Journal of Food Protection, Vol. 45, No. 2, Pages 169-171 (February 1982)

Heat Processing of Oysters Naturally Contaminated with *Vibrio cholerae* Serotype 01

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(Received for publication July 9, 1981)

ABSTRACT

Pathogenic *Vibrio cholerae* 0-Group 1 survived for more than 3 weeks in artificial sea water with little loss in viability. Live oysters placed in such contaminated, artificial sea water took up but did not concentrate *V. cholerae*. Heat treatments provided by an in-can pasteurization process and by preparation of naturally contaminated oysters according to common recipes effectively reduced the numbers of *V. cholerae* by 5 logs/g.

The foodborne pathogen, *Vibrio cholerae*, serotype 01 Inaba, biotype El Tor, has been identified as the causative agent in recent cases of severe gastroenteritis among residents of southern U.S. coastal regions (*2*). These outbreaks plus the increasingly frequent isolation of *V. cholerae* 01 organisms from estuarine waters and shellfish (*3,5*) have raised concern that these organisms may have established endemic foci in U.S. coastal waters.

The potential contamination of oysters taken from approved harvesting areas with *V. cholerae* 01 organisms poses an obvious hazard to public health. One possible remedy for such a hazard would be heat processing of oysters. The feasibility of using an in-can pasteurization process to eliminate hepatitis A virus from shucked oysters has been investigated (*6*). In that study, thermal processing at 60.8 C for up to 42 min was insufficient to inactivate hepatitis A in size 300 × 206 cans of inoculated, shucked oysters.

The objective of this study was to determine whether heat treatment by the proposed pasteurization process (*6*) or by preparation according to common oyster cooking recipes would effectively eliminate the pathogen in shucked oysters naturally inoculated with *V. cholerae* 01.

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MATERIALS AND METHODS

Strain

V. cholerae 01 Inaba, El Tor strain E7629, which had been isolated from a patient's stool during an outbreak of cholera in Louisiana (*2*), was acquired from P. Blake at the Centers for Disease Control, Atlanta, GA. The culture was maintained at ambient temperature with monthly transfer on T_1N_1 medium (*3*).

Oyster maintenance

Refrigerated, live oysters harvested from approved eastern U.S. oyster beds were purchased from a local seafood wholesaler. Oysters were cleaned with a wire brush under warm, running tap water and placed in an aerated, artificial sea water environment at ambient temperature (Aquarium Systems, Inc., Eastlake, OH). After a 24-h depuration period, the fouled sea water was siphoned off and replaced with fresh artificial sea water. Oysters could survive for 5 weeks in such aerated tanks without additional nutrients. In these studies, oysters were used within 2 weeks.

Microbiological methods

Microorganisms indigenous to oysters were monitored by standard plate count methods (*4*). Routine dilutions using phosphate-buffered water were made from oysters blended with alkaline peptone water (APW). Standard plate counts (SPC) were made on standard methods agar after incubation for 48 h at 35 ± 2 C.

V. cholerae 01 organisms were enumerated by MPN and plate count procedures (*4*). Duplicate thiosulfate-citrate-bile salts-sucrose (TCBS) (BBL) plates were prepared at each sampling interval.

Survival of V. cholerae *01 in artificial sea water*

The survival of *V. cholerae* 01 in artificial sea water inoculated with 10^3-10^5 organisms/ml and held at ambient temperature was monitored daily during the first week and weekly for 2 additional weeks.

Survival of V. cholerae *01 in oysters*

Sufficient overnight APW culture was added to artificial sea water to yield an initial concentration $\geq 10^4$ *V. cholerae* 01/ml. To simulate natural inoculation of shellstock by the pathogen, oysters were allowed to rest in contaminated artificial sea water for 2 h before any sampling. Beginning 2 h after oysters were exposed to bacteria and continuing for 48 h, artificial sea water and oysters were monitored for *V. cholerae* 01. Oysters were removed at intervals from aerating tanks and shucked. Next, 25 g of inoculated oyster meats were blended for 1 min with 225 ml of APW and dispensed in triplicate serial dilutions of APW enrichment medium. After 6-8 h of incubation at 35 ± 2 C, 3-mm broth loopfuls from culture enrichment tubes were streaked onto TCBS plates. All TCBS plates were observed after 24 h of incubation at 35 ± 2 C for typical yellow colonies of *V. cholerae* 01.

V. cholerae 01 isolates were verified by agglutination of live organisms in group and Inaba-specific antisera provided by H. Smith (*Vibrio* Reference Laboratory, Philadelphia, PA).

Survival of V. cholerae *01 in canned, heat-treated oysters at 57.2 C*

Shellstock oysters naturally inoculated with *V. cholerae* 01 were shucked into a dry beaker and held under refrigeration for no longer than 1 h while the test cans were prepared. A sufficient quantity of shellstock oysters were shucked to fill ten 300 × 206 cans for each test. Six of the 10 cans were instrumented with 0.16-cm diameter copper-constantan thermocouples (Ecklund Custom Thermocouples, Cape Coral, FL). The sensing tip of the thermocouple was located at the geometric center of the can with entry through the side of the can.

