APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1977, p. 791-801 Vol. 33, No. 4

Printed in U.S.A.

Salmonella in Surface Waters of Central New York State

N. C. DONDERO,* CONSTANCE T. THOMAS,[1] MOHAN KHARE, J. F. TIMONEY, AND G. M. FUKUI

Laboratory of Microbiology, College of Agriculture and Life Sciences, Cornell University, and Department of Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, and Wallace Laboratories, Cranbury, New Jersey 08512*

Received for publication 12 October 1976

Six tributary streams and southern Cayuga Lake in central New York state were sampled for the presence of *Salmonella* on swabs immersed for 4 days. Of a total of 322 swabs, 39% yielded salmonellae. Swabs were cultured in tetrathionate enrichment at 41.5°C. Isolations were made from brilliant green agar. Salmonellae were isolated from many sites on the streams and from some lake sites. Twenty-five serotypes (11 somatic antigen groups) and a distinct biotype of *S. typhimurium* (H_2S negative) were found. Most frequent isolates, in order of decreasing occurrence, were *S. typhimurium*, *S. thompson*, *S. oranienburg*, and *S. enteritidis*. Several uncommon isolates also appeared. When tested for mouse infectivity, the isolates generally showed little or no virulence. The incidence of clinical salmonellosis among humans was low in the area and the variety of serotypes had not been noted among cattle. The presence of *Salmonella* in waters ranging in classification from potable to agricultural and industrial indicated the existence of a low level and undefined reservoirs of the bacteria in the region.

Salmonellosis is more commonly associated with contaminated foods and feeds than with waters; nevertheless, salmonellae have frequently been found in effluents from sewage treatment plants, in industrial wastes, and in streams that receive a variety of sewages and industrial wastes (17, 19).

During a water quality survey begun in 1970 on Taughannock Creek in New York State, salmonellae were found in a small tributary stream on which a cattle feedlot was located; such a result was not unprecedented (23). Sampling was then extended to other streams of the southern part of the Cayuga Lake drainage region to gain some perspective on the occurrence of *Salmonella* in the surface waters of the region.

A variety of *Salmonella* serotypes, some uncommon, was isolated from both urban and rural locations. These isolates possessed apparently low or negligible virulence. The waters from which the salmonellae were isolated ranged in quality, by official New York State classification, from "potable" to "agricultural and industrial." Although *Salmonella* isolations apart from the hosts have been reported repeatedly, virulence tests on such isolates have been rare. This report deals with the distribution of *Salmonella* in surface waters and the possible significance of the findings.

[1] Present address: 128 Muriel St., Ithaca, NY 14850.

MATERIALS AND METHODS

Stream locations and sampling sites. The streams studied included several important tributaries to the southern part of Cayuga Lake. Cayuga, which is one of the Finger Lakes, is a large, deep lake located in central New York State. The characteristics of the lake have been described (15, 36). Taughannock Creek drains approximately 174 km^2 in active agriculture (33%), residential use (0.5%), and former agricultural land (16%) (5).

The streams, sampling stations, and related features are shown in Fig. 1 and 2. Four small, unsewered communities are located on the upper branches of the stream system. The stream passes through a park and discharges at the lake shore about 0.7 km from a bathing beach. At the time of the study, a small feedlot (1,800 head of cattle; no longer in operation) was located on both banks of an upper secondary tributary which joined a larger primary tributary of Taughannock Creek, Reynoldsville Creek, at a point 16 km from the lake. Sampling station no. 1 was just downstream of the feedlot and station no. 2 was just upstream. Station no. 3, on Reynoldsville Creek upstream of the confluence of the feedlot tributary, was selected for testing waters close by but not subject to contamination by the feedlot. The travel time of a slug of water from the feedlot to the lake has been estimated for the wet, spring period to be in the range of 30 to 60 h, with extremes of less than 3 h to about 3 days. During the dry, midsummer months the travel time was 80 to 90 h or longer (T. Doane, M.S. thesis, Cornell University, Ithaca, N.Y., 1971).

