Isolation of *Salmonella enteritidis* Serotype Agona from Eutrophic Regions of a Freshwater Lake

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*Salmonella enteritidis* serotype Agona, which is associated with animal feeds containing fish meal, was isolated consistently from waters influenced by sewage containing poultry processing wastes.

In recent years *Salmonella* sp. have been isolated from various fresh and marine waters contaminated by urban sewage. Spino (12) obtained *Salmonella* as far as 70 miles (ca. 112.6 km) downstream from a source of treated urban sewage effluents. Claudon et al. (4) found 12 serotypes of *Salmonella*, mainly serotypes Anatum, Typhimurium, Thompson, and Derby, in Lake Mendota, a major recreational lake near Madison, Wisc. The presence of the salmonellae was related to contamination by agricultural and urban runoff waters. Several investigators have examined surface waters in North Georgia, a major poultry center. Hendricks (8) recovered serotypes Anatum, Indiana, and Meleagridis from surface waters and sediments of the North Oconee River. Schneider et al. (11) isolated 18 different serotypes, including Anatum, Havana, Heidelberg, and Infantis, from the Chattahoochee River and its feeder streams. From rivers of North Georgia, Cherry et al. (2) obtained salmonellae from 96 of 165 specimens. Serotypes San-diego, Thompson, Montevideo, Give, and Mississipi were among the more common of 29 different serotypes obtained. Serotypes Cubana and Heidelberg were the predominant salmonellae isolated from the environment around a Georgia chicken processing plant (9).

Lake Sidney Lanier, located about 45 miles (ca. 72.5 km) north of Atlanta, Ga., is a 38,000-acre recreational lake which also serves as a water source for metropolitan Atlanta. Portions of this young, man-made reservoir which receive sewage effluents, including wastes from poultry processing plants, are eutrophic and support annual blooms of blue-green algae and algophorous amoebae (5). The established association of salmonellae with poultry products suggested that the lake waters be examined for salmonellae to aid in further detecting the influences of sewage on the eutrophic processes.

In the summer of 1972, Moore swabs (10) were positioned at four stations in Flat Creek and its embayment area, a region receiving urban sewage effluents: at one station in the center of the lake, at one station on Two Mile Creek, a stream draining rural residences on the opposite side of the lake from Flat Creek; and at two stations in Balus Creek, which received textile mill effluents. Physical and chemical characteristics of the lake and a map of the collection area are presented elsewhere (5). Two Mile Creek and the center of the lake contained relatively clean water characterized by a biochemical oxygen demand of less than 1 mg/liter and a fecal coliform number of less than 1/100 ml. The Moore swabs (two per station) were left submerged for 1 week during July, August, and September. After collection, the swabs were placed in sterile plastic bags and iced for transportation. With the exception of the use of the API system for presumptive identification of salmonellae isolates, the methods of isolation and identification were identical to those of Claudon et al. (4). Briefly, one swab from each station was introduced into tetrathionate enrichment broth while the other was incubated in selenite brilliant green sulfa broth. After 24 and 48 h of incubation at 41.5 C, the broths were streaked onto brilliant green, Salmonella-Shigella, and bismuth sulfite agars. Selected isolates were inoculated to triple sugar iron agar (Difco) and lysine iron agar. Isolates typical of *Salmonella* were identified by the API system and serotyped according to the methods of Edwards and Ewing (6).

Twenty-one different serotypes were isolated. Fifteen were obtained from the Flat Creek area, with 12 of these found exclusively in this region (Table 1). Serotype Agona was the only *Salmo- nella* isolated from all stations in Flat Creek on all collections. Its only other occurrence was in a single sample from the embayment station. Six
different serotypes were obtained at the embayment station. Aside from serotype Agona, only serotypes Albany and Minnesota were common to both the embayment and Flat Creek. No salmonellae were obtained from the center of the lake. Three serotypes, Weslaco and Derby obtained from Two Mile Creek and Eimsbuettel from Balus Creek, were isolated exclusively from their respective areas. The limited distribution of certain serotypes may reflect differential host-free survival indexes of the various serotypes.

Densities of fecal coliforms were determined for waters collected from all stations on each collection date according to the membrane filter method of Geldreich (7). In general the higher densities of fecal coliforms (up to 5,000/ml) were found at those sites yielding the greatest variety of serotypes. One exception was at a station located about 100 yards (ca. 91.4 m) below a sewage treatment plant on Flat Creek. Salmonellae were isolated, but no fecal coliforms were obtained. No fecal coliforms were obtained outside Flat Creek, its embayment area, and the Balus Creek site.

The common presence of serotype Agona in the Flat Creek area is significant. In previous studies of Georgia waters, a single isolation of this serotype is noted (2). Agona, rare before 1969, is of increasing epidemiological significance. In 1973 serotype Agona was the seventh most common human isolate of *Salmonella* submitted to the Center for Disease Control for identification (1). Clark et al. (3) associated serotype Agona with fish meal (an ingredient of poultry feeds). Hence, the worldwide emergence of Agona as a significant agent of salmonellosis is currently linked to the need and broad use of fish meal as a protein supplement in animal feeds. Its occurrence in Flat Creek is due presumptively to its presence in poultry wastes. It can be expected that this occurrence of serotype Agona in the feeder streams of a recreational lake will ultimately result in a broader base for its epidemiology.

