD SAMPLES</td>
</tr>
<tr>
<td>10-16</td>
<td>of FY 90 FDA CONSUMER COMPLAINTS</td>
</tr>
</tbody>
</table>

Let's take an example outside of the seafood industry for clarification. Suppose a consumer purchases 20 pounds of ground sirloin at $5.00 per pound, for a total of $100.00. The consumer unwarps the meat at home and finds that ten percent (2 pounds) is, in fact, ground chuck. The price of ground chuck was ten percent less than ground sirloin, i.e., $4.50 per pound. The ground chuck still can be used, but the consumer overpaid for it by $1.00 (total).

Another way to estimate the magnitude of economic fraud is to use per capita consumption figures. In this example, calculating the cost of ten percent economic fraud again leads to over $280 million. Since the per capita consumption in 1989 was 15.9 pounds and the civilian resident population was 246.6 million persons (8), a total of 3.92 billion pounds was consumed in the U.S. With an expenditure of $28.1 billion, the average price paid was $7.20 per pound. If ten percent of the price of the product was related to economic fraud, then $0.72 per pound was economic loss. This equates to only $1.14 per capita but a total of $281 million (390 million pounds of fraudulent product) of economic fraud in 1989.

Thus a $280 million figure, based upon the types of databases, assumptions made and round-off error in computations in the examples given, would be a reasonable estimate of economic fraud. In reality, the loss to the consumer might exceed this figure.

PUBLISHED REPORTS

Published reports usually center on the conduct of a specific survey. For example, species identification of retail red snapper fillets in Florida indicated that 64 percent of the samples labeled red snapper were misbranded (4). Newspapers have run similar studies. One from the Sun-Sentinel in Orlando, FL in December, 1988 concluded that 90 percent of red snapper was misbranded (3). The Asbury Park Press in Asbury Park, NJ in May of 1989 found 78 percent misbranded red snapper (6). If these three surveys were random, then the average indicates that 77 percent of red snapper on the market is in fact some other species.
Consumer costs and processing profit of red snapper substitution has been determined. In 1980 a study performed under contract for National Oceanic and Atmospheric Administration (NOAA) indicated that there were 12 million pounds of red snapper purchased at the point of sale. With the implication that 77 percent of red snapper is mislabeled, this figure indicates that 9.24 million pounds was not red snapper. Based on the ex-vessel prices, the price differential between red snapper and other snappers such as gray, lane, vermilion, etc., is approximately $1.00 per pound. This price differential would be carried through the market place resulting in $9.24 million fraud.

From a processing/distributing viewpoint, such fraudulent practices can be very lucrative. In 1990, if a firm purchased whole pacific rock fish, which is often substituted for red snapper, the firm would pay about $0.32 per pound. Once the purchase of this rock fish was relabeled as red snapper, it could be sold at $4.00 per pound instead of the $1.10 average selling price for a whole rock fish. A firm engaging in such fraudulent practice would have a selling margin of $3.68 per pound, $2.90 of which is fraudulent. Thus, the firm would receive almost five times as much as it should.

OBSERVATIONS AND INTERVIEWS

During the conduct of the MSSP testing, team evaluators made observations related to economic fraud: 1) Deliberate underpacking; 2) Shrimp soaked in a phosphate solution (to enhance moisture absorption) more than 12 hours; 3) Shrimp double soaked, i.e., soaked in phosphate solution, removed, and soaked again; and 4) Scallops soaked in a phosphate solution more than 24 hours, resulting in not only water absorption but an increase in count size as well. When those practices were observed, it was brought to the attention of the firm's management that the practice was questionable. Since the evaluators were there under industry invitation and not as regulators, it was left to the firm's management to correct (or not) the practice.

States were queried on economic fraud investigations. As a rule, the states were aware of the problems but were unable to take action because of limited resources. The exceptions to this was Florida; it has conducted specific surveys on species substitution, as earlier described.

Over 40 large chain grocery (supermarkets) stores also were queried. Only one store responded; it had no information or data, but expressed its concern over the occurrence of economic fraud practices in the seafood industry.

CONCLUSIONS AND RECOMMENDATIONS

As a result of these investigations the following conclusions were drawn:

1) In general, databases contain economic fraud information because of incidental discovery.

2) Not all fraud is intentional.

3) Economic fraud is not sufficiently addressed by regulators because of lack of resources.
4) As with seafood product safety issues, economic fraud issues appear to be product specific.

