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Salmonellae in Fish Meal Plants: Relative Amounts of Contamination at Various Stages of Processing and a Method of Control

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Previous studies have shown that Menhaden fish meal, a common ingredient of animal feeds, is frequently contaminated with salmonellae. Animals that eat contaminated feed may become infected. If they, in turn, are eaten by humans, they may be a means by which salmonellae are introduced into the human population. Epidemiological studies of the fish-meal industry were carried out to determine the sources of salmonellae in fish meal and the factors affecting the persistence and survival of salmonellae during the processing of fish meal. Examination of 190 fish immediately after they came from the Gulf of Mexico revealed no salmonellae, but salmonellae were frequently isolated from samples of fish taken from the boats when they arrived at the plants. Salmonellae were also frequently isolated from dockside water at each of the plants. Approximately 50% of the samples taken in the raw fish processing areas were contaminated with salmonellae. The percentage of samples yielding salmonellae decreased progressively through the various sequences of processing, but more than 15% of the samples taken from the finished products were also positive. Salmonellae were isolated from the raw area of the plant most frequently while the plant was operating and less frequently when the plant was idle, whereas in the processing area of the plant the reverse was true. Salmonellae appeared to survive and multiply in the processing area of the plant while the plant was idle, which resulted in contamination of the first portion of each day's production. Salmonellae in the processed fish meal were reduced to nondetectable levels by reprocessing the first 45 min of each day's production.

Menhaden fish caught in the Atlantic Ocean and Gulf of Mexico, although not eaten because of their high oil content and unpleasant taste, were used as fertilizer by the American Indians and by the white settlers up until the 20th century. Fish meal, a high quality source of protein, is now commonly used as an ingredient of animal feeds.

In recent years, the growth of the food processing industry has been paralleled by an increase of salmonellosis in animals and man. Epidemiological studies of foodborne human salmonellosis have implicated foods of animal origin as the most common source of infection (7). Animal infections, in turn, have been traced to feeds (2), and the feed ingredients of animal origin, such as meat and bone meal, poultry meal, feather meal, and fish meal, are most commonly contaminated (1). It is not surprising to find by-products of animals and poultry contaminated, since animals and fowl are frequently infected with salmonellae. In contrast, contaminated fish meal is processed from ocean fish that are thought to be free from salmonellae.

Studies were undertaken to determine the extent of *Salmonella* contamination in the fish processing industry and the source and pattern of spread of salmonellae within the plants. Three plants were studied during the period from 1962 to 1964; then the plant personnel began using improved clean-up procedures, clean water in pumping fish, and procedures to eliminate rats, flies, and other insects. Follow-up studies were done in the three plants, plus a fourth plant, in 1968. This paper describes the relative contamination of the plants in the two time periods and a method of reducing fish-meal contamination to nondetectable levels of salmonellae.

MATERIALS AND METHODS

The equipment in the plants was sampled with cotton-tipped swabs during idle periods at various points along the processing line. Investigators concentrated on wet areas in the plant and residual fish matter, since they had observed that these areas were most likely to yield salmonellae. The clean dry areas were usually not sampled. In addition, bulk fish-meal samples were collected from the storage warehouse. While the plant was operating, swab samples of the fish product were taken regularly at various stages of processing, and bulk samples were taken occasionally. The plants operated only during the summer, and all the plants had started seasonal operation before the studies.

Swab samples were put directly into 10 ml of tetrathionate broth (Difco) containing a 1:100,000 dilution of Brilliant Green (TET). The cultures were kept at room temperature until taken to the laboratory, usually within 2 days, where they were incubated for 24 to 48 hr and streaked on Brilliant Green agar containing 80 mg of sodium sulfadiazine per liter of agar (BGS). If more than 48 hr had elapsed before the TET cultures reached the laboratory, they were incubated for 24 hr and subcultured to fresh TET broth (1 to 10 ml). This broth was incubated for 24 hr before being streaked on BGS. Samples processed in this manner, utilizing the secondary enrichment broth, yield salmonellae as frequently as or more frequently than samples processed in the usual manner (9; G. K. Morris, J. G. Wells, and C. G. Dunn, *unpublished data*).

The usual incubation temperature for TET enrichments was 37 C; however, because of recent reports in the literature of superior results with higher temperatures (4, 8), duplicate samples were collected in many instances, and half were incubated at 37 C and the others at 43 C. Findings in this study, however, indicated no difference in the effect of the two incubation temperatures. The plating medium in all instances was incubated at 37 C for 24 hr. Colonies that were suspect for salmonellae were carefully picked to triple sugar iron agar (TSI). Cultures that could not be eliminated by the TSI reactions were examined with biochemical media and typed serologically (5, 6).