The other streams drained uplands of similar nature, but their lower reaches and mouths were in

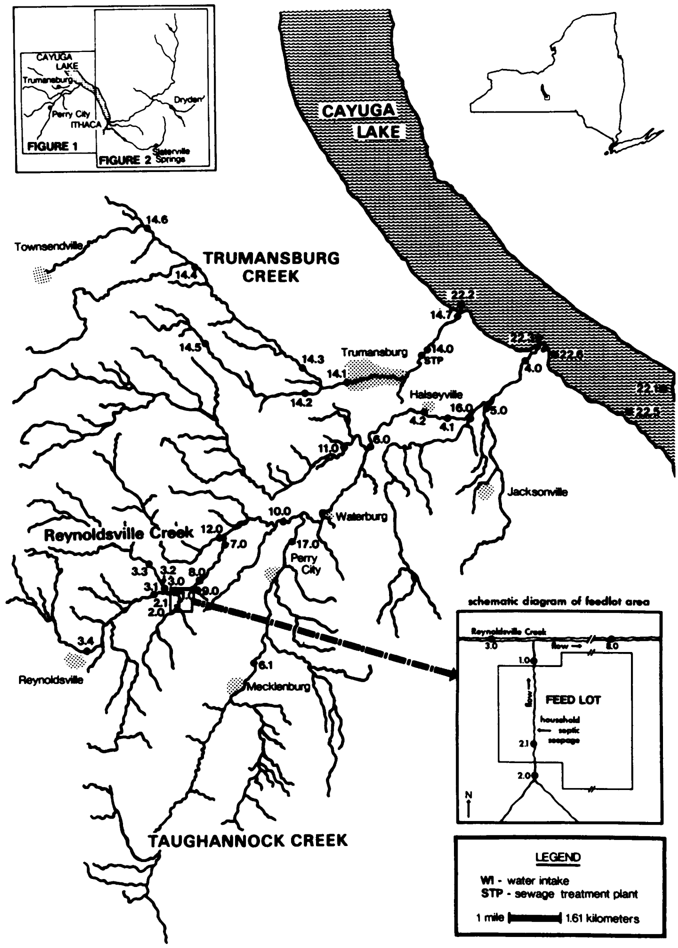

FIG. 1. *Taughannock Creek and Trumansburg Creek stream systems. Sampling stations are shown by numerals. Stippled areas show approximate locations of clusters of habitations.*

urban or residential areas. Fall Creek drains 332 km². It receives treated sewage from the village of Dryden, 24.2 km from the mouth. A public water supply intake is situated 5.5 km from the mouth. On this stream system are several small communities and the city of Ithaca, which surrounds the mouth at the lake. Sixmile Creek drains 128 km², passing through three small communities before traversing Ithaca; water for the city of Ithaca is taken 3.4 km upstream from the confluence with Inlet Creek. Cascadilla Creek was sampled at one station in Ithaca only. Renwick Brook was sampled in the town of Ithaca. Trumansburg Creek drains 35 km² (mostly agricultural land) and flows through the village of Trumansburg (2,000 population). Downstream of the village (1.8 km from the lake) an extended aeration plant (a type of secondary treatment) discharges the treated sewage from the village.

On exploration it was seen that some of the upper reaches of all the streams except Renwick Brook could be exposed, particularly in the spring of the year, to drainage or runoff from grazing areas, dairy farms, a few poultry operations, and fields spread with animal manures.

In spring and autumn, migrating wild ducks and geese come to the fields and the waters of the area. Gulls and other water and shore birds frequent the areas near the lake throughout the year.

Sampling and microbiological methods. Sterile swabs (25) were placed in the streams for 4-day periods. On recovery, the swabs were chilled in sterile plastic pouches for return to the laboratory within 4 h. Entire swabs were incubated in flasks of tetrathionate broth at 41.5°C for 24 h and then subcultured on brilliant green agar (32). When salmonella-like colonies appeared, five or six from a plate were tested in triple sugar iron agar and lysine iron agar and were subjected to cultural tests and serological typing (O and Spicer-Edwards).

