

# Survival of *Vibrio parahaemolyticus* in Shrimp Tissue Under Various Environmental Conditions<sup>1</sup>

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*Vibrio parahaemolyticus* culture O from Gulf Coast shrimp was inoculated into whole shrimp and shrimp homogenate to determine its survival at various temperatures and pH values. Although large decreases in viable population occurred during storage for 2 days at 10 to -18 C, survivors were present even after 8 days. No significant differences were observed in the population changes of inoculated whole shrimp as compared with shrimp homogenates. Low populations ( $5 \times 10^2$  per ml) of *V. parahaemolyticus* were destroyed by heating shrimp homogenates at 60, 80, and 100 C for 1 min. With larger populations ( $2 \times 10^5$  per ml), some survivors were present after heating at 60 and 80 C for 15 min. None survived 1 min at 100 C. *V. parahaemolyticus* was very sensitive to pH values below 6.0. Cells survived for several hours in the contents of the porcine gastrointestinal tract.

A recent review (R. Nickelson and C. Vanderzant, *J. Milk Food Technol.*, *in press*) presents current information on the public health significance, isolation, and enumeration of *Vibrio parahaemolyticus*. Little information is available about the effect on *V. parahaemolyticus* of various environmental conditions, such as pH, refrigeration, freezing, or elevated temperatures. Most of the available data in this area have been obtained with cultures in artificial media. Information on the ability of *V. parahaemolyticus* to survive various environmental conditions in seafood would help to determine its potential role in foodborne illness associated with consumption of seafoods. This paper presents information on the survival of *V. parahaemolyticus* in shrimp tissue under various environmental conditions.

## MATERIALS AND METHODS

**Bacteriological procedure.** *V. parahaemolyticus* O isolated from Gulf Coast shrimp (6) was maintained on Trypticase soy agar (TSA, BBL with 3% NaCl) slants at 25 C. Survival studies were carried out in whole and homogenized shrimp. Homogenates were prepared by blending equal amounts of peeled deveined shrimp and sterile 3% NaCl. Homogenates were inoculated with appropriate dilutions of a 24-hr Trypticase soy broth (TSB, with 3% NaCl) culture to

give approximately 50,000 cells per ml. Whole peeled deveined shrimp were inoculated by injecting 0.1 ml of a TSB culture into three to four sites to give approximately  $10^8$  cells per shrimp. Inoculated shrimp were stored in sterile petri dishes, and homogenates were stored in sterile screw-capped tubes. No *V. parahaemolyticus* could be detected in the test samples before inoculation.

**Inoculation of *V. parahaemolyticus* in porcine stomach and intestinal content.** Porcine gastrointestinal tract was chosen because of its similarity to the human system and its ready availability. The gastrointestinal tract was taken from a freshly slaughtered hog at a local abattoir. Contents of the stomach, small intestine, and large intestine were placed in separate Waring Blenders, and each was inoculated with approximately  $10^6$  cells per ml of *V. parahaemolyticus*.

**Bacterial counts.** Counts of *V. parahaemolyticus* were made by spreading appropriate dilutions on either MT medium (modified Twedt; C. Vanderzant and R. Nickelson, *Bacteriol. Proc.*, p. 20, 1971) or TSA (with 3% NaCl) plates. Plate incubation was at 35 C for 24 hr. The data presented are representative of three independent trials.

## RESULTS

**Survival of *V. parahaemolyticus* under refrigerated and frozen conditions.** In whole shrimp (Fig. 1) stored at 3, 7, 10, and -18 C, there was a sharp decrease in viable cells during the first 2 days of storage. During the

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next 6 days of storage, the viable population remained approximately the same. Unlike the situation in whole shrimp, the population of *V. parahaemolyticus* in homogenized samples increased slightly (except at  $-18^{\circ}\text{C}$ ) during the first 12 hr (Fig. 2). Between 4 and 8 days of storage, there was a slight decrease in viable population. Survival was smallest in the samples stored at 3 and at  $-18^{\circ}\text{C}$ .

**Survival of *V. parahaemolyticus* at elevated temperatures.** Shrimp homogenates were inoculated with *V. parahaemolyticus* at two levels of cell concentration,  $5 \times 10^2$  and  $2 \times 10^6$  per ml, respectively. Counts of survivors were made on MT medium with plate incubation at  $35^{\circ}\text{C}$  for 24 hr. After heating at 60 and  $80^{\circ}\text{C}$  for 15 min and  $100^{\circ}\text{C}$  for 5 min, 1 ml of each homogenate was also placed in an enrich-

ment broth (TSB with 3% NaCl) and incubated at  $35^{\circ}\text{C}$  for 24 hr. The broths were then streaked on MT medium to check for surviving *V. parahaemolyticus*.