Bulk samples were collected aseptically in sterile plastic bags, transported to the laboratory, and held

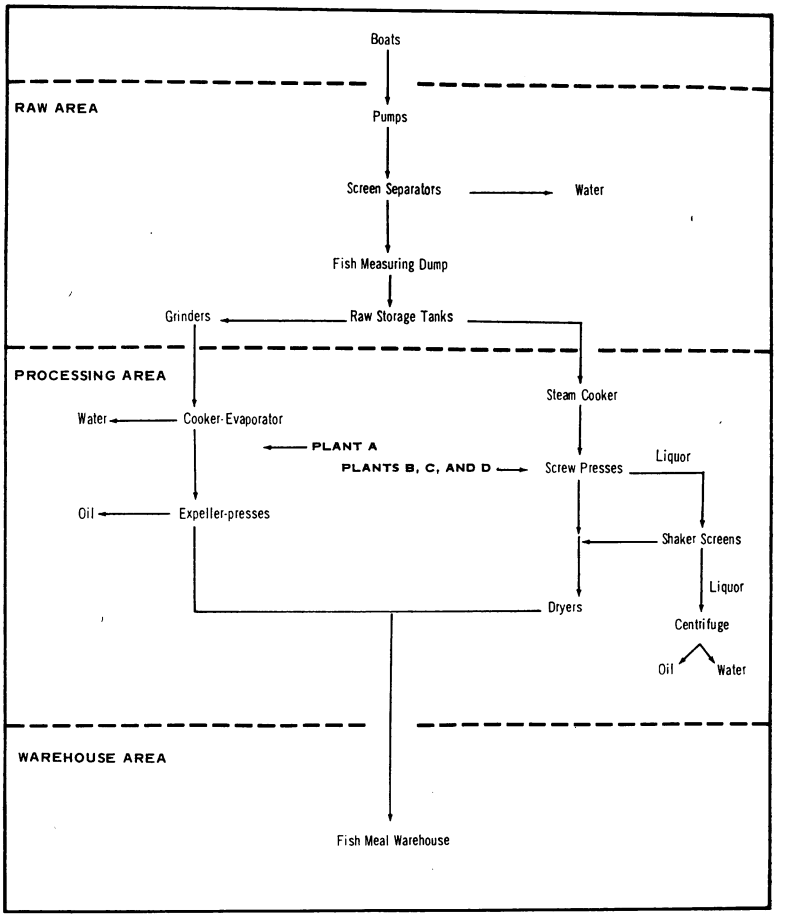

FIG. 1. *Diagram of two methods of processing fish meal.*

TABLE 1. *Salmonella-contaminated samples from plant A during 1968*

Location	May	June	July	August	Total
Raw area					
Pumps	0/10[a]	3/8	3/6	4/6	10/30
Recycled pump water	0/4[b]	0/4[b]	ND[c]	3/3[d]	3/11
Screen separators	0/6	3/8	7/12	6/6	16/32
Fish measuring dump	0/6	1/4	5/6	ND	6/16
Conveyers to raw storage tank	0/11	4/13	10/10	5/6	19/40
Raw storage tank	0/18	8/12	11/12	5/6	24/48
Conveyers, raw tank to processing building	0/8	1/11	10/12	1/9	12/40
Total	0/63	20/60	46/58	24/36	90/217 (41%)
Processing area					
Conveyer before cooking	2/4	4/7	ND	0/3	6/14
Floor in processing building	1/6	0/4	ND	ND	1/10
Conveyers after cooking	1/6	0/6	ND	ND	1/12
Presses	1/7	4/12	0/6	ND	5/25
Conveyers after pressing	5/11	3/13	1/3	2/5	11/32
Total	10/34	11/42	1/9	2/8	24/93 (26%)
Warehouse area					
Environmental (floors, walls, and conveyers)	4/50	2/24	0/24[e]	1/15[e]	7/113
Stored meal	10/17	5/16	0/7	0/6	15/46
Total	14/67	7/40	0/31	1/21	22/159 (14%)
Grand total	24/164	38/142	47/98	27/65	136/469
Per cent	15	27	48	42	29

[a] Positive samples/samples examined.
[b] Samples collected from reservoir while plant was idle.
[c] Sampling not done.
[d] Samples collected while fish were being pumped from boats.
[e] Plant A control program begun after June sampling resulted in fewer isolations from warehouse in July and August.

at room temperature before examination. Samples were examined by adding 30 g to 100 ml of TET and, after 24 and 48 hr of incubation, streaking a loopful to BGS plates.