5) From the consumer viewpoint, short weight does not appear to be as big a problem as species substitution.

6) Economic fraud in the seafood industry is a "shrouded hazard", which could approach $280 million per year.

It is recommended that if a mandatory HACCP-based inspection system is instituted, it should focus resources to control economic fraud hazards.

Additionally, reliable baseline data on economic fraud is needed. Specific surveys on the prevalence of species substitution and problematic species for this practice, the excessive use of food additives such as phosphates that promote water pick-up, and short net weights or counts (again with the definition of problematic species or packaging types) should be performed. The collection of such data can be done within or external to any new mandatory seafood inspection program.

REFERENCES


PROCESSING STRATEGIES TO REDUCE THE NUMBERS OF *Vibrio vulnificus* IN OYSTERS

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Gulf Coast Research Laboratory
Ocean Springs, Mississippi 39564-7000

*Vibrio vulnificus* has been documented to cause primary septicemia in individuals with certain types of underlying disease (1, 6). More recently, *V. vulnificus* has been linked with gastroenteritis in individuals with no underlying disease (4). These illnesses occur primarily during the summer months when oysters are known to contain high levels of *V. vulnificus* (4, 7).

The numbers of *V. vulnificus* cells which must be ingested to produce primary septicemia or gastroenteritis in humans is unknown. However, it is apparent that any steps which can be taken to minimize the level of *V. vulnificus* in shellfish will improve the safety of the shellfish with respect to the organism.

*Vibrios* are known to be cold sensitive and Oliver (5) demonstrated that *V. vulnificus* died quickly in homogenized oyster meats when held in the cold. Ruple et al. (7) observed a marked reduction of *V. vulnificus* in whole oyster meats when stored on ice. These findings have lead us to explore the use of cold treatment as a potential processing step which would reduce the level of *V. vulnificus* in oysters without destroying the raw characteristic of the oysters. This paper also reports findings on the use of mild heat treatments to destroy *V. vulnificus* in raw oysters.

MATERIALS AND METHODS

Source of Oysters

Oysters used in these studies were harvested in Louisiana and transported to Mississippi through normal commercial routes. Oysters were shucked in seafood processing plants as part of normal plant operation. Tests with oysters were conducted on the same day the oysters were shucked.

Pure cultures of *V. vulnificus*

These cultures were isolated from raw oysters and have been maintained in the culture collection at the Gulf Coast Research Laboratory.

Cold temperature studies with pure cultures

Cultures were grown on a shaker overnight in TY broth (1% tryptone, 1% NaCl). Cultures were diluted in phosphate buffered saline (PBS) and inoculated into the suspension medium at a final concentration of approximately 1,000,000 colony forming units (CFU) per mL. Suspension media were tryptic soy broth (TSB), PBS and PBS+2% NaCl. Tubes of the inoculated suspension media were held at 4°C, 0°C, and -20°C. At desired intervals, the tubes were warmed or thawed in cold running tap water, diluted in PBS and plated on tryptic soy agar using a spread plate technique. Plates were counted after 48 hrs incubation at room temperature.

Thermal death studies with pure cultures

Cultures were grown as stated above and diluted to a concentration of approximately 1 billion CFU per mL. Ten mL of PBS+2% NaCl was placed into a 22 X 175 mm tube which contained a spin bar. The tubes were held in a water bath while mixing to equilibrate the
temperature. Tubes were inoculate with 0.1 mL of the diluted culture to achieve a final concentration of 1,000,000 CFU per mL. At predetermined time intervals, 1 mL samples were removed from the tubes, quickly cooled to 10°C, diluted as necessary and plated as described above. D-values were determined from semi-log plots of the data (2).

**Procedure for V. vulnificus counts in oysters**

Analyses were performed on duplicate 100 g samples of oyster meats. Meats were homogenized in phosphate buffered saline and enriched for *V. vulnificus* in alkaline peptone water using a 3 tube MPN. Following 12 to 16 hours incubation at 35°C, all tubes showing growth were streaked onto CPC agar plates and incubated for 24 hours at 40°C (3). Typical and atypical colonies were picked from CPC plates for confirmation as *V. vulnificus* using the ELISA procedure described by Tamplin et al. (8). Results were reported as an average of the duplicate samples, rounded to two significant figures, and expressed as MPN of *V. vulnificus* per gram of oyster tissue.