At lake stations, swabs were anchored to lie on the sediment. On recovery of the swabs, bottom deposits for separate culturing were taken with an

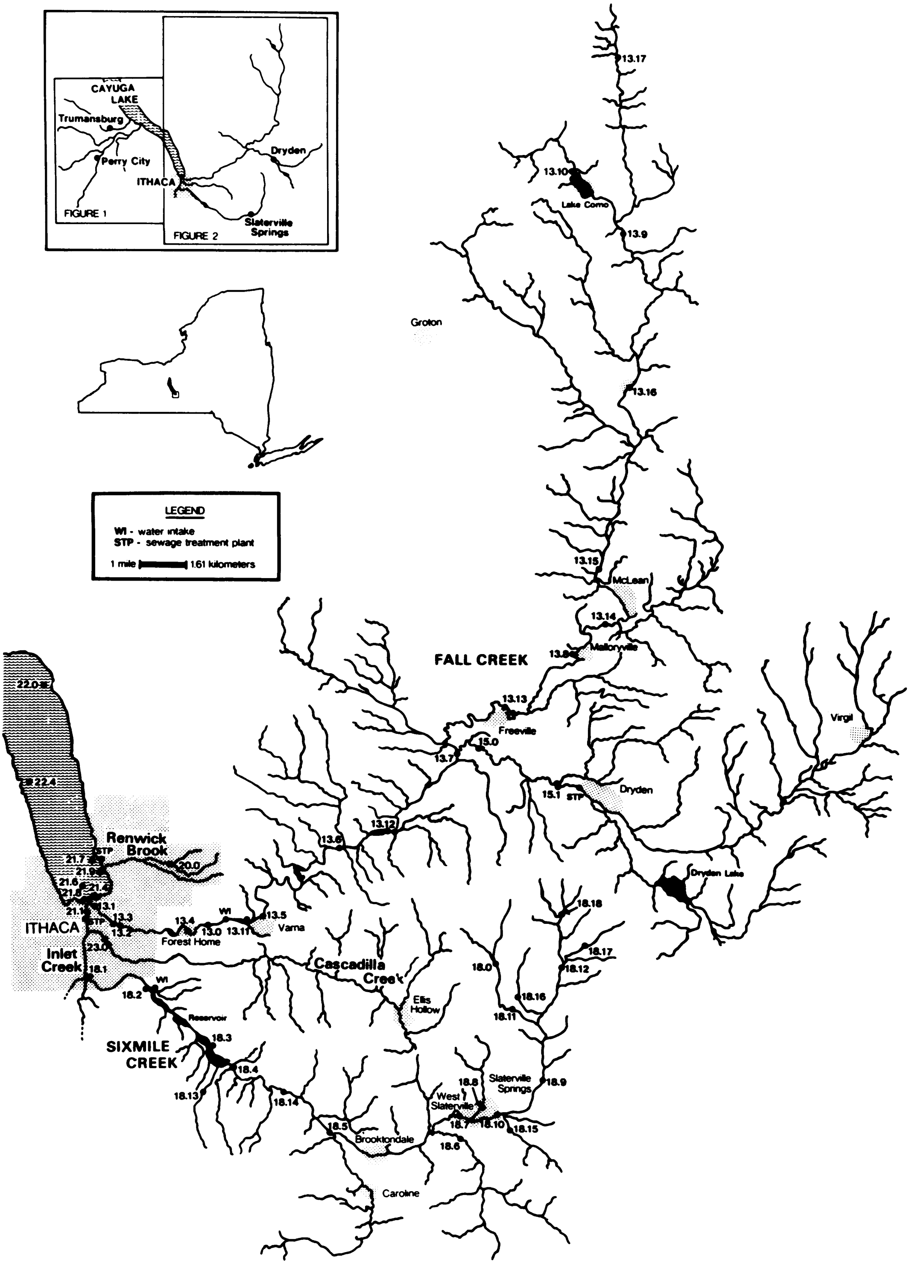

FIG. 2. *Stream systems of Sixmile, Cascadilla, and Fall Creeks, and Renwick Brook.*

Ekman dredge disinfected by immersion in alcohol. Surface film samples were taken at lake stations by applying a sterilized square of paper towel (about 37 cm^2) to the water with sterile forceps. Lake samples were cultured in the same manner as stream samples. With one exception lake samples were taken near shore in water 0.6 to 4.6 m deep. The offshore station was at a channel marker 0.3 km from shore.