The dockside river water was sampled by submerging a cotton gauze swab (10) in the water for 24 hr. The swab was then put in a sterile plastic bag with 100 ml of TET broth. Enrichment and isolation procedures for the Moore swab cultures were similar to the procedures for the cotton-tipped swab samples above.

Three of the four fish-meal plants (plants B, C, and D) were well established and similar in construction. Processing equipment consisted of a steam cooker, presses to separate the oil and water, and hot air dryers to dry the fish meal (Fig. 1). The fourth plant (plant A) was newer, and its processing equipment consisted of a cooker, an evaporator to dispose of the water from the fish, and expeller-type presses to separate the oil from the fish meal. The raw (wet) areas were similar in plants A, C, and D; they were of predominantly steel construction. The raw area of plant B was older and was constructed primarily of wood. Plants B, C, and D were included in both the 1962 to 1964 study and in the 1968 study, but the new plant A was examined in 1968 only.

RESULTS

In 1968, plants A and B were examined most extensively. Salmonellae were isolated from 136 of 469 samples (29%) collected from plant A (Table 1). Salmonellae were isolated most often from raw-area samples (41%), then from the processing-area samples (26%), and finally from warehouse samples (14%). Some fluctuation was noted in the frequency with which salmonellae were isolated from the same areas as the summer progressed; fewer were isolated in May and June than in July and August. However, a control procedure initiated in this plant between June and July may have contributed to the low values for the warehouse samples.

Salmonellae were isolated from 218 of the 474 samples (46%) from plant B (Table 2). As in plant A, salmonellae were isolated from samples collected in the raw area of plant B most frequently (57%), followed by samples collected in the processing area (45%), and then those collected in the warehouse (26%). Salmonellae were

TABLE 2. *Salmonella-contaminated samples from plant B during 1968*

Location	May	June	July	August	Total
Raw area					
Pumps	0/6[a]	9/10	10/12	8/9	27/37
Screen separators[b]	3/6	5/6	5/6	3/6	16/24
Fish measuring dump	2/12	4/6	6/6	6/6	18/30
Conveyers to storage tank	4/12	2/14	ND[c]	4/8	10/34
Raw storage tank	8/18	11/18	6/6	7/18	32/60
Shaker screens[b]	1/12	3/6	4/6	8/9	16/33
Drain trough below shaker screens	ND	4/4	6/6	6/9	16/19
Total	18/66	38/64	37/42	42/65	135/237 (57%)
Processing area					
Conveyer after cooking	1/6	4/4	ND	ND	5/10
Presses	4/14	4/6	6/6	3/9	17/35
Drain trough below presses	ND	2/2	ND	6/6	8/8
Shaker screens before centrifuging	1/6	0/4	ND	4/6	5/16
Conveyer after pressing	1/6	2/2	6/6	1/3	10/17
Conveyer after dryers	0/10	3/8	1/2	0/2	4/22
Total	7/42	15/26	13/14	14/26	49/108 (45%)
Warehouse area					
Environmental (floors, walls, and conveyers)	5/24	2/18	2/24	2/12	11/78
Stored meal	4/12	9/12	2/14	8/13	23/51
Total	9/36	11/30	4/38	10/25	34/129 (26%)
Grand total	34/144	64/120	54/94	66/116	218/474
Per cent	24	53	57	57	46

[a] Positive samples/samples examined.
[b] Used to regain fish particles from water.
[c] Sampling not done.

isolated more consistently from plant B samples than from plant A samples. This is probably because plant B is built primarily of wood and plant A is mostly steel, which is easier to clean.

Salmonellae were isolated from 41 of 120 samples from plant C (34%) and 33 of 82 samples from plant D (40%; Table 3). Salmonellae were isolated from 2 of 11 samples collected from the warehouse of plant D and from none of the 15 samples collected from the warehouse of plant C. Many samples (83%) collected from the raw area of plant D contained salmonellae, but only 13% of the samples from the raw area of plant C were contaminated. In the processing areas, the reverse was true; i.e., in plant D 3% of the samples were contaminated and in plant C 73% of the samples were contaminated.