**Cold temperature studies with oysters**

In each study, oyster meats or shell stock oysters were obtained from a single shipment of oysters. Shucked oyster meats were divided into 100 g portions and placed in sterile wide-mouth glass bottles. Oyster meats to be stored at 0°C were held in melting crushed ice. Those stored at -1.9°C were held in a circulating bath of refrigerated 50% ethylene glycol. Ice crystal formation was not observed in oyster meats held at this temperature.

Shellstock oysters were held in a refrigerator at 4°C, placed in plastic bags and stored between layers of melting ice (0°C) or held at -1.9°C. The latter temperature was achieved by placing the oysters in an insulated chest equipped with cooling coils. Refrigerant chilled to -1.9°C was pumped through the coil. A thermocouple placed in the center of one oyster in the chest was used to verify the temperature.

**Studies with frozen oysters**

Oysters meats were frozen by three different procedures. A commercial liquid carbon dioxide freezer set for -30°C was used to produce individually quick frozen (IQF) oysters. After freezing, the oysters were divided into portions of approximately 100 g and placed in zip-seal freezer grade plastic bags for storage. A commercial blast freezer adjusted to -23°C was used to freeze 100 g portions of oyster meats in zip-seal freezer grade plastic bags. Oysters remained in the freezer for 24 hrs before they were transferred to the storage freezer. A third freezing technique similar to what may be used in the home consisted of placing the oysters in plastic bags in 100 g amounts and freezing them in a laboratory freezer at -20°C. All frozen oysters were stored in the laboratory freezer at -20°C. On a predetermined schedule, bags of frozen oysters were removed from the freezer and placed at 4°C overnight to thaw before testing for *V. vulnificus*.

**Heat treatment of oyster meats**

Oyster meats were heat treated in a circulating water bath constructed from a insulated plastic container equipped with a heater, thermoregulator and submersible pump. The water was heated to the desired temperature before introduction of the oyster meats. Exposure times were either 5 or 10 minutes. During the heating period, the meats remained suspended due to the action of the moving water. At the end of the heating period, the meats were immersed in ice water for five minutes to promote rapid cooling. In some experiments, the control oysters were subjected to the same exposure routine except the water was kept at 5°C.
RESULTS AND DISCUSSION

Cold Studies
When pure cultures of V. vulnificus were placed in suspension media and held at temperatures of 4°C and 0°C, all cultures experienced a rapid time dependent decrease in numbers (data not shown). However, V. vulnificus could be cultured from 0.1 mL portions of all media after 14 days of storage at both temperatures. A similar pattern of decreasing numbers was observed with naturally occurring V. vulnificus in cold stored shucked oyster meats (table 1) and in cold stored shellstock oysters (table 2).

Table 1. Effect of cold storage on the numbers of V. vulnificus in oyster meats.

<table>
<thead>
<tr>
<th>DAYS IN STORAGE</th>
<th>STORAGE TEMPERATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IN MELTING ICE (0°C)</td>
</tr>
<tr>
<td>DAY 0</td>
<td>&gt;110,000*</td>
</tr>
<tr>
<td>DAY 2</td>
<td>15,000</td>
</tr>
<tr>
<td>DAY 4</td>
<td>3,900</td>
</tr>
<tr>
<td>DAY 6</td>
<td>430</td>
</tr>
</tbody>
</table>

* MPN of V. vulnificus per gram of oyster meat.

Table 2. Effect of storage time on the numbers of V. vulnificus in shell oysters held at different storage temperatures.

<table>
<thead>
<tr>
<th>DAYS IN STORAGE</th>
<th>SHELLSTOCK STORAGE CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>REFRIGERATED (4°C)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>DAY 0</td>
<td>2300*</td>
</tr>
<tr>
<td>DAY 2</td>
<td>2300</td>
</tr>
<tr>
<td>DAY 7</td>
<td>590</td>
</tr>
<tr>
<td>DAY 14</td>
<td>930</td>
</tr>
</tbody>
</table>

* MPN of V. vulnificus per gram of oyster meats.

Freezing pure cultures of V. vulnificus in suspension media at -20°C reduced the number of culturable cells more quickly than did holding the cultures at 0°C. However, cells could be easily recovered after 19 days of frozen storage (data not presented). The freezing and storage of oyster meats containing naturally occurring V. vulnificus resulted in a decrease in the numbers of but not the elimination of V. vulnificus (table 3).
Table 3. The effect of freezing by different techniques and frozen storage time on the numbers of *V. vulnificus* in oyster meats. Frozen oysters were stored in zip-seal plastic freezer storage bags at -20°C.