To each test can was added 80 g of a 1% NaCl brine followed by approximately 120 g of drained oyster meats. This procedure yielded an oyster-to-brine fill ratio of 60/40 in the canned product. One oyster in each of the six instrumented cans was impaled onto the sensing tip of the thermocouple. The cans were hermetically sealed on a hand-operated seamer (Automatic Canning Devices, Inc., Manitowoc, WI) and washed in an Iodophor solution to destroy any *V. cholerae* 01 that might have splashed onto the outside of the can during the seaming operation.

The test cans of oysters in brine were then heated in a waterbath at 48.9, 54.4 and 57.2 C, with holding times of 15, 30, 45 and 60 min at each temperature. Two cans were processed at each holding time and two cans were used as unprocessed controls. Internal can temperatures were monitored during processing on a multi-point potentiometer (Bristol Series 760 Dynamaster, Waterbury, CT). After heat processing, the cans were removed from the waterbath and cooled in ice water until the internal can temperature was about 21 C. The processed cans were refrigerated until microbiological analyses were initiated.

Survival of V. cholerae *01 in oysters during simulated home preparation*

Shellstock oysters naturally inoculated with *V. cholerae* 01 were shucked and prepared according to recipes for oyster stew and oysters Rockefeller (*1*). Two batches of oyster stew were prepared in the following manner: (a) butter was melted in a pan; (b) flour, seasonings, and undrained oysters were added to melted butter; (c) the oyster mixture was simmered and stirred for 3-4 min until the edges of the oysters curled; (d) scalded milk was added and the mixture was removed from heat. Internal oyster temperatures were measured at the end of simmering and stew temperatures were measured at the center of the pot.

Four batches of oysters Rockefeller were prepared in the following manner: (a) a shucked oyster naturally inoculated with *V. cholerae* 01 was placed on a half-shell; (b) a mixture of chopped spinach, onion, parsley, bread crumbs, butter and spices was placed over and around the oyster; (c) the oyster was impaled on a thermocouple to monitor the internal temperature during baking; (d) oysters were baked for 10 min in a 232.2 C oven on a bed of hot rock salt.

Our analyses of the canned- and recipe-prepared oysters were restricted to bacteriological quality.

RESULTS AND DISCUSSION

Public Health authorities faced with recent outbreaks of cholera (*2*) have been concerned that these incidents might indicate the establishment of an endemic focus of *V. cholerae* 01 in the coastal regions of the United States. Long-term persistence of the pathogen could be accomplished by a life-cycle mechanism involving sequential contaminations from human, to water, to shellfish and back to human. The ability of *V. cholerae* 01 to survive sea water exposure sufficiently for natural inoculation of shellfish to occur was critical to this study.

Figure 1 illustrates the survival of *V. cholerae* 01 in artificial sea water at ambient room temperature. From an initial concentration of 7.5×10^4/ml, the pathogen declined gradually (approximately 2 logs in about 3 weeks). It must be realized that this long-term survival reflects the absence of competitive microflora that would be present in the marine environment. However, this result does suggest that no strongly vibriocidal activity could be attributed to sea water constituents *per se*.

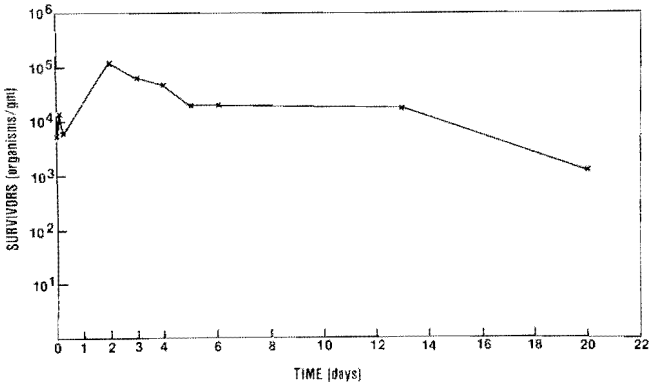

Figure 1. V. cholerae *01 survival in artificial sea water.*

When *V. cholerae* 01 in artificial sea water is ingested by living oysters, there appears to be neither a concentration nor an inhibitory effect. The simultaneous uptake of bacteria from artificial sea water and their appearance in shellfish at 2 h are then followed by a slow parallel decline in each substrate (Fig. 2).