The frequency with which salmonellae were isolated from the raw area or processing area of a plant appeared to depend on the operational status of the plant (Table 4). Salmonellae were isolated more frequently in the raw areas when plants were in operation than during idle periods (plants A, B, and D), whereas salmonellae were isolated more frequently from samples collected from the processing area during idle periods than during operation. However, only 14 samples were obtained during processing because of the heat and danger from the machinery.

Table 5 shows the results of the examination of newly processed samples collected in the warehouse at 5-min intervals from the beginning of processing in plant A. In May, examination of fish-meal samples collected for the first 50 min indicated that the fish-meal product was contaminated with salmonellae for only the first 35 min. During the June survey, the samples were contaminated consistently for the first 15 min and intermittently for the next 35 min.

After the June survey, the conveyer linking the processing building to the warehouse was modified so that processed fish meal could be diverted back to the raw area of the plant. To eliminate contamination, meal processed in the first 45 min of each production day was diverted and reprocessed. Samples were collected at intervals in July and August, and no contaminated meal was detected going into the warehouse. During the August survey, examination of fish meal collected by interval sampling of the diverted meal indicated that eight of nine samples were positive for salmonellae. (This material was reprocessed.)

TABLE 3. *Isolation of salmonellae from plants C and D during 1968*

Location	Plant C	Plant D
Raw area		
Pump	2/12[a]	10/12
Recycled pump water	ND[b]	6/6[c]
Screen separators	2/12	8/12
Fish measuring dump	2/6	ND
Conveyers to raw storage tank	0/6	6/6
Raw storage tank	2/24	ND
Total	8/60 (13%)	30/36 (83%)
Processing area		
Conveyers after cooking	ND	0/14
Screen separators	3/3	ND
Presses	8/12	0/12
Drain trough below presses	6/6	ND
Centrifuge	ND	0/6
Conveyers after pressing	14/15	1/3
Conveyers after dryers	2/9	ND
Total	33/45 (73%)	1/35 (3%)
Storage warehouse		
Environmental (floors, walls, and conveyers)	0/12	ND
Fish meal (stored)	0/3	2/11
Total	0/15 (0%)	2/11 (18%)
Grand total	41/120 (34%)	33/82 (40%)

[a] Positive samples/samples examined.
[b] Sampling not done.
[c] Samples collected while fish were being pumped from boats.

Samples collected from the storage warehouse in each of the above months were examined with the following results (Table 1): May, 14 of 67 (21%) contained salmonellae; June, 7 of 40 (18%); July, 0 of 31 (0%); and August, 1 of 21 (5%). The one positive sample in August was actually collected from meal next to a post and did not represent meal involved in rapid turnover.

The 1968 survey findings were compared with the 1962 to 1964 survey (Table 6). Despite extensive sanitation programs in the intervening years, salmonellae were isolated as frequently in 1968 as in the period from 1962 to 1964. In addition to the samples in this table, 190 fish were collected directly from the Gulf of Mexico and examined, and no salmonellae were isolated.

TABLE 5. *Salmonellae isolated from samples collected at 5-min intervals from the beginning of processing*

Time (min)	May	June	July[a]	August
0	+	+	−	−
5	+	+	−	−
10	+	+	−	−
15	+	+	−	−
20	+	−	−	−
25	+	−	−	−
30	+	−	−	−
35	+	+	−	−
40	−	−	−	−
45	−	−	−	−
50	−	+	−	−
55	NT[b]	−	−	−
60	NT	−	−	−
65	NT	−	−	−
70	NT	−	−	−
75	NT	−	−	−

[a] The control procedure, initiated after the June sampling, consisted of reprocessing all the meal from the first 45 min of production.
[b] Sample not taken.

TABLE 4. *Salmonellae isolated from four fish-meal plants while idle and while operating during 1968*

Plant	Sample area	Operational status: Idle			Operating		
		Positive/total	Proportion	Standard error	Positive/total	Proportion	Standard error
A	Raw area	31/154[a]	0.20	0.03	99/113	0.88	0.03
	Processing area	23/68	0.34	0.06	0/6	0	0
B	Raw area	132/237	0.56	0.03	15/15	1.00	0
	Processing area	64/134	0.48	0.04	ND[b]		
C	Raw area	8/60	0.13	0.04	ND		
	Processing area	31/36	0.86	0.06	ND		
D	Raw area	13/18	0.72	0.11	29/30	0.97	0.03
	Processing area	1/16	0.06	0.06	0/8	0	0

[a] Positive samples/samples examined.
[b] Sampling not done.