<table>
<thead>
<tr>
<th>STORAGE TIME AFTER FREEZING</th>
<th>IQF FROZEN -30°C</th>
<th>BLAST FROZEN -23°C</th>
<th>LAB FROZEN -20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEFORE FREEZING</td>
<td>150,000*</td>
<td>150,000</td>
<td>150,000</td>
</tr>
<tr>
<td>AFTER FREEZING</td>
<td>930</td>
<td>93</td>
<td>430</td>
</tr>
<tr>
<td>WEEK 1</td>
<td>93</td>
<td>750</td>
<td>430</td>
</tr>
<tr>
<td>WEEK 2</td>
<td>7.5</td>
<td>93</td>
<td>230</td>
</tr>
<tr>
<td>WEEK 4</td>
<td>0.4</td>
<td>93</td>
<td>4.3</td>
</tr>
<tr>
<td>WEEK 8</td>
<td>0.9</td>
<td>1.5</td>
<td>15</td>
</tr>
<tr>
<td>WEEK 12</td>
<td>0.9</td>
<td>43</td>
<td>23</td>
</tr>
</tbody>
</table>

* MPN of *V. vulnificus* per gram oyster meat.

These findings document that cold treatment will bring about a significant reduction in the numbers of *V. vulnificus* in oysters, but the reductions are time dependent. Oysters have a defined shelf life whether in the shell, as shucked meats stored on ice or frozen. Therefore, it would be impractical to hold oysters for the time period necessary to insure that the *V. vulnificus* had been eliminated.

Heat studies

Preliminary studies with pure cultures indicated that temperatures above 45°C were necessary to bring about the rapid death of *V. vulnificus*. D-values were measured at 47°C on 52 pure cultures of *V. vulnificus*. The average D₄₇-value was 78 sec. (s.d. ± 30 sec.). Eighteen of these cultures which had the longest survival time were tested at 50°C and the resulting D₅₀-value was 38 sec. (s.d. ± 12 sec.). The low D-values indicate that *V. vulnificus* is very heat sensitive.

The effect of heat treatment on the numbers of *V. vulnificus* in oyster meats is shown in table 4. A 10 min. treatment at 50°C proved adequate to reduce *V. vulnificus* to a non-detectable level.

Table 4. Effect of heating time and temperature on the numbers of *V. vulnificus* in oyster meats.

<table>
<thead>
<tr>
<th>HEATING TIME</th>
<th>45°C</th>
<th>47°C</th>
<th>50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 MIN.</td>
<td>59,000*</td>
<td>59,000</td>
<td>59,000</td>
</tr>
<tr>
<td>5 MIN.</td>
<td>29,000</td>
<td>14,000</td>
<td>220</td>
</tr>
<tr>
<td>10 MIN.</td>
<td>870</td>
<td>4</td>
<td>&lt;0.3</td>
</tr>
</tbody>
</table>

* MPN *V. vulnificus* per gram oyster meat.
Oysters which had been heated at 50°C for 10 min. were stored on ice for a 14 day period and examined periodically for *V. vulnificus* (table 5). A comparison was made with oysters that had been exposed to the same treatment time but at a water temperature of 5°C. The 50°C reduced the levels of *V. vulnificus* in the oyster meats to a non-detectable level immediately. Further, there was no evidence of resuscitation of any *V. vulnificus* cells during storage on ice for 14 days.

Table 5. The effect of heat treatment and storage time on the numbers of *V. vulnificus* in oyster meats. Meats were packed in containers and held in melting ice.

<table>
<thead>
<tr>
<th>STORAGE AFTER TREATMENT</th>
<th>NO HEAT TREATMENT</th>
<th>OYSTERS HEATED AT 50°C FOR 10 MIN.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEFORE TREATMENT</td>
<td>4300*</td>
<td>4300</td>
</tr>
<tr>
<td>AFTER TREATMENT</td>
<td>2300</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>DAY 2</td>
<td>450</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>DAY 6</td>
<td>4</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>DAY 9</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>DAY 12</td>
<td>4</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>DAY 14</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
</tr>
</tbody>
</table>

* MPN of *V. vulnificus* per gram of oyster meat.

SUMMARY

The fact that *V. vulnificus* is a significant public health problem for a portion of the population justifies the study of processing steps that may result in the reduction or elimination of this organism from raw molluscan shellfish. Oysters are the molluscan shellfish most frequently associated with cases of primary septicemia caused by *V. vulnificus*. This study has verified the sensitivity of *V. vulnificus* to the cold by showing that their levels are reduced with time when held at temperatures below 4°C. These authors are aware of the research concerning "the viable but not culturable cells" that result from cold treatment of pure cultures of *V. vulnificus*. At present, techniques are not available to allow us to assess the significance of this phenomenon, if it exists, in oysters during cold storage.

