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Prolonged *Salmonella* Contamination of a Recreational Lake by Runoff Waters[1]

DAVID G. CLAUDON,[2] DONALD I. THOMPSON, ELEANOR H. CHRISTENSON, GERALD W. LAWTON, AND ELLIOT C. DICK[3]

Department of Preventive Medicine and the Wisconsin State Laboratory of Hygiene, University of Wisconsin Medical School, Madison, Wisconsin 53706

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In the summer and fall of 1968, various *Salmonella* serotypes were isolated from a portion of Lake Mendota, the major recreational lake for Madison, Wis. The apparent sources of these organisms were a residential storm sewer and a University of Wisconsin Experimental Farms' washwater drain. Salmonellae were isolated with regularity from a swimming beach located approximately 0.5 mile (0.8 km) from these sources.

In recent years recovery of *Salmonella* sp. from relatively unpolluted waters has been greatly facilitated by a revival of the time-honored selective effect of incubation at elevated temperatures (8). By using this technique, several investigators have reported that sewage-contaminated waters may contain salmonellae. Spino (11) obtained *Salmonella* isolates from as great a distance as 73 miles (117.5 km) downstream from the treated effluent of Moorehead, Minn. Grunnet and B. Nielsen (7) isolated many *Salmonella* sp. from a Danish bay receiving effluent from a city of 100,000 inhabitants, and Brezenski and Russomanno (2) obtained salmonellae from several Staten Island beaches and from shellfish in New York Harbor. There seems little doubt, therefore, that waters containing dilute sewage may contain these fecal pathogens and are, accordingly, a potential danger to bathers. There have been some suggestions that urban and agricultural runoff also may be a danger to health. Evans et al. (3) isolated *S. thompson* from a Cincinnati, Ohio, storm sewer, and Miner et al. (9) found *S. infantis* in several samples of cattle feedlot runoff.

The present study was undertaken to assess the contribution of agricultural or urban runoff, or both, to a portion of a northern lake which includes a swimming beach. It will be shown that such runoff, even though greatly diluted, can be a regular contributor of *Salmonella* to recreational water.

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[2] Third year medical student and Public Health Service student trainee. D. Claudon is the recipient of the University of Wisconsin Medical School's Theobald Smith Award for his part in this research.

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MATERIALS AND METHODS

Lake Mendota and its tributaries. Although the lake was sampled at various locations on its periphery, major emphasis for study was confined to the University Creek-University Bay-Willow Beach area (Fig. 1). Lake Mendota is a large basin-shaped lake with a maximum depth of 82 ft (25 m), a diameter of 4 to 5 miles (6.4 to 8.0 km), and a surface area of 9,730 acres. The lake is fed by numerous springs and small streams and by the Yahara River flowing in from the north. Because of its size and of the presence of the city of Madison on its southern shores, the lake is used heavily for recreation, including swimming at the several beaches around its periphery. Some treated sewage may reach the northern shores of the lake from the small communities located on the influent rivers and streams and from a few residences, but there are no influent streams on the southern shores other than University Creek. The south-shore residences are nearly all serviced by the Madison Metropolitan Sewage system.

University Creek is a man-made bayou approximately 0.3 mile (0.5 km) long, and its flow rate (usually very slow) into University Bay depends chiefly upon height of the lake water, winds, and the flow of storm-sewer effluent from Madison. A city of Madison storm sewer (station 1M) is located at the "headwaters" of the creek and, after rainfall, appears to provide considerable impetus to water flow. Also located on the creek is a drainage outlet for University of Wisconsin (UW) Farms washwater (station 4M). This drain does not receive heavy runoff from the animal quarters located approximately 0.5 mile (0.8 km) from the creek. All animal excrement is removed manually or by machine before the barns and pens ware washed, and a collecting and settling basin precedes the outlet to the creek.

