Salmonellae in the Environment Around a Chicken Processing Plant

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Studies have been conducted over a 2-year period to determine the extent to which a poultry processing plant served as a reservoir of salmonellae reaching the external environment, to examine the question of the importance of salmonellae in the environment, and to consider how best to control the spread of the organisms. The studies have been undertaken at a chicken processing plant handling between 75,000 and 80,000 birds per day. Populations of salmonellae and indicator bacteria were estimated in the raw wastes, through the waste treatment plant, and in the receiving stream waters. The results demonstrate that salmonellae are present in poultry processing wastes in a surprisingly constant relation to fecal coliforms (in excess of 1 Salmonella per 500 fecal coliforms), that serotypes in the environment are constantly changing, and that they may reflect unusual conditions in the processing plant, or a possible source of infection among human and animal residents of the environment. Disinfection of poultry processing wastes is recommended.

During December 1969, populations of fecal coliforms and fecal streptococci were determined in the wastes from a chicken processing plant. A lagoon system for the treatment of the wastes had been placed into operation during the previous year, and the purpose was to determine the effect of treatment on the number of indicator bacteria being discharged to the receiving stream which consisted largely of plant effluent and which flowed ultimately into a large recreational lake.

Populations of fecal coliforms and fecal streptococci in the raw wastes were 1.7×10^6 bacteria per 100 ml and 1.45×10^6 bacteria per 100 ml, respectively. During treatment the population of fecal coliforms was reduced by 90% to $1.7 \times$ 10^6 bacteria per 100 ml in the effluent and $4.3 \times$ 10^4 bacteria in the stream. At the same time the population of fecal streptococci was reduced by 97% to 4.4×10^4 bacteria per 100 ml in the effluent and 4.3×10^4 bacteria in the stream. In spite of the reduction in the populations of indicator bacteria, which were consistent with reductions observed in stabilization ponds receiving domestic wastes (for instance see 19),

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the numbers of indicators discharged to the stream were high. In view of the populations of indicator bacteria in the effluent and the receiving stream, and in view of the well-documented association of salmonellae with poultry (2, 11-13, 16, 21, 25, 35, 38), a study was undertaken to determine the extent to which the plant did, indeed, serve as a reservoir of salmonellae reaching the external environment, to examine the question of the importance of salmonellae in the environment, and to consider how best to control the spread of the organisms. It should be pointed out, however, that although salmonellae generally are considered to be associated with poultry, this is not necessarily the case. In a study of many animals undertaken at Haryana Agricultural University (28) between 1968 and 1972, relatively few poultry yielded salmonellae.

MATERIALS AND METHODS

Description of plant. Studies have been conducted at a chicken processing plant handling between 75,000 and 80,000 birds per day. On the grounds of the plant were located a hatchery, the processing plant, a freezing plant, and a waste treatment plant. During that portion of the study when antibiotic susceptibility testing was undertaken, newly-hatched chicks were provided feed containing furazolidone for the first 10 days of life. When they were 1 day old they were injected with turkey blood containing oxytetracycline and shipped to chicken farms which employed

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feed produced by the parent company. At chicken farms, antibiotics were said not to be administered except to combat disease outbreaks when they occurred. The chickens were returned to the plant for processing when they were between 8 and 9 weeks old. For the 10 days preceding processing, chickens were treated with coccidiostats, but not with antibacterial drugs unless they were needed.

Chickens were returned to the plant by truck and were killed and processed on the day of their return. Offal, feathers, and blood were separated and were loaded onto trucks which carried them to another site where they were processed by another company for use in feeds. Liquid wastes were discharged to the stabilization pond system described schematically in Fig. 1.

Prior to late 1971, approximately 0.725 million gallons of liquid wastes per day, including overflow from a septic tank which received human wastes from employees at the plant, passed directly into the aerated stabilization pond in which the mean residence time was about 3.5 days. From the aerated pond the waste passed into a facultative pond in which it was retained another 3.5 days. The effluent from the second pond passed over an effluent weir and



FIG. 1. Schematic diagram of treatment facilities at a chicken processing plant. Sampling points are designated by the symbol \otimes . Direct connection between the two ponds was eliminated when settling facilities were added in 1971.

was discharged without further treatment to the receiving stream. The stream above the point of discharge consisted of a small trickle, the size of which was small when compared with the size of the stream below the point of discharge where it was comprised mainly of effluent from the plant.

After late 1971, liquid wastes passed through a flotation unit in which grease was removed prior to discharge to the aerated pond. Furthermore, partially treated wastes leaving the aerated pond were passed through a settling tank prior to entering the unaerated pond, and the pipeline joining the two ponds was plugged. Otherwise the plant operated as before.

At least one further source of wastes existed upstream from the treated discharge. During daily clean-up of the plant and its associated receiving area, drainage entered the stream at the point identified in Fig. 1 and perhaps other points as well.

Sampling procedures. Samples were obtained on 12 occasions between August 1970 and March 1973 in sterile Mason jars at points indicated in Fig. 1. In addition, samples were obtained on two occasions in July 1972 from the receiving stream at points 1, 3, and 5 km downstream from the point of discharge of the treated effluent. On 11 and 12 August 1970, samples were obtained at 5 sampling stations at approximately 6-h intervals over a 24-h period and were processed immediately. On all other occasions grab samples were returned on ice to the laboratory for processing.