Mild heat treatment of oysters for short periods of time have proven successful to reduce the numbers of *V. vulnificus* in shucked oyster meats to a level where they can no longer be detected. Following refinement, techniques using mild heat treatments may prove an acceptable addition to oyster processing to insure the safety of the product with respect to *V. vulnificus*.

ACKNOWLEDGEMENT

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REFERENCES


SURVIVAL AND CULTURABILITY OF VIBRIO VULNIFICUS IN ARTIFICIAL SEAWATER MICRO COSMS

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Department of Food Science and Human Nutrition  
University of Florida  
Gainesville, FL 32611

INTRODUCTION

Vibrio vulnificus is one of several types of Vibrio bacteria occurring world-wide. It occurs naturally along United States coastal waters, including the Atlantic and Pacific Coasts, and the Gulf of Mexico. It has also been isolated from seawater, sediment, plankton, and animals; mainly oysters (10).

Systemic infection involving this organism can result in fever, chills, nausea, and abdominal pain. V. vulnificus infections can be sometimes life threatening and can progress rapidly due to its highly invasive nature. In individuals with preexisting liver disease who reported consumption of raw seafood, greater than 50% mortality has been reported (4). In addition to being foodborne, V. vulnificus can cause severe infections by exposing a skin lesion, an open wound with seawater. Skin lesions are characterized by redness, swelling, and intense pain and can result in amputations (9).

Another interesting aspect of the pathogenesis of V. vulnificus is the discovery that two colony morphotypes exist, the "translucent" and the "opaque". The presence of an acidic polysaccharide layer at the cell surface, which has antiphagocytic properties, accounts for the opacity of the colonies and the virulence of V. vulnificus (7,11).

Infections with V. vulnificus occur mainly in the summer months, and it is only during these months that the organism can be recovered from seawater or oysters. This marked seasonality was attributed to the die-off of the V. vulnificus in seawater during the cold months. It now appears, however, that this apparent decrease in cell culturability may not be due entirely to cell death, but to a situation where the organism enters into a viable but non-culturable (VBNC) state. This may be the result of sublethal injury to the cell or a strategy of the cell to conserve energy and survive. A viable but non-culturable stage of bacteria has been documented (1,2), allowing for a possible persistence of human pathogens in the aquatic environment (5).

MATERIALS AND METHODS

1- Preparation of artificial seawater (ASW):

Artificial seawater was prepared as follows: Artificial sea salt (Forty Fathoms) was dissolved in distilled water to a specific gravity of 1.022 (30 parts per thousand, ppt). The solution was then passed through a 0.22 um filter and autoclaved at 1210°C for 15 min.

2- Preparation of V. vulnificus microcosms:

Stock cultures of strain C7184, opaque (O) and translucent (T), V. vulnificus were grown on heart infusion (HI) (100 ml) broth and grown overnight at room temperature. The cells were then harvested by centrifugation at 10,000 x g for 20 min. and washed twice with 50 ml of ASW and centrifuged (20 min. at 10,000 x g). The cells were then suspended in ca. 10 ml of ASW and aseptically transferred to a 1 l flask, prewashed with 6N HCl, containing 750 ml of ASW (8). The V. vulnificus microcosms were incubated at various temperatures and the survival of the organisms was monitored over time.
3- Incubation of microcosms at various temperatures:
Opaque and translucent morphotype microcosms of V. vulnificus were incubated at 37o, 25o, and 4oC. Aliquots from the microcosms were examined at time 0 and weekly thereafter. Total bacterial numbers were determined by the acridine orange direct count (AODC) (3) whereas the actively metabolizing cells were counted using the direct viable count (DVC) method of Kogure et al. (6). Ten microscopic fields were counted per sample. Culturable cell counts were determined by plating on the non-selective HI agar and the vibrio-selective thiosulfate-citrate-bile salts-sucrose (TCBS) and cellobiose polymyxin colistin (CPC) agar media in duplicates. ASW was used for making dilutions throughout the experiment. Conversions from one morphotype to the other were monitored on HI agar plates.