TABLE 6. *Salmonellae isolated from the various fish-meal plants in the two studies*

Source of samples	Plants						
	A	B		C		D	
	(1968)	1962–1964	1968	1962–1964	1968	1962–1964	1968
River or bay water	5/13[a] 38%	NS[b]	9/13 69%	11/28 39%	1/13 8%	95/237 40%	46/64 72%
Boats	5/8 63%	NS	3/16 19%	31/112 28%	NS	NS	NS
Plant raw area	120/270 44%	3/19 16%	149/261 57%	0/21 0%	8/60 13%	31/71 44%	47/54 87%
Plant processing area	24/93 26%	12/61 20%	69/139 50%	40/198 20%	33/45 73%	34/230 15%	1/35 3%
Warehouse area	22/159 14%	20/59 34%	34/129 26%	21/175 12%	0/15 0%	5/75 7%	2/11 18%

[a] Positive samples/total samples examined.
[b] No samples examined.

The river or bay water adjacent to the plants was examined to determine the relationship between salmonellae in the plants and in the dockside water. Some relationship was expected, since the clean-up water drained into the river or bay. In the period from 1962 to 1964, river water was used to pump the fish into the plant, but, in the 1968 survey, city water was used. Salmonellae were frequently isolated from dockside water samples during both time periods.

Salmonella serotypes isolated from water generally reflected the serotypes isolated from the plants except in the case of plant D. *S. inverness* was isolated at various points along the river from 1 mile upstream from the plant to 2 miles downstream during both the 1962 to 1964 survey and the 1968 survey, but this serotype was never isolated from the plant itself.

Twenty-three serotypes were isolated during 1968 (Table 7). Only four were isolated from all four plants, i.e., *S. thomasville*, *S. bareilly*, *S. kentucky*, and *S. senftenberg*. *S. senftenberg* and *S. bareilly* were the only serotypes isolated from every plant in both surveys.

When the 10 most commonly isolated serotypes during 1962 to 1964 were compared with the 10 most commonly isolated serotypes in 1968, 6 appeared on both lists (Table 8).

DISCUSSION

Samples taken in all stages of processing, from when the fish were caught to the finished product, yielded salmonellae. Fish taken directly from the Gulf of Mexico yielded no salmonellae; therefore, ocean fish appear to be free from salmonellae or contaminated infrequently. Other investigators have also found that some freshwater fish and fish collected from seawater near the coast are infected with enteric bacteria, but fish caught in the open sea are not (3). Samples collected on the boats were frequently contaminated, but the fish were washed out of the boats with pump water from the plants and the boats were not thoroughly cleaned subsequent to unloading. The pumps and pump water were frequently contaminated; therefore, we could not determine whether the initial source of contamination was the pump water or the boats. Salmonellae were isolated from samples of fish on the boats when they arrived at different plants, and pump water used to wash out the boats when a plant was active yielded salmonellae in every instance. Thus, it appears that the boats may be contaminated with salmonellae while being unloaded, and, since they are not cleaned thoroughly between loads, salmonellae subsequently contaminate the next load of fish. Cleaning the holds of the boats between loads may reduce contamination. It is also possible, however, that salmonellae multiply in isolated locations of the raw area and pumping system of a plant when it is idle. When the plants start operating, the bacteria may uniformly spread to all of the raw areas and the boats by the conveyers and pumping systems.

The raw areas of the plants were frequently contaminated with salmonellae. The cleaned equipment in the raw areas yielded salmonellae less frequently during idle periods than during periods of operation. In the processing areas, however, the opposite was true. Samples collected during idle periods frequently yielded salmonellae, but no samples collected from the processing areas during operation were positive. The moist fish matter left in the presses and conveyers of the

processing area apparently provided a good growth medium. The source of salmonellae in the processing areas was not determined. Quantities of fish taken from the cookers during operation were apparently too hot for salmonellae to survive, but salmonellae inside a large piece of fish may survive this cooking and serve as an inoculum in the fish left in the presses after shutdown. In addition, moist fish left in the processing areas may be inoculated with salmonellae from the air or by flies or other insects. It is obvious that sanitation in these plants in 1968 had greatly improved, but flies and other insects were still present and may be difficult to completely eliminate. At any rate, it appears that the only solution is to clean all fish matter from the presses and conveyers after each operation.