Enumeration of indicator bacteria. Samples processed in the field or returned to the laboratory for processing were mixed in a Waring blender for 1 min to break up clumps of bacteria prior to dilution and inoculation of appropriate media for the enumeration of fecal coliforms and fecal streptococci. Dilutions were made in sterile phosphate buffer (pH 7.2) as described in reference 1.

Prior to May 1972, populations of fecal coliforms were estimated as most probable numbers (MPNs). Appropriate 10-fold dilutions of sample were inoculated into five replica tubes of sterile lauryl tryptose broth (Difco) which were incubated at 35 C for 24 h. The presence of fecal coliforms in tubes containing gas was confirmed by a test for gas production after incubation for 24 h at 44.5 C in EC medium (Difco). Most probable numbers were obtained from MPN tables (1). After May 1972, counts of fecal coliforms were determined on membrane filters incubated in triplicate at 44.5 C for 24 h on m-FC broth (Difco) as described by Geldreich et al. (20).

Counts of fecal streptococci were made on membrane filters. Appropriate dilutions of sample were filtered in triplicate through membrane filters (0.45 μ m mean pore size, Millipore Corp.) which were incubated for 48 h at 35 C on M-Enterococcus agar (BBL).

Identification and enumeration of salmonellae. Salmonellae were isolated from chicken processing wastes and their receiving waters after enrichment in tetrathionate enrichment broth (BBL) to which was added 10 ml of a 1:1,000 aqueous solution of brilliant green dye per liter (15). Grab samples either were inoculated directly into 5 replica volumes of tetrathionate broth or were filtered through membrane filters (0.45 μ m mean pore size, Millipore Corp.), which in turn were added to tetrathionate broth. The protocol was altered somewhat throughout the study in an effort to improve the recovery of salmonellae. In August 1970, inocula of 100, 10, 1, and 0.1 ml were employed. Inocula (100 ml and 10 ml) were added to 100- and 10-ml volumes of double-strength broth. The tetrathionate broth was incubated at 37 C for 24 and 48 h.

In the processing of subsequent samples, 100 ml of inocula and double-strength broth were not employed except with several samples of stream water obtained downstream from the point of discharge. Volumes of inocula were held to between 5 and 10% of the volume of enrichment medium (13). Furthermore, incubation was carried out at 41.5 C as recommended by Spino (40).

In the processing of samples of wastes after the beginning of 1972, most samples were inoculated directly into tetrathionate broth and also filtered through membrane filters, which in turn were added to 10-ml volumes of tetrathionate broth. Stream samples were not filtered, but rather were inoculated directly into tetrathionate broth since 100-ml volumes of stream water could not be filtered easily. MPNs obtained by filtering samples and adding the filters to tetrathionate broth were from four to eight times the MPNs obtained when samples were added directly to enrichment broth.

After incubation of the enrichment medium for 24 and 48 h, a loopful of broth was streaked on brillant green agar containing 0.08 g of sulfadiazine per liter of agar (BGS) (BBL). Streaked plates were incubated at 37 C. After 24 h of incubation at least two pink colonies were picked to slants of triple sugar iron (TSI) agar (BBL) and to slants of lysine iron agar (LIA, BBL), which in turn were incubated at 37 C for 24 h. Cultures which produced alkaline slants and acid butts, with or without H_2S , in TSI, and which produced an alkaline reaction throughout the LIA medium with or without signs of H_2S production were subjected to serological testing.

All cultures exhibiting characteristic reactions on TSI and LIA were subjected to slide agglutination tests employing polyvalent O and 15 O group antisera (Difco) reacting with the serotypes most frequently isolated in the United States (34). Of 365 isolates assignable to a specific O group, flagellar antigens were determined on 88. H broth (BBL) was inoculated from TSI agar slants and incubated at 37 C for 24 h. Cultures were killed with an equal volume of 0.6% formalized saline, and H antigens were determined employing Spicer-Edwards pooled antisera (Difco). Serotypes were confirmed employing single-factor antisera (Difco). The second phase was isolated in the semisolid medium of Edwards and Bruner (BBL) and tested in the same manner. Twenty cultures were sent to the Georgia Department of Public Health for identification and for confirmation.

MPNs of salmonellae were obtained employing MPN tables (1) or were approximated from the following formula:

$$MPN/ml = \frac{\text{no. of positive tubes}}{\sqrt{(\text{no. of ml in negative tubes})}} \times (\text{no. of ml in all tubes})$$

Enrichments were considered positive if they yielded isolates which could be assigned to a *Salmonella* O group. All such isolates, when their flagellar antigens were analyzed, were found to be typable as salmonellae.

Antibiotic susceptibility testing. Ninety-four Salmonella strains isolated during 1972 and 1973 were tested for their resistance to 14 antibiotics by the method of Bauer et al. (3) employing Sensi-Discs (BBL). Although the value of the resulting data is somewhat limited because of a delay between the times of isolation and testing, they do suggest changing patterns of resistance and support other data included in this paper.