After the organisms became non-recoverable on agar media plates, 10 ml samples from the microcosms were inoculated into 3 tubes of double-strength alkaline peptone broth (APB) (10 ml) pH 8.4, and incubated at 37oC for 24 hr. When these alkaline peptone tubes were all negative, the microcosms were presumed to be in the non-culturable state.

4- Resuscitation of the VBNC cells of V. vulnificus:
After 5 weeks of incubation at 4oC, aliquots from the VBNC V. vulnificus microcosms were drawn and incubated at room temperature for 48 hr. Samples for total cell counts (AODC), direct viable counts (DVC), and media plate counts were examined.

RESULTS AND DISCUSSION

1- Microcosms incubated at 37oC:
V. vulnificus cells went partially into the VBNC but remained culturable for the duration of the experiment (11 weeks). The direct viable counts (DVC) rather stabilized in the fourth week of incubation. The encapsulated and the non-encapsulated forms (T & O) had similar profiles (Graph 1), and conversions between morphotype were not observed.

2- Microcosms incubated at 25oC:
Similar results were observed with microcosms incubated at 25oC where the agar media counts decreased steadily but did not reach 0 after 11 weeks. DVC also decreased at least 3 logs which indicates that some of the V. vulnificus cells went into the VBNC state (Graph 2). In contrast to incubation at 37oC, at 25oC a conversion from the avirulent (T) to the virulent (O) form was observed, but the converse (virulent to avirulent) was not observed. This conversion started on the fourth week of incubation and at the end of the experiment (week 11) all the translucent cells had transformed to the opaque form. This might be explained by the fact that the polysaccharide capsule helps in the adsorption of nutrients to the cell. As the organisms were incubated in a nutrient deprived environment, this could have triggered the formation of the capsule. It should be noted that this effect might be temperature related also, because it did not occur at 37oC.

3- Microcosms incubated at 4oC:
V. vulnificus cells incubated in microcosms at 4oC went into the VBNC state in 4-5 weeks. The total cell count (AODC) remained rather constant and the organisms changed from rods to round cells. The direct viable count (DVC) has decreased by 3 logs on week 4 and the cells changed from being very long and filamentous in fresh cultures to slightly enlarged (double the size of a normal rod) upon incubation in yeast extract and nalidixic acid. The culturability of the cells on agar media decreased quickly and was <1 CFU/ml microcosm on the fourth week.

Culturability on TCBS and CPC selective media decreased quicker than on the non-selective heart infusion media. This indicated that as cells get stressed due to starvation, they are unable to grow on selective or inhibitory media and later they fail to be recovered on non-selective media.
Graph 1. Survival of *V. vulnificus* in Artificial Seawater Microcosms upon Incubation at 25°C.

Graph 2. Survival of *V. vulnificus* in Artificial Seawater Microcosms upon Incubation at 37°C.
Graph 3. Survival of \textit{V. vulnificus} in Artificial Seawater Microcosms upon Incubation at 4°C.

Graph 4. Resuscitation of \textit{V. vulnificus} VBNC artificial seawater microcosms after incubation at RT
Both morphotypes had similar profiles (Graph 3) as they went into the VBNC state. No conversions between the two forms were observed.

4- Resuscitation of VBNC V. vulnificus:

As mentioned above, microcosms were considered to be in the VBNC state when they were negative on 3/3 double strength APB tubes after incubation at 37°C for 24 hr. When aliquots of these microcosms were incubated at room temperature in the same ASW, they became culturable again after 24 hr, and increasing to reach the original number of total cell count (Graph 4). When the DVC were examined the resuscitated cells changed from being slightly enlarged, which is typical of a VBNC cell, to being very long which characterizes fresh culture cells. It is worth mentioning that when the cells are in a normal metabolizing state, they increase in number upon the addition of yeast extract even in the presence of nalidixic acid which inhibits cell division of gram negative bacteria. This might be due to incomplete inhibition by nalidixic acid or due to breaking of the very long cells during the addition of stain and filtration (DVC procedure). This would explain the increase in DVC above the total cell count (AODC) at 24 hr. This was a problem with fresh cultures and it was solved by decreasing the incubation time suggested by Kogure to 2-3 hr for actively growing cells.

It should also be noted that the total cell count (AODC) was stable throughout the experiment. This could rule out any cell division upon incubation. These cells, which were round when in the VBNC state, became rod shaped similar to fresh cultures. After 48 hr the plate counts increased reaching the total cell count. This raises the question whether the DVC was a true enumeration of VBNC cells or if all the V. vulnificus cells went into the VBNC state including those that were presumed dead (AODC - DVC).