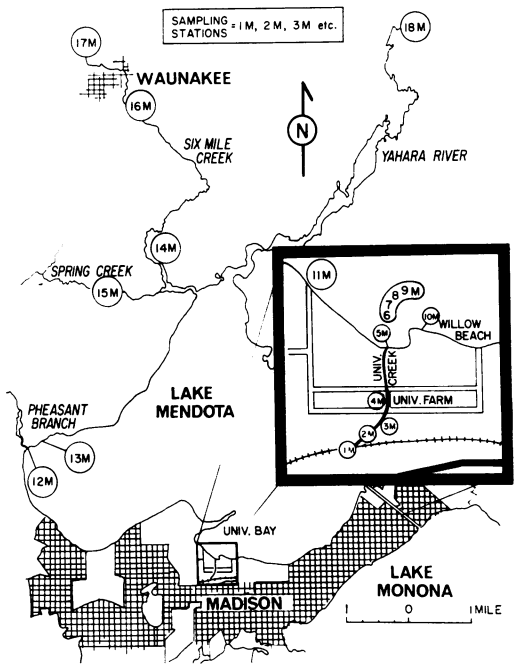

FIG. 1. *University Creek, University Bay, and Lake Mendota.*

University Creek flows into University Bay, and within the bay and approximately 0.2 miles (0.3 km) from the mouth of University Creek is Willow Beach (station 10M), a city of Madison facility heavily used by UW student families.

Sampling technique. Specimens for *Salmonella* culture were obtained chiefly by Moore swab (10), but at stations 3M, 5M, 6M, and 7M, 250- or 500-ml grab samples were taken as well. The Moore swabs consisted of 12 gauze layers [9 by 9 inch (22.9 by 22.9 cm)] cut into five strips connected at one end by an inch (0.6 cm) of uncut material. The uncut ends were tied together forming a swab which appeared like a gauze feather-duster. The swabs were secured in pairs 1 ft (0.3 m) below the water's surface by attachment to an anchored float. The swabs were retrieved after 4 to 7 days, placed by pairs in individual plastic bags, and dropped into an ice chest. When the swabs were picked up, a 250-ml water sample usually was taken for fecal coliform analysis.

Bacteriology. All media were Difco dehydrated. The swab pairs were separated, and one was transferred to 260 ml of tetrathionate enrichment broth and the other to an equal volume of SBG-sulfa enrichment broth. The broths were prepared sufficiently concentrated to allow 40-ml dilution by the swab. The inoculated broths were rapidly warmed to 41 C in a water bath at 44.5 C and then transferred to a dry air incubator at 41.5 C. At 24 and 48 hr of incubation, an inoculum from each enrichment was streaked onto Brilliant Green, SS, and bismuth sulfite agars. After incubation suspicious colonies were picked to Triple Sugar Iron (TSI) Agar, lysine-iron agar, and urea agar slants and incubated at 35 to 37 C. At 24 hr, H_2S-positive, lysine decarboxylase-positive, urea-negative organisms fermenting only glucose on the TSI slant were transferred to phenylalanine agar to further rule out the Providence group organisms. Phenylalanine-negative cultures were then subcultured into various substrates to accomplish partial speciation. "Sugars" used were lactose, glucose, sucrose, maltose, mannitol, and dulcitol broths. Other biochemical tests utilized were indol, KCN, tartrate, mucate, and malonate broths, and Simmons' citrate agar. Final identification was made with specific H and O agglutinins (sera were obtained from Difco and the Center for Disease Control).

Water samples were tested for fecal coliforms by the membrane-filter, elevated-temperature technique of Geldreich et al. (6).

The six water samples tested for salmonellae were put through membrane filters, and the filter was placed in tetrathionate broth. These broths were then treated as were those for Moore swabs.