RESULTS AND DISCUSSION

Studies of indicator bacteria through the treatment plant confirmed the earlier observation of reductions generally in excess of 90% (Table 1). By the addition of a flotation unit, the populations of indicator bacteria entering the pond system were reduced by a factor of approximately 10. The effect of the addition of a clarifier between the aerated and facultative ponds is not clear, however. The efficiency of removal of fecal coliforms was enhanced although no effect on the removal of fecal streptococci was apparent. Populations of fecal coliforms and fecal streptococci in the treated effluent were close to 10⁵ bacteria per 100 ml before the improvements to the treatment plant, and 10³ and 10⁴ bacteria per 100 ml, respectively, after the additions to the plant. Similar levels were demonstrated in the stream, and the effects of the discharge were detected for a distance of 5 km downstream from the plant (Table 2). High counts of indicator bacteria and salmonellae were determined in the stream above the point of discharge, at times exceeding counts in the stream below the discharge. These counts reflect the effects of drainage from the processing plant, which was examined on one occasion, exhibiting an MPN of 94 salmonellae per 100 ml. The volume of flow was small compared with that of the effluent, however.

The essential question relates to the significance to be assigned to the discharge of wastes containing large numbers of indicator bacteria. Salmonellae generally were demonstrated without difficulty. It should be stressed, however, that salmonellae ordinarily were demonstrated only in dilutions of the raw waste during the period August 1970 to January 1971. During the

| | | Prior to installation of flotation unit and clarifier | | | | | | | | | | | | | | | | | |
|---|---------------------------------------|---|-------------------|---------------------|--------------|--------------------------------------|------------------|--------------------|-------------------|-----------------------|----------------|----------------|----------------|-------------------|-------------------|--------------------|--------------|---------|--|
| | | Fecal coliforms | | | | | | Fecal streptococci | | | | | | Salmonellae | | | | | |
| Sampling | | MPN/100 ml (×10 ⁵) du | | | % duc | % re- luction (×10 ^s) | | | % re- duction | | MPN/ 100 ml | | | | % re- duction | | | | |
| location | Aug.ª | Oct. | Jan. ^b | Geometric mean | 1 Pond | 2 Ponds | Aug.ª | Oct. | Jan.° | Geometric mean | 1 Pond | 2 Ponds | Aug.ª | Oct. | Jan.* | Geometric mean | 1 Pond | 2 Ponds | |
| Raw waste Effluent from aer- ated pond Discharge to stream | 26 4.2 1.8 | 92 24 17 | 9.1 1.9 .28 | 27.9 5.8 2.05 | 79.2 64.6 | 92.6 | 38 8.0 .95 | | 10 1.6 .52 | 19.5 3.68 0.703 | 81.2 80.9 | 96.4 | 139 81 2 | 410 49 31 | 82 4.7 | 238 71.8 6.6 | 69.8 90.8 | 97.0 | |
| | | | | | | Afte | er insta | llatio | n of flo | tation u | nit an | d clar | fier | | | | | | |
| | | | Fecal | coliform | s | | | F | 'ecal st | reptococ | ci | | | | Sal | monellae | , | | |
| Sampling | MPN/100 ml (×104) % re- duction | | | | | MPN/100 ml (×10 ⁴) | | | | % re- duction | | MPN/ 100 ml | | | % re- duction | | | | |
| location | May ^c | Juned | June | Geometric mean | 1 Pond | 2 Ponds | Mar.* | Apr., May/ | June ^d | Geometric mean | 1 Pond | 2 Ponds | Mar.* | Apr. ^e | June ^a | Geometric mean | 1 Pond | 2 Ponds | |
| Raw waste from flota- tion unit Effluent | 36 1.0 | 40 .5 | .6 5 | 38.2 0.74 | 98.2 | 99.3 | 4 3.2 | 9.4 3.8 | 12.1 .33 | 7.7 1.6 | 79.2 | 84.4 | 2 | 27 | 13 | 8.9 | | | |
| from aer- ated pond Discharge to stream | .5 | .1 | 6 | 0.283 | 61.8 | | 2.0 | 3.2 | .27 | 1.2 | 25.0 | | 2 | 8 | 4.5 | 4.16 | 63.2 | | |

TABLE 1. Populations of fecal coliforms, fecal streptococci, and salmonellae in the pond system

^a Geometric mean of four samples taken over a 24-h period. Temperature range, 20.5 to 25.0 C. ^b Geometric mean of samples taken on two dates. Temperatures: raw waste, 12.5 C, pond effluents, 7.0 C. ^c Temperatures, 20 to 22 C. ^d Geometric mean of samples taken on two dates. Temperatures 22.5 to 25 C. ^c Temperatures, 15.5 to 17.5 C. ^f Geometric mean of samples taken on three dates. Temperatures 16 to 22 C. ^d Temperatures, 16 to 18 C. ^b Temperatures, 24 to 25 C.

later portion of the study, March 1972 to June 1972, salmonellae never were recovered from enrichments inoculated with raw wastes or with material removed from the raw wastes on membrane filters. In the early portion of the study, the removal of salmonellae (97%) was somewhat in excess of the removal of fecal coliforms and about equal to the removal of fecal streptococci. The populations demonstrated in the waste discharges were similar throughout the study and varied about means of 6.6 and 4.2 salmonellae per 100 ml in the early and late portions of the study, respectively.