When cells were added at the rate of 500 per ml, no survivors were detected in heated homogenates by direct plating on MT medium. Inoculated homogenates heated at 60 or  $80^{\circ}\text{C}$  for 15 min or at  $100^{\circ}\text{C}$  for 5 min did not yield *V. parahaemolyticus* when placed in enrichment broth and subsequently plated on MT medium. When cells were added at a level of  $2 \times 10^6$  per ml, *V. parahaemolyticus* was recovered by the direct plating and enrichment procedure from samples heated at 60 or  $80^{\circ}\text{C}$  for 15 min. No survivors were noted in the homogenates heated at  $100^{\circ}\text{C}$  for 1 and 5 min by direct plating or by the enrichment procedure.

**Survival of *V. parahaemolyticus* in shrimp homogenates at various pH values.** Shrimp homogenates were adjusted to pH values from 1 to 10 with HCl or NaOH. Each sample was inoculated with approximately  $10^2$  to  $10^3$  cells of *V. parahaemolyticus* per ml and sampled after 5, 15, 30, 60, and 120 min. Homogenates were held at  $37^{\circ}\text{C}$ . In homogenates adjusted to pH 1, 2, 3, and 4, no survivors could be detected in 0.1-ml quantities of the homogenate (Fig. 3). At pH 5.0, a sharp drop in viable count took place immediately, with no survivors detectable after 15 min. The viable population of homogenates with pH values of 6, 7, 8, 9, and 10 remained about the same for 2 hr.

**Survival of *V. parahaemolyticus* in porcine stomach and intestinal contents.** Counts were determined after various times to approximate the time food would have re-

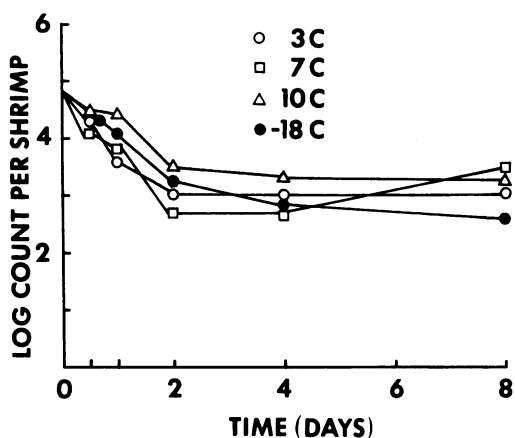


FIG. 1. Survival of *Vibrio parahaemolyticus* in whole shrimp stored at various temperatures.

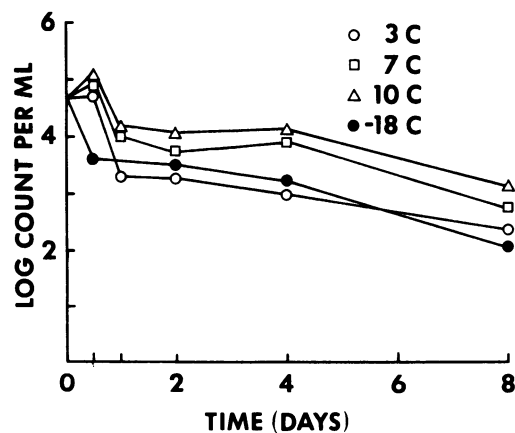


FIG. 2. Survival of *Vibrio parahaemolyticus* in shrimp homogenates stored at different temperatures.

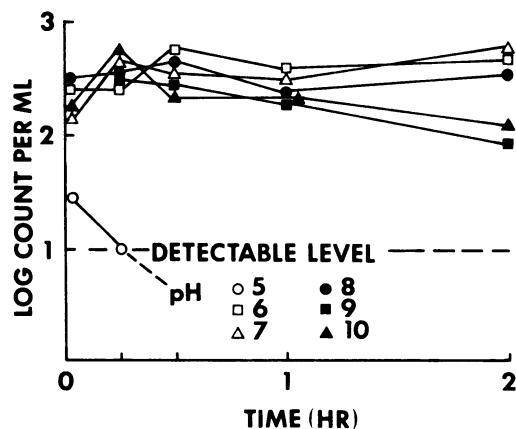


FIG. 3. Survival of *Vibrio parahaemolyticus* in shrimp homogenates at various pH levels.

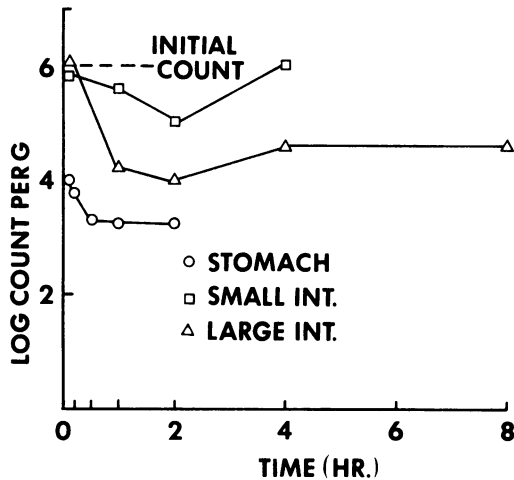


FIG. 4. Changes in population of *Vibrio parahaemolyticus* inoculated into contents of various parts of porcine gastrointestinal tract.