Several simple, quantitative checks for *Salmonella* were made by centrifuging measured portions of grab samples of water. The pellets were cultured with tetrathionate broth and brilliant green agar.

Determinations for coliforms, standard plate counts, and chemical analyses were performed according to standard methods (1). Grab samples were taken at weekly intervals on Taughannock Creek throughout the year. The weekly sampling dates of these determinations did not coincide in time or necessarily in location with those of the swabs. The results, however, represent the chemical and microbiological conditions during the period of study. Similar determinations were performed twice on Sixmile Creek, but the results, being similar, are not presented. The chemical and microbiological studies on the effects of the feedlot on Taughannock Creek are reported in detail elsewhere (18).

The numbers of samples and the periods when they were collected were as follows: Taughannock Creek, 15 times, 2 February to 24 February 1970, 18 May to 2 November 1970, and 23 March to 10 May 1971; Sixmile Creek, twice, 25 May and 24 July 1971; Trumansburg Creek, 7 times, 25 August to 21 July 1971; Fall Creek, 6 times, 11 August 1970 to 6 July 1971; Cascadilla Creek, once, 25 October 1971; Cayuga Lake, 3 times, 2 September to 11 November 1970; Renwick Brook, once, 6 August 1971.

Fresh fecal specimens from deer were individually collected from the ground in late winter into sterile plastic pouches and were cultured by the same methods used for the swabs.

Virulence tests were performed by intraperitoneal injection and by oral gavage to white mice. Groups of 20 male Swiss-Webster mice weighing approximately 20 g were injected intraperitoneally with graded doses of culture suspension in nutrient broth. After 18 to 24 h at 37°C, cells growing on nutrient agar were washed off the agar with nutrient broth and adjusted to appropriate concentrations by optical density. The actual dose was determined from triplicate plate counts of the suspension. In tests for enhancement of infectivity by iron, 5 mg of ferric ammonium citrate per mouse was also injected intraperitoneally when cells were administered. The lethal dose (LD_{80}) was the number of viable cells killing 80% of the mice in 7 days.

Salmonella serotyping was performed by the usual methods (8).

RESULTS

The location and number of isolations of *Salmonella* from surface waters are shown in Fig. 1 and 2 and Tables 1 and 2, respectively. The high totals from Taughannock Creek are partly the result of the more intensive testing at that location, since the investigation originated relative to the effects of the feedlot on water quality and was only later extended to the other streams. Swabs from just below the feedlot were positive at a high frequency (86%); however, the high rate of positive swabs (46%) at station 3, which was not influenced by the feedlot, and at stations upstream of station 3 prompted investigation of 5 other nearby streams and of 13 locations in Cayuga Lake. Of a total of 332 swabs, 39% yielded salmonellae. Only *S. typhimurium* was isolated just below the feedlot (station 1). This serotype was by far the most frequently isolated (60%) of the 28 distinguishable types from all stations. It was isolated from the lake and all of the streams except Renwick Brook and Trumansburg Creek. *S. enteritidis* and *S. livingstone* were isolated from both Sixmile and Taughannock Creeks, and *S. thompson* was isolated from Taughannock and Fall Creeks and from Cayuga Lake. The remainder were isolated from various points on the several waters.

For each stream about twice as many positive samples (57%) occurred when high stream levels were recorded after snow melt or rain as during dry weather flow (24%). There were high water levels on 6 of the 14 sampling days on Taughannock Creek.

In the quantitative checks only *S. typhimurium* was found, occurring only twice at levels of one or more cells per 100 ml of sample. Both instances were days of heavy runoff due to snow melt and rain, and the samples were from immediately below the feedlot. Salmonellae were not detected by this method at other stations when most-probable-number (MPN) levels of fecal coliforms and fecal streptococci were 10^4 cells per 100 ml.