The populations of salmonellae demonstrated in the treatment facilities appear low in view of studies of municipal waste treatment facilities reported by Cheng et al. (6), but were not inconsistent with populations reported by McCoy (33). MPNs of salmonellae demonstrated in the waters of the receiving stream confirm that salmonellae can be isolated at some distance from a source of pollution, and indicate that a poultry processing plant may indeed serve as a reservoir of salmonellae reaching receiving waters and exert an influence upon them for a distance of at least 5 km. The data included in Table 2 are consistent with those reported by Cheng et al. (6), Claudon et al. (8), and Cherry et al. (7).

A comparison has been made between MPNs

of salmonellae and of fecal coliforms in those samples on which both determinations were completed. The results presented in Table 3, although drawn from an admittedly small sample, suggest a surprising degree of consistency. When sample was inoculated directly into tetrathionate broth, the apparent number of salmonellae in relation to fecal coliforms was lower than when material filtered from the sample was added to the enrichment medium. Results obtained prior to mid-January 1970 indicated a population of about 5.6 salmonellae per 100,000 fecal coliforms. Individual ratios of salmonellae to fecal coliforms varied between 1.11 and 19.3. The mean ratio obtained after mid-January 1970 was 41.0 salmonellae per 100,000 fecal coliforms, and individual values lay within a fivefold range (14 to 74.1). The consistency of

nate broth from a single lot was used and the medium was unusually toxic to salmonellae, although the possibility of a shift in the populations of salmonellae in relation to fecal coliforms cannot be dismissed. No record remains of the lot numbers of media employed in this study to confirm the hypothesis, but media probably should be evaluated prior to use.

MPNs obtained for samples which were filtered, the filters being added subsequently to tetrathionate broth, were on the average five times as great as MPNs obtained on samples added directly to the enrichment medium, suggesting a ratio of 216 salmonellae per 100,000 fecal coliforms (or about 1 Salmonella per 500 fecal coliforms). Again, the range of individual values, less than 10-fold from 78 to 671, was narrow. The consistency of the overall results results obtained before and after mid-January suggests that, in an industrial waste of the kind 1970 suggests that, prior to that time, tetrathio- investigated during the present study (but not

TABLE 2. Populations of fecal coliforms, fecal streptococci, and salmonellae in drainage and the receiving stream

| | | Prior to installation of flotation unit | | | | | | | | | and clarifier | | | | | |
|--|------------------------|---|------------|---------------------------------|--------------------|--------------------------|--------------------|---|-------------------|---------------------------------|------------------|----------------|-----------------------|-------------------|---------------------------------------|--|
| | | Fee | cal co | oliforn | ns | | Fecal streptococci | | | | Salmonellae | | | | | |
| | (MPN/100 ml) (×10*) | | | | | (count/100 ml) (×104) | | | | (MPN/ 100 ml) | | | | | | |
| Sampling location | Aug." | Oct b | | Jan. ^b | Geometric | mean | Aug.ª | Jan.° | Geometric | mean | Aug.ª | | Oct.° | Jan. ⁶ | Geometric mean | |
| Drainage 50 m upstream from main discharge 300 m downstream from main discharge 1 km downstream from main discharge 3.5 km downstream from main discharge 5 km downstream from main discharge | 25 | 5 | 40 | 24 14 | 54 2 14 | 0 4 1 | 12.2 | 19. 2.3 | 23 | 15.3 2.33 | 94 11 | | 110 | 2 | 94 110 4.7 | |
| | | After installati | | | | | | llation of flotation unit and clarifier | | | | | | | | |
| | Fecal coliforms | | | rms | Fecal streptococci | | | | | | Salmonellae | | | | | |
| | | (MPN/100 ml) (×10 ⁴) | | | | (count/100 ml) (×10*) | | | | | (MPN/ 100 ml) | | | | | |
| Sampling location | May ^c | June" | Julye | Geometric mean | Mar.′ | Apr. ^s | May ^e | June ^a | July ¹ | Geometric mean | Mar.' | June 9/ | June 26 | July' | Geometric mean | |
| Drainage 50 m upstream from main discharge 300 m downstream from main discharge 1 km downstream from main discharge 3.5 km downstream from main discharge 5 km downstream from main discharge | .15 .7 .49 | .86 .47 .29 | .08 .03 | .36 .57 .38 .08 .03 | 1.6 | .98 3.8 3.1 | .5 .55 .47 | 8.8 3.15 3.6 | 1.3 .54 | 1.6 1.8 1.7 1.3 .54 | 93 | 49 13 17 | 7.8 <.018 <.018 | <1.8 | $19.5 \\ 13' \\ 8.5' \\ <.018 \\ 0.2$ | |

^a Median of three samples taken over a 24-h period, temperature 24.5 to 25 C. ^o Temperatures not recorded. ^d Temperatures, 20 to 23 C. "Temperatures, 20.5 to 21 C. ^c Temperatures, 19 to 22 C. 'Temperature, 17 C. ^a Geometric mean of samples taken on 2 days. Temperature, 19.5 to 24.5 C. 'Tempera-"Temperatures, 19 to 22 C. tures. 20.5 to 21 C. ¹Temperatures, 19.5 to 24.5 C. * Temperatures, 20 to 23 C. ' Median.