CONCLUSION

Vibrio vulnificus was able to survive a nutrient deprived environment (ASW) when incubated at room temperature and 37°C and remained culturable on laboratory media for 11 weeks. Conversion from the avirulent type (T) to the virulent type (O) was observed only when the bacteria were incubated at room temperature. However, the organism went into the viable but non-culturable state in 5 weeks, when incubated at 4°C in the same medium. The V. vulnificus were able to resuscitate and became culturable again in 24 hr when the temperature of incubation was changed from 4°C to room temperature (ca 25°C).

REFERENCES


GAMMA IRRADIATION OF VIBRIO PARAHEMOLYTICUS 
IN THE BLUE CRAB (CALLINECTES SAPIDUS)

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INTRODUCTION

_Vibrio parahemolyticus_ is a naturally occurring pathogen found in the Gulf of Mexico year round due to warmer temperatures (4) and as a result, _V. parahemolyticus_ has been responsible for several food poisoning incidents in recent years (1).

_V. parahemolyticus_ is also a known pathogen in blue crabs (2,3) and could easily be introduced into the processing plant as part of its natural flora. It is possible for cross contamination to occur while simultaneously processing crayfish (Procambaris clarkii) with blue crab (Callinectes sapidus). In addition to contamination of seafood, it could also pose a health problem to the consuming public and processing employees. In order to protect consumer safety and provide an economically viable product, it is necessary to control the presence of this pathogen.

Low dose gamma irradiation has been shown to be both safe and effective in the pasteurization of foods. Irradiation of fruits, vegetables and spices is already wide-spread and approved. Irradiation has also been shown to be highly effective in preventing sprouting, molds and larva maturation in fruits and vegetables (7).

The longer shelf life of fresh foods resulting from low dose gamma irradiation would allow greater distribution and thus provide a greater economic impact in marketing fresh products (5,6). Therefore, the purpose of this study was to determine the effect of pasteurization levels of low dose gamma irradiation on _V. parahemolyticus_ in crab and the effect of refrigeration storage (4°C) on the survival of the _V. parahemolyticus_ in Louisiana blue crab.

MATERIALS AND METHODS

Organisms

_Vibrio parahemolyticus_ (serotype 05:17) strain 116 was obtained from the microbial collection of the Department of Food Science at Louisiana State University. This culture was donated to the department by R. Sakazaki, National Institute of Health, Tokyo, Japan.

The organism was maintained on trypticase soy agar with 3% NaCl on slants. The organism was transferred to fresh media on a weekly basis.

Preparation of Crab Homogenate

Freshly processed crab meat were donated by a local Baton Rouge seafood processor. The nonsterile crab meat was prepared by blending one part crab with one part sterile saline (3% NaCl) solution in a Waring blender to form a smooth paste. The sterile crab meat was prepared in the same manner, except that the crab meat was autoclaved at 121°C for 15 minutes before blending with 3% sterile saline solution.
Preparation of Inoculum

Stationary log phase cells of *V. parahemolyticus* (serotype 05:17) were grown for 18 hours at 37°C and transferred for 3 successive days to insure log phase growth. A loopfull of the organism was inoculated into 50 ml of tryptic soy broth (3% NaCl), placed on a rotary water bath shaker at 125 rpm, and incubated for 12 hours at 37°C and served as the starter culture.

One ml of the starter culture was placed into 99 ml of tryptic soy broth and grown until a reading of 0.6 absorbance (600 nm) was obtained on the Bausch & Lomb Spectronic 70 (Bausch & Lomb Inc., Rochester, NY). The media suspension was then representative of a population of 1.0 X 10^7 CFU (colony forming units) *Vibrio parahemolyticus*/ml.

Ten ml of the broth was added to 10 gram samples of crab homogenates to achieve a final concentration of 1.0 X 10^7 *V. parahemolyticus*/g sample.

Irradiation of the Samples

The whirl packs containing the inoculated sterile and nonsterile samples were transported to the LSU Nuclear Science Center and placed in a water tight diving bell, which was filled with ice and sealed. The diving bell was then lowered into the irradiation pit to expose the samples to the cobalt-60 source which emitted 0.68 Gy/minute.

The samples were exposed to 0.10, 0.20, and 0.35 kGy, respectively. The packs were then removed, placed in ice and transferred back to the laboratory at the Department of Food Science for analysis and storage. Control samples were treated in the same manner, except the low dose gamma irradiation treatments were omitted.