With an initial inoculum of 2.47×10^5 *V. cholerae* 01/g in a 300 × 206 can of shucked oysters, a heat treatment of 48.9 C for 60 min reduced the count of *V. cholerae* 01 to 5.42×10^3/g. Duplicate thermal treatment trials were conducted at water bath temperatures of 54.4 and 57.2

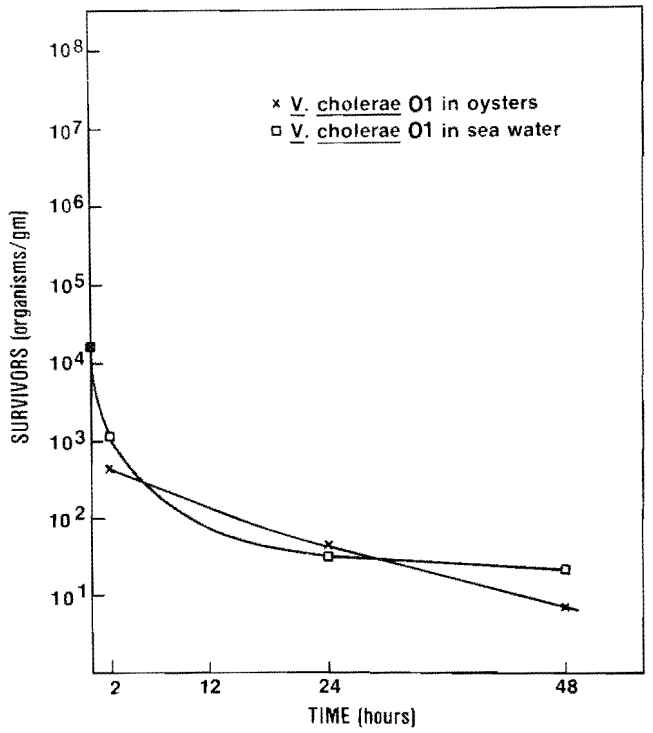

Figure 2. V. cholerae *01 survival in live oysters and sea water.*

C. In the first trial at 54.4 C, the *V. cholerae* 01 count was reduced from 9.45×10^4/g to 5.5/g, with a processing time of 60 min. In the second trial at 54.4 C, no *V. cholerae* 01 was recovered with a processing time of 60 min from an initial inoculum of 3.2×10^4/g. Neither trial conducted at 57.2 C yielded any *V. cholerae* 01 with processing times ≥ 30 min and initial inocula of 1.20×10^5 and 1.68×10^4 of *V. cholerae* 01/g, respectively. Data for heat processing at 57.2 C are shown in Table 1. Data from the study of Peterson et al. (*6*) indicate that hepatitis A is substantially more heat resistant than *V. cholerae* 01 in shucked oysters. In the light of present evidence, hepatitis A would be the controlling microorganism of public health significance in the design of any pasteurization process for shucked oysters.

The internal oyster temperatures in the 300 × 206 cans were measured at the point of minimum heating for all tests. However, the relatively large sample (200 g) with the resulting large temperature gradient within the can precluded the calculation of accurate D-values from these data.

Shucked oysters naturally inoculated with *V. cholerae* 01 were prepared according to published recipes for oyster stew and oysters Rockefeller (*1*). *V. cholerae* 01 was not recovered from oyster samples taken after the simmering phase and from the final stew. Internal oyster temperatures were approximately 80 C after simmering for 3 min. The oysters Rockefeller were baked in a 232.2 C oven for 10 min on a bed of hot rock salt. *V. cholerae* 01 was not recovered from samples of oysters Rockefeller. Internal oyster temperature at the end of baking was approximately 94 C, but this final temperature was variable because of thermocouple slippage. Accordingly, the thermal treatment that a consumer would give shucked oysters in typical recipe preparations would eliminate *V. cholerae* 01 at levels expected from natural contamination.

We conclude from our studies that *V. cholerae* 01 can survive for at least 3 weeks in artificial sea water with little loss of viability. These pathogens can be taken up but not concentrated by live oysters. Our heat studies indicate that *V. cholerae* 01 is significantly more sensitive to a stringent pasteurization process that is nevertheless insufficient to eliminate the well-recognized health hazard of hepatitis A virus.

Traditional recipes such as oyster stew and broiled oysters used in home and restaurant cooking are entirely sufficient to eliminate *V. cholerae* 01.

ACKNOWLEDGMENTS

The authors acknowledge and appreciate the guidance, suggestions and support of R. M. Twedt, E. P. Larkin, R. G. Dickerson, Jr. and R. B. Read, Jr.; and the editorial and typing assistance of J. C. Williams.

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TABLE 1. *Recovery of* V. cholerae *01 and indigenous microflora from naturally inoculated oysters heated at 57.2 C for various times.*

Duration of heat (min)	Trial 1		Trial 2	
	SPC/g[a]	*V. cholerae* 01/g	SPC/g	*V. cholerae* 01/g
0	4.90×10^3	1.20×10^5	2.07×10^5	1.68×10^4
15	1.07×10^3	1.03×10^4	2.00×10^5	1.06×10^4
30	2.91×10^3	0	3.65×10^4	0
45	1.99×10^4	0	8.90×10^3	0
60	1.81×10^1	0	1.10×10^3	0

[a]SPC = total plate count on standard plate count agar.