sewage, in which numbers of salmonellae may fluctuate in response to the changing incidence of acute infection in the community, [33]), a relationship may exist between the pathogens and indicators of fecal contamination, and this relationship can be employed in conjunction with MPNs of fecal coliforms to estimate numbers of the pathogen released to, and existing in, the environment. It should be stressed, however, that the actual ratio of salmonellae to fecal coliforms probably is very much higher than 1:500. Cherry et al. (7) reported that the number of positive enrichments is about 63% higher than the number yielding growth of salmonellae on the selective medium (BGS). The results support a rational basis for increasingly frequent qualitative demonstrations of salmonellae associated with increasing populations of fecal coliforms in samples reported by numerous workers (9, 17, 18, 24; D. J. Van Donsel and E. E. Geldreich, Bacteriol. Proc., 1970, p. 18).

During the present study 365 Salmonella strains were isolated and assigned to O groups. Of these, 88 selected strains were serotyped either in our laboratories or in the laboratories of the Georgia Department of Public Health. The distribution of serotypes examined is presented in Table 4. Although the distribution of serotypes presented is not representative of the

 TABLE 3. Relationship between most probable numbers of salmonellae and most probable numbers of fecal coliforms^a

| | Salmonellae per 100,000 fecal coliforms | | | | | | | | | | |
|--|---|--------------------|-------------------|--------------------|-------------------|--------------------|-------------------|--|--|--|--|
| Sampling location | 11-12 Aug. 1970 | 16 Oct. 1970 | 5 Jan. 1971 | 18 Jan. 1971 | 9 June 1972 | 26 June 1972 | 6 July 1972 | | | | |
| Raw waste (or waste after flotation unit) | 5.35 | 4.45 | | | | | | | | | |
| Effluent from aerated pond (or from clarifier) | 19.3 | 2.04 | | 27.9 | (34.2) | (250) | | | | | |
| Discharge to receiving stream | 1.11 | 3.93 | 5.45 | 47.9 | 46.9 | | | | | | |
| Stream upstream from main discharge | | | | | (671) | 14 (78) | | | | | |
| Stream 50 m downstream from main discharge | 4.4 | 2.04 | 7.5 | | (130) | | | | | | |
| Stream 1 km downstream from main discharge | | | | | 34.6 (131) | 31.74 | | | | | |
| Stream 3.5 km downstream from main discharge | | | | | | | | | | | |
| Stream 5 km downstream | | | | | | 74.1 | | | | | |
| Stream 5 km downstream | | | | | | (4.1 | | | | | |

^a Figures not in parentheses represent direct enrichments of water samples. Figures in parentheses represent enrichments from materials filtered from the samples on membrane filters.

| | No. of isolates serotyped | | | | | | | | | | |
|----------------------|---------------------------|-----------------|-------------------------|----------------|----------------|-----------------|--|--|--|--|--|
| Serotype | 11-12 Aug. 1970 | 16 Oct. 1970 | 22 Mar . 1972 | 9 June 1972 | 6 July 1972 | 12 Mar. 1973 | | | | | |
| Group B | | | | | | | | | | | |
| S. heidleberg | | 17 | 1 | | | 5 | | | | | |
| S. saint-paul | | 2 | | | | | | | | | |
| S. der by | | | | 3 | | | | | | | |
| Group C ₁ | | | | | | | | | | | |
| S. infantis | 3 | 7 | | | | | | | | | |
| S. thompson | | 1 | | | | | | | | | |
| Group C ₂ | | | | | | | | | | | |
| S. blockley | | | | 1 | | | | | | | |
| Group E ₁ | | | | | | | | | | | |
| S. anatum | | | | 2 | | | | | | | |
| S. bornum | | | | 2 | | | | | | | |
| Group E ₂ | | | | | | | | | | | |
| S. new brunswick | | | | | 1 | | | | | | |
| Group E ₃ | | | | | | | | | | | |
| S. thomasville | 1 | | | | | | | | | | |
| Group E4 | | 0 | | 0 | | | | | | | |
| S. senftenderg | | Z | | 2 | | | | | | | |
| Group G | 20 | 0 | | 1 | | | | | | | |
| S. cubana | 29 | 0 | | 1 | | | | | | | |

TABLE 4. Serotypes of selected Salmonella strains isolated

actual distribution of serotypes in the environment of the chicken processing plant, several features of the table are notable, particularly when considered in conjunction with Table 5. Certain serotypes such as S. enteriditis ser. typhimurium which often figure prominently on lists of frequently isolated salmonellae are absent from Table 4. On the other hand, S. enteriditis ser. cubana, which seldom figures prominently on such lists, is conspicuous in Table 4. However, frequent isolates from poultry processing plants differ from plant to plant and from day to day at given plants (14, 23, 30, 31, 36, 37, 42).