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**LITERATURE CITED**

1. Center for Disease Control. 1974. Salmonella surveillance, first, second and third quarters, 1973. Report no. 118, U.S. Department of Health, Education and Welfare, Public Health Service, Atlanta.

2. Cherry, W. B., J. B. Hanks, B. M. Thomason, A. M. Murlin, J. W. Biddle, and J. M. Croom. 1972. *Salmonella* as an index of pollution of surface waters. Appl. Microbiol. 23:334–340.

3. Clark, G. M., A. F. Kaufmann, and E. J. Ganga ros. 1973. Epidemiology of an international outbreak of *Salmonella agona*. Lancet 2:490–493.

4. 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.

5. Cook, W. L., D. G. Ahearn, D. J. Reinhardt, and R. J. Reiber. 1974. Blooms of an algalphonous amoeba associated with *Anabaena* in a fresh water lake. Water Air Soil Pollut. 3:71–80.

6. Edwards, P. R., and W. H. Ewing. 1972. Identification of Enterobacteriaceae. 3rd ed. Burgess Publishing Co., Minneapolis.

7. Geldreich, E. E., H. F. Clark, D. B. Huff, and L. C. Best. 1965. Fecal-coliform-organism medium for the membrane filter technique. J. Amer. Water Works Ass. 57:208–214.

8. Hendricks, C. W. 1971. Increased recovery rate of salmonellae from stream bottom sediments versus surface waters. Appl. Microbiol. 21:379–380.

9. Hoadley, A. W., W. M. Kemp, A. C. Firmin, G. T. Smith, 

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**Table 1. Occurrence of serotypes of *Salmonella enteritidis* in Lake Sidney Lanier, Georgia**

| Serotype     | Area of sampling |
|--------------|-----------------|
|              | Flat Creek      | Flat Creek embayment | Center of lake | Balus and Two Mile Creeks |
| Agona<sup>b</sup> | 9/9<sup>h</sup> | 1/3 | 0/2 | 0/8 |
| Alachua<sup>c</sup> | 1/9 | 0/3 | 0/2 | 0/8 |
| Albany<sup>b</sup> | 1/9 | 1/3 | 0/2 | 0/8 |
| Anatum<sup>a, d, r, i</sup> | 2/9 | 0/3 | 0/2 | 0/8 |
| Blockley<sup>c</sup> | 0/9 | 1/3 | 0/2 | 0/8 |
| Braenderup<sup>b</sup> | 0/9 | 1/3 | 0/2 | 0/8 |
| Derby<sup>c, r</sup> | 0/9 | 1/3 | 0/2 | 1/8 |
| Eimsbuettel<sup>b</sup> | 0/9 | 0/3 | 0/2 | 1/8 |
| Havana<sup>a</sup> | 2/9 | 0/3 | 0/2 | 0/8 |
| Heidelberg<sup>a, r</sup> | 4/9 | 0/3 | 0/2 | 0/8 |
| Infantis<sup>a, c</sup> | 2/9 | 0/3 | 0/2 | 0/8 |
| Kottbus | 1/9 | 0/3 | 0/2 | 0/8 |
| Minnesota<sup>a</sup> | 1/9 | 1/3 | 0/2 | 0/8 |
| Montevideo<sup>a, i</sup> | 0/9 | 1/3 | 0/2 | 0/8 |
| New Brunswick<sup>c</sup> | 2/9 | 0/3 | 0/2 | 0/8 |
| Oranienburg<sup>b</sup> | 1/9 | 0/3 | 0/2 | 0/8 |
| Saint-Paul<sup>c</sup> | 1/9 | 0/3 | 0/2 | 0/8 |
| Sieburg | 1/9 | 0/3 | 0/2 | 0/8 |
| Simsburg | 1/9 | 0/3 | 0/2 | 0/8 |
| Typhimurium<sup>a, d</sup> | 1/9 | 0/3 | 0/2 | 0/8 |
| Weslaco | 0/9 | 0/3 | 0/2 | 1/8 |

Total serotypes per area: 15/7/0/3

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<sup>a</sup> One set of subs was lost.
<sup>b</sup> Found in Georgia waters by Cherry et al. (2).
<sup>c</sup> Number of positive samples/number of attempts.
<sup>d</sup> Found in Georgia waters by Hendricks (8).
<sup>e</sup> Found in Georgia waters by Hoadley (9).
<sup>f</sup> Found in Georgia waters by Schneider et al. (11).
and P. Schelhorn. 1974. Salmonellae in the environment around a chicken processing plant. Appl. Microbiol. 27:848-857.

10. Moore, B. 1948. The detection of paratyphoid carriers in towns by means of sewage examination. Mon. Bull. Min. Health (London) 7:241-248.

11. Schneider, R. F., D. W. Hill, M. R. Weldon, and R. E. Gentry. 1972. Preimpoundment study of West Point Lake, Georgia. Environmental Protection Agency technical study no. JS-03-71-208-001.2, Southeastern Water Laboratory, Athens, Ga.

12. Spino, D. F. 1966. Elevated-temperature technique for the isolation of Salmonella from streams. Appl. Microbiol. 14:591-596.