Enumeration of Vibrio parahemolyticus

The crab meat were examined for the presence of *V. parahemolyticus* at 0, 7, 14, and 21 days, respectively, after cold storage at refrigeration (4°C) temperature. Both sterile and nonsterile samples were enumerated by the most probable number method (MPN) on tryptic soy agar (3% NaCl) and on thiosulfate-citrate-bile salts- sucrose agar (TCBS) media. The sterile and nonsterile plating and counting of *V. parahemolyticus* were performed in triplicate.

The plates were incubated for 24 hours at 35°C after streaking. At that time, the plates were removed and counted employing the MPN methodology. Further biochemical tests were run on TCBS positive plates to confirm the presence of *V. parahemolyticus*.

The number of surviving organisms were calculated by determining the average number of the triplicate samples of *V. parahemolyticus* recovered from samples that were exposed to the same low dose gamma irradiation dosages, and the same storage time and refrigeration temperature treatments.

RESULTS AND DISCUSSION

Effects of Radiation

In the sterile homogenates an original inoculation 1.2 X 10^7 CFU (colony forming units)/g *V. parahemolyticus* were reduced 4 log cycles at 0.10 kGy, 6 log cycles at 0.20
kGy, and no *V. parahemolyticus* were recovered following irradiation with 0.35 kGy as seen in Figure 1.

In nonsterile crab meat the original inoculum 4.4 X 10^7 CFU (colony forming units)/g *V. parahemolyticus* were reduced 4 log cycles at 0.10 kGy, 5 log cycles at 0.20 kGy, and 6 log cycles at 0.35 kGy of gamma irradiation as seen in Figure 2.

The reduction of *V. parahemolyticus* in both the sterile and nonsterile samples with low dose gamma irradiation can be easily seen in Figures 1 and 2. Therefore, low dose gamma pasteurization dosages can be used to reduce the number of *V. parahemolyticus* in blue crab meat.

**Effect of Time**

There was a general reduction in numbers of *V. parahemolyticus* during the three-week trial period at refrigeration temperature (4°C) in the nonirradiated, sterile and nonsterile crab meat samples. In the sterile crab meat, the *V. parahemolyticus* declined from 1.2 X 10^7 CFU/g at 0 kGy on day 0 to 9.0 X 10^1 CFU/g at day 21. At 0.10 kGy the number of colonies in the sterile homogenate was reduced from 1.2 X 10^7 CFU/g to no growth evident on day 21. With a pasteurization dose of 0.35 kGy there was no growth of *V. parahemolyticus* detected on any of the testing days (days 0, 7, 14, and 21) thus complete elimination and destruction.

With a low gamma irradiation dose of 0.1 kGy, the population in the nonsterile crab meat was reduced from the original inoculation of 4.7 X 10^7 CFU/g to 3.0 X 10^1 on day 21. At a 0.2 kGy dose the *V. parahemolyticus* in the nonsterile crab meat homogenate was reduced from the original inoculation of 4.7 X 10^7 CFU/g to no growth evident on day 21. At a 0.35 kGy dose the *V. parahemolyticus* in the nonsterile crab meat was reduced from 4.7 X 10^7 CFU/g to no growth evident on days 7, 14 and 21 thus complete elimination and destruction.

**CONCLUSIONS**

The results indicate that at a low gamma irradiation dosage of 0.35 kGy, *V. parahemolyticus* can be reduced approximately 4 log cycles during the first 7 days of storage on ice at refrigeration temperature (4°C) in nonsterile crab meat and reduced an additional 3 logs by day 21. In sterile crab meat, at 0.35 kGy irradiation, *V. parahemolyticus* was reduced immediately to 0 after irradiation and remained at 0 during the entire 21-day storage period at refrigeration temperature (4°C). It appears that some protection is offered to *V. parahemolyticus* by the presence of other microorganisms to *V. parahemolyticus* by low dose gamma irradiation, but only up to 14 days of storage at refrigeration temperatures (4°C).

**REFERENCES**


FIG 1: Survival of V. parahaemolyticus in STERILE blue crab meat after irradiation
FIG 2: Survival of *V. parahaemolyticus* in NONSTERILE blue crab meat after irradiation

![Graph showing survival of V. parahaemolyticus in nonsterile blue crab meat after irradiation. The graph plots log 10 CFU/g against storage period (days). Different lines represent different doses of irradiation: 0 kGy, 0.10 kGy, 0.20 kGy, and 0.35 kGy.]