A striking feature of populations of salmonellae in the external environment of the chicken processing plant was their variable composition. This is demonstrated best in Table 5. Strains in group B often, but not always, predominated. Other groups comprised a significant component of the population for short periods, as group C₁ on 9 June and 26 June 1972 (note presence of group B isolates on 9 June and their absence on 26 June), group E₄ isolates on 9 June, or group E₂ isolates on 6 July 1972. These data suggest that serotypes occurring in the during the second half of 1970 and January 1971 environment of a chicken processing plant may (Fig. 2) suggests an unusual condition in the not persist for such periods as does S. enteriditis plant and perhaps the industry or a segment of ser. paratyphi B in sewers (26). Other sero- the industry as a whole. Among isolates from groups of interest may comprise a significant human sources throughout the United States component of the population for relatively long received at the Center for Disease Control, this periods and then subside. Group G isolates, serotype comprises less than 1%. Among nonhuprobably all S. enteriditis ser. cubana, were man isolates the serotype comprises slightly prominent among strains isolated between Au- more than 1% of the isolates, animal feeds being gust 1970 and January 1971. The continual shift the most significant source (5). The very high in predominant serotypes undoubtedly reflects, proportion of isolates of S. enteriditis ser. cuover the longer run, shifts which may occur bana in the chicken processing wastes, immediwithin a processing plant from hour to hour as ately preceding and coincident with a sharp rise the source of poultry changes. Morris and Wells in the number of isolates from human sources (37), for instance, demonstrated shifts in the received at the Center for Disease Control from Salmonella serotypes present in a chicken proc- most of the United States with the primary

Similar shifts would be anticipated at the plant described in the present study which processed chickens that, although hatched on the premises, were raised on many farms off the site. Morris et al. in an earlier study (36) reported that, whereas the serotypes associated with a flock of breeder hens remained constant through the growing period, these changed at maturity, perhaps in relation to salmonellae present in feeds.

Of note also are shifts in patterns of antibiotic resistance illustrated in Table 6. To some extent these are associated with particular serogroups. But the important point to be made is that there exists further evidence of shifts in the history of salmonellae occurring in the environment of the chicken processing plant and of the transient nature of such populations.

Salmonellae in the external environment of a poultry processing plant may be significant both as an indication of unusual conditions in the processing plant and as a reservoir of salmonellae which may affect animal and human residents of that environment. The predominant position of S. enteriditis. ser cubana essing plant as new flocks were processed. exception of a number of western states, Alaska,

| | | | | | N | o. of isola | tes | | <u>.</u> | | |
|---------------------------------------|-----------------------|--------------------|-------------------|--------------------|--------------------|-------------------|-------------------|--------------------|-------------------|--------------------|--------------------|
| O Group | 11–12 Aug. 1970 | 16 Oct. 1970 | 5 Jan. 1971 | 18 Jan. 1972 | 22 Mar. 1972 | 6 Apr. 1972 | 9 June 1972 | 26 June 1972 | 6 July 1972 | 16 Jan. 1973 | 12 Mar. 1973 |
| B C ₁ C ₂ | 12 7 1 | 65 14 | | 2 | 10 3 | 15 1 | 20 28 2 | 24 | | 6 3 | 12 3 |
| E1 E2 E3 E4 G | 9 1 36 | 2 27 | 3 | 7 | 4 | 5 | 9 12 5 | 1 | 5 | 1 | 6 |

TABLE 5. Distribution of O groups among Salmonella isolates

| | No. of isolates | | | | | | | | | | | |
|-----------------------------------|-----------------|----------------|----------------|-----------------|----------------|----------------|-----------------|--|--|--|--|--|
| Resistance pattern ^{abc} | 22 Mar. 1972 | 6 Apr. 1972 | 9 June 1972 | 26 June 1972 | 6 July 1972 | 9 July 1972 | 12 Mar. 1973 | | | | | |
| Te, S, CL, PB, NA, C, CF | | | 1 | | | | | | | | | |
| Te, S, CL, PB, NA, SSS | | | 1 | | | | | | | | | |
| Te, CL, PB, C Te, S, CL, PB | | 1 | 1 | | | | 2 | | | | | |
| Te, CL, PB | 2 | 7 | | 1 | | | 1 | | | | | |
| Te, S, K Te, S, CF | | | | | | | 1 | | | | | |
| Te, N, K S, N, K | | | | 8 | | 1 | | | | | | |
| CL, NA, AM | | | 1 | | | | | | | | | |
| Te, S | 1 | | 17 | 1 | 1 | | 1 | | | | | |
| Te, N Te, NA | | | 1 | | | | | | | | | |
| S, K CF, AM | | | 1 | | | | 2 | | | | | |
| Te | | | 1 | | | | | | | | | |
| NA S | | | | | 1 | 6 | | | | | | |
| SSS | | | | | 1 | | | | | | | |
| Sensitive | 14 | 11 | 42 | 10 | 2 | 3 | 26 | | | | | |

| I ABLE 6. Drug resistance patterns of Salmonetia isolate | TAI | BLE | 6. | Drug | resistance | patterns o | f | Sai | lmonel | la | isol | ates | 3 |
|---|-----|-----|----|------|------------|------------|---|-----|--------|----|------|------|---|
|---|-----|-----|----|------|------------|------------|---|-----|--------|----|------|------|---|

^a Abbreviations: Te, tetracycline; S, streptomycin; CL, colistin; PB, polymyxin-B; NA, nalidixic acid; C, chloramphenicol; CF, cephalothin; SSS, triple sulfa; N, neomycin; K, kanamycin; AM, ampicillin. No strains were resistant to gentamicin.