mained in each section of the gastrointestinal tract (4). At the time of inoculation, the stomach was full of fresh green feed. The pH of this material was 6.0. The pH values of the contents of the small and large intestines were, respectively, 7.2 and 8.1. All samples were kept at 37 C. Figure 4 shows the survival of *V. parahaemolyticus* in the contents of various parts of the gastrointestinal tract. The number of viable organisms in the contents of the small and large intestines decreased during the first 2 hr, increased between 2 and 4 hr, and remained the same from 4 to 8 hr of storage. The viable population in the stomach decreased immediately 100-fold, showed a gradual decline during the next 30 min, and then remained about the same for 1.5 hr.

### DISCUSSION

To determine the potential role of *V. parahaemolyticus* in foodborne illness in the United States, it is necessary to evaluate its ability to survive under different environmental conditions. It has been reported that *V. parahaemolyticus* is not psychrotrophic (1, 5). Temmyo (5) found no survivors in peptone water or fish extract after 5 days at 4, -2, and -18 C.

The present study indicates that this organism is sensitive to refrigeration and freezing. Storage of inoculated whole shrimp for 8 days at 10 to -18 C caused reductions in *V. parahaemolyticus* of 1 to 2 logs. However, even after 8 days at -18 C, about  $10^3$  cells per shrimp survived when the initial population

was  $10^5$  cells per shrimp. This observation agrees with reports that *V. parahaemolyticus* can be recovered from frozen shrimp (6) and from frozen marine sediments (7). Digirolamo et al. (2) suggest that bacterial survival in food homogenates should be interpreted cautiously. In their study, survival of *Salmonella* in oyster homogenates differed from that in whole oysters. In the present study, the only difference in behavior of *V. parahaemolyticus* in refrigerated whole shrimp and shrimp homogenate was a slight increase of the population in the latter during the first 12 hr of storage.

Low population levels of *V. parahaemolyticus* in shrimp homogenates were destroyed by heating at 60, 80, and 100 C for 1 min. With large populations, a few survivors were present in samples heated at 60 and 80 C for 15 min. No survivors were present after heating at 100 C. Temmyo (5) reported that *V. parahaemolyticus* was destroyed after 5 min at 60 C in peptone water. It is possible that food homogenates afforded some protection. *V. parahaemolyticus* in shrimp homogenates was very sensitive to pH values below 6.0. Little change in viable population occurred when stored for 2 hr at pH values ranging from 6 to 10. According to Fisher et al. (3), the effectiveness of stomach acidity as a bacterial inhibitor depends on the type of protein present in the stomach. The pH of the stomach contents increases considerably when food is present. In this study, the pH of the contents of the porcine stomach was 6.0. Under these conditions, *V. parahaemolyticus* could probably overcome the inhibitory effect of the stomach. Although a 100-fold decrease in viable population took place immediately, followed by another 10-fold decrease in 30 min, there still remained a viable population (about  $10^3$  per g) for the next 1.5 hr. Food begins to leave the human stomach in 30 min and its removal is completed in 4 to 5 hr (4). The live organisms which pass the stomach possibly could begin to multiply in the small intestine.

### ACKNOWLEDGMENT

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### LITERATURE CITED

- Baross, J., and J. Liston. 1970. Occurrence of *Vibrio parahaemolyticus* and related hemolytic vibrios in marine environments of Washington State. *Appl. Microbiol.* **20**:179-186.
- Digirolamo, R., J. Liston, and J. Matches. 1970. The effects of freezing on the survival of *Salmonella* and *E. coli* in Pacific oysters. *J. Food Sci.* **35**:13-16.

3. Fisher, L. H., D. H. Strong, and C. L. Duncan. 1970. Resistance of *Clostridium perfringens* to varying degrees of acidity during growth and sporulation. *J. Food Sci.* **35**:91-94.
4. Gregory, R. A. 1955. Digestion, p. 156-180. *In* F. R. Winton and L. E. Bayliss (ed.), *Human physiology*. Little, Brown and Co., Boston.
5. Temmyo, R. 1966. Studies on the prevention of outbreaks of food poisoning caused by *Vibrio parahaemolyticus*. *Bull. Tokyo Med. Dent. Univ.* **13**:489-510.
6. Vanderzant, C., R. Nickelson, and J. C. Parker. 1970. Isolation of *Vibrio parahaemolyticus* from Gulf Coast shrimp. *J. Milk Food Technol.* **33**:161-162.
7. Ward, B. Q. 1968. Isolations of organisms related to *Vibrio parahaemolyticus* from American estuarine sediments. *Appl. Microbiol.* **16**:543-546.