J. Milk Food Technol. Vol. 39, No. 5, Pages 322-324 (May, 1976)

Validity of Members of the Total Coliform and Fecal Coliform Groups for Indicating the Presence of *Salmonella* in the Quahaug, *Mercenaria mercenaria*

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(Received for publication September 26, 1975)

ABSTRACT

To determine the relationship of most probable number (MPN) of the total and fecal coliform groups in shellfish and shellfish-growing waters to the presence of *Salmonella* in quahaugs (*Mercenaria mercenaria*), a microbiological survey of 214 samples of the quahaug, or hard-shell clam, was done over 24 months. For purposes of this study, waters were classified as safe for shellfish harvesting by one of two criteria: (*a*) a total coliform MPN of $\leq$ 70/100 ml of water or (*b*) a fecal coliform MPN value of $\leqslant$14/100 ml of water. None of the quahaug samples harvested from waters meeting these standards contained *Salmonella*. Additionally, *Salmonella* was not detected in any of the quahaug samples meeting the wholesale market quality standard of 230 fecal coliforms per 100 g of shellfish as specified by the National Shellfish Sanitation Program. An increase in the total coliform and fecal coliform MPN of the waters more closely paralleled an increase in the fecal coliform MPN, as compared to the total coliform MPN, of the quahaug meats. Five *Salmonella* serotypes, including *Salmonella parathyphi* B, were found singly and in combination in five (2.3%) of the quahaug samples in this survey.

During a recent 2-year interval, a microbiological survey was conducted of shellfish-growing waters and shellfish samples harvested from such waters. On the primary bases of economic importance and availability, this survey was restricted to the investigation of two types of shellfish: the Eastern oyster, *Crassostrea virginica*, and the quahaug, *Mercenaria mercenaria*. The project was concerned not only with updating the determination of the safety of the shellfish-growing waters and of the microbiological quality of shellfish harvested from these waters, but was also involved in investigating: (*a*) the relative reliability of the total coliform and fecal coliform groups in the growing waters to indicate the presence of *Salmonella* in the shellfish themselves, (*b*) the comparative efficiency of various analytical procedures for recovering *Salmonella* from shellfish, and (*c*) the relative occurrence of the various *Salmonella* serotypes in shellfish. The findings with the oyster samples have been reported previously (*2*); the data of the quahaug samples are given here.

MATERIALS AND METHODS

This survey, conducted during a consecutive 24-month interval, was initiated on July 1, 1972, and ended on June 30, 1974. The collection of quahaug samples and the water samples overlying these harvesting areas was done by the Northeast Technical Services Unit (NETSU), a field unit of the Food and Drug Administration's Shellfish Sanitation Branch, located at Davisville, Rhode Island. Sampling was dependent upon such factors as tidal movements, climatic conditions, and estuary size. From each of 8-10 sampling sites, enough shellstock was collected to yield a sample of approximately 500 g of shellfish meat and liquor. Additionally, water samples consisting of approximately 200 ml were collected above each quahaug sampling site. The shellfish and water samples were collected monthly from shellfish-producing estuaries in Rhode Island, Massachusetts, Connecticut, and New Jersey. Collection of samples was equally divided between areas approved for harvesting and adjacent conditionally approved, restricted, or prohibited areas. All areas were classified in accordance with the specifications of the *Manual of Operations* (*4*) of the National Shellfish Sanitation Program (NSSP).

During sampling operations, water depth over the actual sampling areas ranged from $\leq$ 1 to 30 ft. Salinities ranged from 5.4 to 32.6 parts per thousand, and water temperatures ranged from 2.6 to 8.7 C during November-April and from 10.9 to 27.9 C during May-October.

To effect a more even distribution of the analytical workload, all water samples and most of the quahaug samples from approved growing areas were examined by NETSU, whereas all shellfish samples from non-approved areas and the balance of the shellfish samples from the approved areas were sent for analysis by air express to the Division of Microbiology laboratories in Washington, D.C. Definition of samples, specification of sizes of analytical units, procedures for collection and shipment of water and quahaug samples, and techniques for recording hydrographic data have been detailed previously (*2*). The recommended procedures of the American Public Health Association were used in the determination of coliform numbers in waters and shellfish (*1*). To effect maximum recovery of *Salmonella* from the shellfish, both the lactose pre-enrichment and direct selective enrichment procedures of the *Bacteriological Analytical Manual* (*BAM*) (*5*) were used. Each of three 100-g portions of the shellfish sample was blended in a high-speed blender for 60-120 sec at 14,000 rev/min with 150 ml of 35 C-tempered lactose, selenite cystine (SC), or tetrathionate (TT) broth containing 10 mg of brilliant green dye per liter. The homogenates were poured into flasks containing the remainder (750 ml) of the 900 ml of the respective broths. After adjusting the pH of the broth mixtures to 6.8 $\pm$ 0.2, flasks were incubated in a walk-in incubator at 35 C. After 24 h of incubation, 100