⁶ Results recorded as sensitive or resistant. Inhibition zones lying within the resistant and intermediate zones (Bauer et al. [3]) recorded as resistant.

^c Testing with chlortetracycline and furazolidone on isolates obtained on 12 March 1973 indicated that five of seven resistant strains were resistant to both antibiotics. Later testing with selected strains isolated earlier suggested that of the sensitive strains about $\frac{1}{3}$ (16.67%) were resistant to chlortetracycline and $\frac{1}{3}$ (0.33%) were resistant to furazolidone, and of resistant strains, all were resistant to chlortetracycline and $\frac{1}{3}$ (25.7%) were resistant to furazolidone.



FIG. 2. Isolation of S. enteriditis ser. cubana from human sources reported by the National Center for Disease Control (solid line) and proportion of Salmonella isolates from the environment of a chicken processing plant identified as S. enteriditis ser. cubana (bars). Numbers in parentheses represent total number of isolates examined.

and Hawaii, is highly suggestive of a causative relation between human salmonellosis and the poultry industry, perhaps related to its association with feeds or breeder hens. Similar relationships have been reported by Harvey and Price (27) and by McConnell (32). No follow-up was undertaken during the present study, so no inference may be drawn concerning the role of the plant or the industry as a whole.

The potential significance of a poultry processing plant as a reservoir of salmonellae which may affect man or animals in the nearby community is suggested by their consistent occurrence in the receiving stream and the accessibility of the stream to children and to animals which might carry the bacteria into the home. Support for this concern can be found in the literature (4, 10, 22, 24, 29, 39, 41).

In the meantime, for the reasons discussed, disinfection of treated wastes from poultry processing plants is to be recommended as suggested by Edel et al. (10) and by Will et al. (44). This should be undertaken with care, however, since the efficacy of chlorination of such wastes is highly unpredictable (Hoadley, unpublished data).

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LITERATURE CITED

- American Public Health Association. 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Inc., New York.
- Aserkoff, B., S. A. Schroeder, and P. S. Brachman. 1970. Salmonellosis in the United States—a five year review. Amer. J. Epidemiol. 92:13-24.
- Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Tech. Bull. Reg. Med. Technol. 35:493-496.
- Bicknell, R. R. 1970. Salmonella aberdeen infection in cattle associated with human sewage. J. Hyg. 70:121-126.

- Center for Disease Control. 1965-73. Salmonella Surveillance Reports. U.S. Department of Health, Education, and Welfare, Atlanta, Ga.
- Cheng, C. M., W. C. Boyle, and J. M. Goepfert. 1971. Rapid quantitative method for *Salmonella* detection in polluted waters. Appl. Microbiol. 21:662-667.
- Cherry, W. B., J. B. Hanks, B. M. Thomason, A. M. Murlin, J. W. Biddle, and J. M. Croom. 1972. Salmonellae as an index of pollution of surface waters. Appl. Microbiol. 24:334-340.
- Claudon, D. G., D. I. Thompson, E. H. Christenson, G. W. Lawton, and E. C. Dick. 1971. Prolonged Salmonella contamination of a recreational lake by runoff waters. Appl. Microbiol. 21:875-877.
- Dutka, B. J., and J. B. Bell. 1973. Isolation of salmonellae from moderately polluted waters. J. Water Pollut. Contr. Fed. 45:316-324.
- Edel, W., P. A. M. Guinée, M. Van Schothorst, and E. H. Kampelmacher. 1972. The role of effluents in the spread of salmonellae. Zentralbl. Bakertiol. Parasitenk. Infektionsk. Hyg. I. Abt. Orig. 221:547-549.
- Edwards, P. R. 1958. Salmonellosis: observations on incidence and control. Ann. N.Y. Acad. Sci. 70:598-613.
- Edwards, P. R., D. W. Bruner, and A. B. Moran. 1948. The genus Salmonella: its occurrence and distribution in the United States. Ky. Agric. Exp. Sta. Bull. no. 525.
- Edwards, P. R., and M. M. Galton. 1967. Salmonellosis. Advan. Vet. Sci. 11:1-63.
- Galton, M. M., D. C. Mackel, A. L. Lewis, W. C. Haire, and A. V. Hardy. 1955. Salmonellosis in poultry and poultry processing plants in Florida. Amer. J. Vet. Res. 16:132-137.
- Galton, M. M., G. K. Morris, and W. T. Martin. 1968. Salmonellae in foods and feeds: review of isolation methods and recommended procedures. U. S. Public Health Service, Communicable Disease Center, Atlanta, Ga.
- Galton, M. M., J. H. Steele, and K. W. Newell. 1964. Epidemiology of salmonellosis in the United States, p. 421-444. In World problem of salmonellosis. W. Junk, Publishers, The Hague.
- Gallagher, T. P., and D. F. Spino. 1968. The significance of numbers of coliform bacteria as an indicator of enteric pathogens. Water Res. 2:169-175.
- Geldreich, E. E. 1970. Applying bacteriological parameters to recreational water quality. J. Amer. Water Works Ass. 62:113-120.
- Geldreich, E. E., H. F. Clark, and C. B. Huff. 1964. A study of pollution indicators in a waste stabilization pond. J. Water Pollut. Contr. Fed. 36:1372-1379.
- Geldreich, E. E., H. F. Clark, C. B. Huff, and L. C. Best. 1965. Fecal-coliform-organism medium for the membrane filter technique. J. Amer. Water Works Ass. 57:208-214.
- Gerichter, C. B., J. Yofe, and I Sechter. 1972. Salmonellosis in Israel, 1963, 1967. Israel J. Med. Sci. 8:1921-1931.
- Gibson, E. S. 1965. Reviews of the progress of dairy science. E. Diseases of dairy cattle. Salmonella infection in cattle. J. Dairy Res. 32:97-134.
- Glezen, W. P., M. P. Hines, M. Kerbaugh, M. E. Green, and J. Koomen, Jr. 1966. Salmonella in two poultry processing plants. J. Amer. Vet. Med. Ass. 148:550-552.
- 24. Grunnet, K., and B. Brest Nielsen. 1969. Salmonella types isolated from the Gulf of Aarhus compared with types from infected human beings, animals, and feed products in Denmark. Appl. Microbiol. 18:985-990.