RESULTS

Salmonellae were recovered consistently from University Creek, from the lake area into which

TABLE 1. *Salmonella incidence for the University Creek-University Bay sampling stations, 1968*

Sampling station	Duration of observation (dates)	Proportion of *Salmonella*-positive samples
1M	7/29–11/3	2/8
2M	10/19–12/1	2/3
3M	12/1	0/1
4M	8/9–10/19	2/5
5M	7/1–9/11	4/5
6M	8/9–11/3	3/5
7M	8/9–11/3	4/4
8M	9/4–9/28	3/5
9M	9/4–9/28	3/3
10M	7/29–10/19	4/8
11M	7/29–9/28	0/6
Totals		27/53

TABLE 2. *Salmonella serotypes isolated from the University Creek-University Bay area, 1968*

Salmonella serotypes (including *Arizona*)	No. of samples positive
S. anatum	6
S. typhimurium	6
S. thompson	7
S. derby	5
S. saint paul	4
S. java	4
S. kentucky	2
S. barielly	2
S. binza	1
S. blockley	1
S. livingstone	1
S. tennessee	1
Arizona hinshawii	1

the creek drains, and from the nearby swimming beach (Fig. 1, Table 1). In University Creek, 4 of the 12 samples taken at the three stations above the UW Farms drain were positive (presumably from the storm sewer at the "head-waters"), and 2 of the 5 samples obtained at the UW Farms drain were positive. However, salmonellae were most regularly recovered at the mouth of the creek, only one of five specimens being negative. Judging from recovery frequencies, there was little immediate dilution of the salmonellae as sampling moved northeast toward station 10M located approximately 0.2 miles (0.3 km) from the mouth of University Creek. At this latter station four consecutive samples taken between 29 July and 12 August yielded *Salmonella*. Station 11M located approximately 0.4 miles (0.6 km) northwest of the creek mouth was, however, *Salmonella*-free for all of its six samples.

Including the antigenically related *Arizona hinshawii*, 13 *Salmonella* serotypes were isolated from the University Creek and Bay area (Table 2). *S. anatum*, *S. typhimurium*, and *S. thompson* accounted for nearly one-half of the isolates. In general, the same serotypes were found throughout the chain of sampling stations; for example, *S. anatum* was found at stations 2M, 4M to 7M, and 10M, and *S. thompson* was obtained from stations 1M, 2M, 5M, and 7M to 10M. Since station 10M is a municipal swimming beach, its salmonellae are of special public health interest; *S. anatum*, *S. thompson*, *S. java*, *S. derby*, *S. kentucky*, and *S. saint-paul* were found.

The stations (12M to 18M) on the north side of the lake were sampled only once each, for a total of seven specimens. Four of the seven were positive, but only one positive northside station, 12M, was actually in the lake; it yielded *S. infantis* and *S. tuindorp*.

Water sampling by either Moore swab or grab sample was successful in obtaining salmonellae. All 6 grab samples yielded at least 1 serotype, and, of 58 Moore swabs, 29 were *Salmonella*-positive.

Salmonellae were not usually obtained in the absence of an elevated fecal coliform count; however, *S. thompson* was obtained from station 7M (3 Nov.), and *S. derby*, *S. kentucky*, *S. thompson*, and *S. typhimurium* were isolated from station 9M (11 and 28 Sept.) at times when fecal coliforms were not detected.

DISCUSSION

Although there is now abundant evidence that *Salmonella* may be found in recreational waters contaminated with sanitary sewage (2, 7, 11), to our knowledge, there is no direct evidence for contamination of recreational waters by urban and agricultural runoff as has been described in the present study. However, some studies have given clear indications that such land runoff waters may contain fecal pathogens and therefore be a hazard to recreational waters. Evans et al. (3) isolated *S. thompson* in a concentration of 4,500 per 100 ml (5) from a storm sewer serving downtown Cincinnati; Miner et al. (9) reported 26 *Salmonella* isolates from cattle feedlot runoff in Kansas; and Bidwell and Kelly (1) found many salmonellae in runoff from Long Island duck pens.

Although the sources of contamination in the present study are somewhat analagous to those cited above, they would seem quantitatively much less significant. The residential Madison storm sewer is presumably much smaller than that serving downtown Cincinnati, and, similarly, the settled washwater of the UW Farms does not seem as potent a source of *Salmonella* as direct feedlot runoff. Nevertheless, these effluents were sufficient to distribute salmonellae to areas about 0.5 mile (0.8 km) from the points of discharge. We feel that our findings provide a clear practical example for the warnings of Geldreich and his colleagues (4, 5) of the danger to health which may result from urban and agricultural runoff pollution of recreational waters.

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