ml of lactose pre-enrichment mixture was subcultured to flasks containing 900 ml of fresh, 35 C-tempered SC or TT broth. After 24 and 48 h of incubation, a portion of the contents of the flasks containing the selective enrichment broths was streaked with a 3-mm loop to plates of brilliant green, bismuth sulfite (BS) and *Salmonella-Shigella* agar (Difco). All plates were incubated and examined after a 24-h incubation period. The BS agar plates were examined after 24 h, but were incubated an additional 24 h at which time they were re-examined. When present, at least two colonies suspicious for *Salmonella* were picked per plate in tandem to triple sugar iron agar and lysine iron agar. Cultures giving reactions suspicious for *Salmonella* were submitted to biochemical screening and, if necessary, were definitively serotyped.

RESULTS AND DISCUSSION

The relationship of levels of total coliforms in the waters to the levels of total and fecal coliforms and the presence of *Salmonella* in the quahaugs harvested from these waters is shown in Table 1. Except for the first category of 0-70 coliforms/100 ml of water, the categories of water MPN were arbitrarily established. The upper limit of 70 coliforms/100 ml of water was chosen as the first category since the present standard for approved shellfish-growing waters specifies that the total coliform median MPN of the water does not exceed 70/100 ml, and that not more than 10% of the samples ordinarily exceed an MPN of 230/100 ml for a 5-tube decimal dilution test, or 330/100 ml for the 3-tube decimal dilution test, in those portions of the area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions (*4*).

In addition to this growing-area standard, there is a quality standard established by the NSSP for shellfish at the wholesale market level. Shellfish having a fecal coliform MPN of $\leq$230/100 g and a total plate count not in excess of 500,000 organisms/g of shellfish are considered to be of acceptable quality at the wholesale market (*4*). The range of fecal coliforms in quahaugs harvested from waters with a total coliform MPN of $\leq$ 70 was from < 20 to 260. Only one of these samples had a fecal coliform MPN in excess of 230. Of the 40 quahaug samples examined in this category, 33 had indeterminant fecal coliform MPNs of < 20, reflecting a bacteriologically high-quality product.

Salmonella was not recovered in quahaug samples harvested from waters of the 0-70 and the 71-200 total coliform MPN categories. It was not until the total coliform MPN of the waters exceeded 200 that *Salmonella* was recovered from the quahaugs. The range of total coliform MPN of the waters at which *Salmonella* could be recovered was from 490 to 11,000.

Table 2 shows the relationship between level of fecal coliforms in the waters to the levels of total and fecal coliforms and the presence of *Salmonella* in quahaugs taken from these waters. Sixty-seven quahaug samples were harvested from waters having a fecal coliform MPN of $\leq$ 14. Fourteen was chosen as the upper limit of the first fecal coliform category since the recently recommended standard at the Eighth NSSP Workshop proposed that "the median fecal coliform MPN value for a sampling station shall not exceed 14/100 ml of sample and not more than 10% of the samples shall exceed 43 for a 5-tube, 3-dilution test or 49 for a 3-tube, 3-dilution test" (*3*). Forty-eight of the quahaug samples in the $\leq$ 14 category had indeterminant fecal coliform MPN of < 20 and none had MPN in excess of 230 fecal coliforms/100 g of quahaugs.

No *Salmonella* were recovered from any of the 67 samples in the $\leq$14 category. The range of fecal coliform MPN of waters at which *Salmonella* could be recovered in the quahaug meats was from 33 to 2300. Tables 1 and

TABLE 1. *Correlation of coliforms and* Salmonella *in quahaugs to the total coliform densities of overlying waters*

Total coliform MPN/100 ml of overlying waters	Quahaug samples examined		Total coliform MPN/100 g quahaug meats		Fecal coliform MPN/100 g quahaug meats		*Salmonella*-positive	
	No.	Percent[a]	Range	Median	Range	Median	No.	Percent
0-70	40	18.7	$<20-2.30 \times 10^3$	46	<20-260	<20	0	0
71-200	26	12.2	$<20-4.90 \times 10^3$	230	$<20-1.09 \times 10^3$	20	0	0
201-1,000	60	28.0	$<20-7.90 \times 10^3$	330	$<20-3.30 \times 10^3$	<20	2	3.3[b]
>1,000	88	41.1	$<20-1.72 \times 10^5$	130	$<20-3.30 \times 10^4$	49	3	3.4[b]
Total	214	100.0					5	
Overall average								2.3[a]

[a]Expressed as percentage of 214 samples.
[b]Expressed as percentage of samples in this category.