- Guinée, P. A. M., E. H. Kampelmacher, and J. J. Valkenburg. 1967. Salmonella isolations in the Netherlands, 1961-1965. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. I. Orig. 204:476-485.
- Harvey, R. W. S., and W. P. Phillips. 1955. Survival of Salmonella paratyphi B in severs. Its significance in the investigation of paratyphoid outbreaks. Lancet ii:137-139.
- Harvey, R. W. S., and T. H. Price. 1970. Sewer and drain swabbing as a means of investigating salmonellosis. J. Hyg. 68:611-624.
- 28. Haryana Agricultural University. 1972. Studies on Salmonella infections in domestic animals and birds to provide information on the natural reservoirs and mode of transmission of infections from animal to animal and animal to man. Haryana Agricultural University, Department of Bacteriology and Hygiene, College of Veterinary Medicine, Hissar, Haryana, India.
- Hughes, L. E., E. A. Gibson, H. E. Roberts, E. T. Davies, G. Davies, and W. J. Sojka. 1971. Bovine salmonellosis in England and Wales. Brit. Vet. J. 127:225-238.
- Kaufmann, A. F., and J. C. Feeley. 1968. Culture survey of Salmonella at a broiler-raising plant. U.S. Publ. Health Rep. 83:417-422.
- Kraft, D. J., C. Olechowski-Gerhardt, L. Berkowitz, and M. S. Finstein 1969. Salmonella in wastes produced at commerical poultry farms. Appl. Microbiol. 18:703-707.
- McConnell, G. W. 1973. The epidemiology of an emergent serotype: Salmonella agona. Epidemic Intelligence Service Conference, 9-13 April. Epidemiology Program, Center for Disease Control, Atlanta, Ga.
- McCoy, J. H. 1971. Sewage pollution of natural waters, p. 35-50. *In* G. Sykes and F. A. Skinner (ed.), Microbial aspects of pollution. Academic Press Inc., New York.
- Martin, W. J., and W. H. Ewing. 1969. Prevalence of serotypes of Salmonella. Appl. Microbiol. 17:111-117.
- Moran, A. B. 1961. Occurrence and distribution of Salmonella in animals in the United States, p. 441-448 In Proc. U.S. Livestock Sanit. Ass.
- Morris, G. K., B. L. McMurray, M. M. Galton, and J. G. Wells. 1969. A study of the dissemination of salmonellosis in a commercial broiler chicken operation. Amer. J. Vet. Res. 30:1413-1421.
- Morris, G. K., and J. G. Wells, 1970. Salmonella contamination in a poultry-processing plant. Appl. Microbiol. 19:795-799.
- Pietkiewicz, K., and Z. Buczowski. 1969. Salmonellosis in man in Poland, 1957–66. U. S. Publ. Health Rep. 84:712–720.
- Schaal, E. 1963. Über eine durch Bachwasser verursachte Salmonella-Enzootie in einem Rinderbestand, Deutsche Tieräztliche. Wochenschrift 70:267-268.
- Spino, D. F. 1966. Elevated-temperature technique for the isolation of *Salmonella* from streams. Appl. Microbiol. 14:591-596.
- Watt, J., and T. DeCapito. 1950. The frequency and distribution of Salmonella types isolated from man and animals in Hidalgo, Texas. Amer. J. Hyg. 51:343-352.
- Wilder, A. N., and R. A. MacCready. 1966. Isolation of Salmonella from poultry. Poultry products and poultry processing plants in Massachusetts. N. Engl. J. Med. 274:1453-1460.
- Will, L. A., S. L. Diesch, and B. S. Pomeroy. 1973 Survival of Salmonella typhimurium in animal manure in a model oxidation ditch. Amer. J. Publ. Health 63:322-326.