TABLE 7. *Serotypes of salmonellae isolated from fish-meal samples and plant environments*[a]

Serotypes	1962–1964 Plants				1968 Plants				
	B	C	D	Total	A	B	C	D	Total
S. anatum	4	2	0	6	2	4	0	0	6
S. bareilly	1	4	1	6	29	47	2	28	106
S. bredeney	10	24	3	37	0	3	13	0	16
S. cerro	1	0	21	22	20	9	0	10	30
S. cubana	0	2	4	6	0	2	6	1	9
S. derby	0	0	0	0	0	2	0	0	2
S. eimsbuettel	0	0	0	0	9	28	0	4	41
S. give	0	0	1	1	0	1	0	0	1
S. illinois	0	33	4	37	0	3	1	0	4
S. infantis	0	0	0	0	1	0	0	0	1
S. kentucky	0	32	9	41	12	55	13	1	81
S. livingstone	0	0	0	0	0	0	0	6	6
S. meleagridis	0	4	0	4	0	0	0	0	0
S. menhaden	0	1	0	1	0	0	0	0	0
S. minnesota	0	0	4	4	0	0	0	1	1
S. montevideo	0	2	1	3	7	9	1	0	17
S. newbrunswick	0	0	2	2	35	34	0	2	71
S. newington	0	0	0	0	10	5	0	0	15
S. oranienburg	6	6	6	18	2	14	2	0	18
S. schwarzengrund	0	0	4	4	0	1	0	0	1
S. senftenberg	24	11	21	56	17	23	3	11	54
S. simsbury	1	0	0	1	0	0	0	0	0
S. taksony	0	1	0	1	0	0	0	0	0
S. tennessee	1	7	0	8	3	1	0	1	5
S. thomasville	0	0	18	18	101	95	8	2	206
S. thompson	0	0	0	0	0	3	0	0	3
S. wildwood	0	2	0	2	0	0	3	0	3
S. worthington	2	0	4	6	0	0	0	0	0
Total	50	131	103	284	248	339	52	67	706

[a] The number of isolates is influenced by the number of samples taken and the per cent positive. Multiple serotypes were isolated from some samples.

TABLE 8. *Ten most commonly isolated serotypes in 1962–1964 and in 1968*

Order of frequency	1962–1964	Per cent of total isolates	1968	Per cent of total isolates
1	*S. senftenberg*	21.2	*S. thomasville*	30.2
2	*S. kentucky*	12.9	*S. bareilly*	15.0
3	*S. bredeney*	9.7	*S. kentucky*	11.4
4	*S. illinois*	9.4	*S. newbrunswick*	10.3
5	*S. thomasville*	8.1	*S. senftenberg*	7.9
6	*S. cerro*	6.2	*S. cerro*	5.4
7	*S. oranienburg*	5.1	*S. eimsbuettel*	5.4
8	*S. give*	2.5	*S. newington*	2.5
9	*S. muenchen*	2.1	*S. montevideo*	2.4
10	*S. newbrunswick*	2.1	*S. oranienburg*	2.2

Fish meal in the warehouse was contaminated, but only the first 30 to 45 min of each day's production yielded salmonellae. This indicates that once the equipment in the processing area heated up, salmonellae were destroyed. However, meal processed early apparently contaminated the rest of the meal in the warehouse when they were blended to prevent overheating.

The fact that only the first portion of each day's production was positive, however, indicated a means of controlling salmonellae in the finished product, i.e., reprocessing the first 30 to 50 min of production. Almost 50% of the samples of stored fish meal taken from plant A in May and June were positive for salmonellae, but all samples of stored meal collected in July and August, after a control procedure had been instituted in this plant, were negative for salmonellae.

There was some similarity in serotypes of salmonellae isolated in the four plants during both periods. There was also similarity between the serotypes of salmonellae isolated from the dockside water and those isolated from the plants, possibly due to clean-up water draining into the dockside water. Other fish-meal plants were located in the general vicinity of each of these plants, and plant D was located 2 miles upstream from a city sewage outlet. These neighboring industries may account for part of the *Salmonella* flora in the dockside water.

A comparison of the results from 1962 to 1964 and the results from 1968 indicated no decrease in salmonellae in these plants.

Several things can be done to improve sanitation in fish-meal plants: (i) use chlorinated water to pump fish so as to avoid introducing pollution from other industries, (ii) avoid reusing pumping

water, (iii) clean boats and plant machinery thoroughly after each use, (iv) reprocess the first portion of each day's fish meal production, and (v) use decontaminated railroad cars and trucks to ship meal.

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