TABLE 2. *Correlation of coliforms and* Salmonella *in quahaugs to the fecal coliform densities of overlying waters*

Fecal coliform MPN/100 ml of overlying waters	Quahaug samples examined		Total coliform MPN/100 g quahaug meats		Fecal coliform MPN/100 g quahaug meats		*Salmonella*-positive	
	No.	Percent[a]	Range	Median	Range	Median	No.	Percent
0-14	67	31.3	$<20-5.42 \times 10^3$	70	<20-230	<20	0	0
15-70	51	23.8	$<20-7.00 \times 10^3$	220	$<20-3.30 \times 10^3$	<20	2	3.9[b]
71-200	29	13.6	$<20-7.90 \times 10^3$	330	$<20-1.10 \times 10^4$	<20	0	0
201-1,000	44	20.6	$<20-1.30 \times 10^5$	230	$<20-3.30 \times 10^4$	20	1	2.3[b]
>1,000	23	10.7	$<20-1.72 \times 10^5$	130	$<20-3.30 \times 10^4$	70	2	8.7[b]
Total	214	100.0					5	
Overall Average								2.3[a]

[a]Expressed as percentage of 214 samples.
[b]Expressed as percentage of samples in this category.

2 show that an increase in the total and fecal coliform MPN of the waters more closely paralleled an increase in the fecal coliform MPN, as compared to the total coliform MPN, of the quahaug meats. This finding would support the NSSP use of the fecal coliform indicator group, rather than the total coliform indicator group, in establishing the present wholesale market quality standard for quahaugs and other shellfish as well.

Of the total of 214 quahaug samples examined, 182 had fecal coliform MPN of ≤ 230 (Table 3). Even though

TABLE 3. *Correlation of* Salmonella *in quahaugs to the fecal coliform densities in quahaugs*

Fecal coliform MPN/100 g quahaug meats	Quahaug samples examined		*Salmonella*-positive	
	Number	Percentage[a]	Number	Percentage
0-230	182	85.0	0	0
>230	32	15.0	5	15.6[b]
Total	214	100.0	5	
Overall average				2.3[a]

[a]Expressed as percentage of 214 samples.
[b]Expressed as percentage of samples in this category.

some of these shellfish samples had been harvested from waters having a total coliform MPN exceeding 70 or a fecal coliform MPN exceeding 14, many of these samples were able to meet the NSSP wholesale market standard. The finding that *Salmonella* was not isolated from any of the 182 samples meeting the market quality standard substantially supports the validity and bacteriological safety of the NSSP standard. The range of total coliforms and fecal coliforms in the quahaug samples containing *Salmonella* was from 1,300 to 172,000 and from 490 to 33,000, respectively.

The occurrence of such few numbers of *Salmonella*-positive samples led to an investigation of the sensitivity of the BAM method for recovering *Salmonella* from both oysters and quahaugs. As few as 8-10 *Salmonella* organisms/100 g of artificially contaminated shellfish could consistently be recovered, indicating an acceptable level of sensitivity. Additionally, the possibility of the methodology used being inadequate is further minimized when one considers that the methodology for recovering *Salmonella* from quahaugs was the same as that used in the analysis of oysters, where 11.1% of the oyster samples examined were positive for *Salmonella* (2). Of the five *Salmonella*-positive quahaug samplers, three were positive exclusively by direct enrichment in TT broth with added brilliant green dye, whereas the remaining two samples were positive by both the lactose pre-enrichment and direct selective enrichment procedures. *Salmonella typhimurium* and *Salmonella paratyphi B* were found individually in two of the samples, *Salmonella anatum* and *Salmonella thompson* were both found in each of two samples, and one sample contained *S. anatum, S. thompson,* and *Salmonella derby. Salmonella* was chosen as the representative of the potential pathogen load since the methodology for its detection is established, relatively standardized, and sensitive. In determining the overall significance of the data from this study, it must be kept in mind that other pathogenic bacteria, viruses, and parasites are capable of existing and proliferating in shellfish.

In this survey, *Salmonella* was not found in the quahaug when either the coliform or fecal coliform densities were within the recommended limits. Because of the relatively low number of *Salmonella*-positive quahaug samples, however, no conclusion can be made about the comparative efficacy of these two indicator groups to indicate the presence of *Salmonella* in quahaugs. It can be concluded, however, that both the present total coliform standard and the proposed fecal coliform growing-area standards were acceptable from the standpoint that the use of each resulted in the harvesting of a shellfish product capable of meeting or surpassing the present wholesale market quality standard and indicated the absence of *Salmonella* in this marketed product. The findings of this study support the conclusion that the bacteriological content of quahaugs is a reflection of the bacteriological conditions of the waters overlying the sites from which the shellfish were harvested.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Captain James Verber, Chief, Northeast Technical Services Unit, and his staff for the collection of samples. Appreciation is expressed to Aida Romero and Paul Poelma, Division of Microbiology, for serotyping of *Salmonella* isolates. Special thanks are expressed to Daniel Hunt, Assistant Chief, Shellfish Sanitation Branch, for co-ordination of field activities.

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