When the MPN of indicator bacteria (fecal coliforms) was estimated, there was evidence of fecal contamination, usualy 10^2 to 10^3 per 100 ml. Fecal streptococci were often more numerous than fecal coliforms; however, when *S. agona* and *S. heidelberg* were isolated, all of the coliforms were the fecal type (1.1×10^4 per 100 ml) and were approximately fivefold the fecal streptococcus value. Station 18.1, in a sewered area near the confluence of Sixmile and Inlet Creeks, was subject to urban street drainage.

The samples from Cayuga Lake were obtained at the southern end near the mouths of streams. After heavy rains or snow melt, these waters became turbid. Only two of the stream-mouth samples were positive: Taughannock and Fall Creeks, both streams from which salmonellae were commonly isolated. At three lake stations, subsamples of about 25 ml of

TABLE 1. *Locations and frequency of isolation of salmonellae from surface waters*

Station	No. of samples		No. of sero- or biotypes
	Positive	Total	
Taughannock Creek			
1	12	14	2
2	0	13	0
2.1	3	7	1
3	6	13	6
3.1	5	11	3
3.2	1	4	1
3.3	1	9	1
3.4	0	3	0
4	7	12	6
4.1	4	8	2
4.2	3	8	2
5	5	13	5
6	6	13	5
6.1	1	8	1
7	8	13	2
8	10	13	4
9	0	1	0
10	5	9	2
11	1	9	1
12	4	10	1
16	1	3	1
17	0	3	0
Fall Creek			
13.0	1	5	1
13.1	2	3	4
13.2	0	2	0
13.3	4	4	4
13.4	1	3	1
13.5	2	3	2
13.6	0	3	0
13.7	2	3	1
13.8	0	3	0
13.9	0	3	0
13.10	0	1	0
13.11	1	2	1
13.12	0	2	0
13.13	0	2	0
13.14	0	2	0
13.15	0	2	0
13.16	0	2	0
13.17	0	1	0
15	1	1	1
15.1	1	1	1
Sixmile Creek			
18.0	1	2	1
18.1	1	2	2
18.2	1	2	1
18.3	0	2	0
18.4	2	2	1
18.5	1	2	1
18.6	0	2	0
18.7	1	2	1
18.8	1	2	1
18.9	1	2	1
18.10	0	2	0
18.11	1	2	1
18.12	2	2	2
18.13	0	2	0
18.14	2	2	1
18.15	1	2	2
18.16	1	2	1
18.17	1	2	2
18.18	0	2	0
21.1	0	2	0
Trumansburg Creek			
14.0	2	8	2
14.1	4	8	3
14.2	0	1	0
14.3	0	1	0
14.4	0	1	0
14.5	0	1	0
14.6	0	1	0
14.7	0	1	0
Cascadilla Creek			
23.0	1	1	2
Renwick Brook			
20	1	1	1
Cayuga Lake			
21.4	1	1	1
21.6	1	1	1
21.7	2	2	1
21.8	0	1	0
21.9	0	1	0
22.0	0	1	0
22.1	0	1	0
22.2	0	1	0
22.3	1	1	1
22.4	0	1	0
22.5	0	1	0
22.6	0	1	0

dredged benthal material were positive when swabs from the same locations were negative. Since the lake stations yielded isolates that differed from station to station or were negative, and all surface film samples were negative, there was no evidence of *Salmonella* being carried between these stations by us. The offshore station was positive for *S. thompson*, and the dredge samples taken on two occasions near the outfall of a sewage treatment plant discharging chlorinated, primary effluent were positive once for *S. saint-paul* and once for *S. typhimurium*. By this it is not implied that the sewage treatment plants were necessarily the sources of the salmonellae found nearby, although treatment plants have been implicated by other workers (11, 17). In the case of the lakeside plant, other possible sources in the

TABLE 2. *Distribution of Salmonella serotypes in surface waters*

Salmonella serotype	Location[a]
agona	Sm 18.1
anatum	Fl 13.3
cubana	Tb 14.0
denver	Sm 18.17
derby	Tg 4, 5
drypool	Rn 20
enteritidis	Tg 6, 3, 3.1, 4.2; Sm 18.12
eppendorf	Tg 3
heidelberg	Sm 18.1
holcomb	Tb 14.0, 14.1
infantis	Fl 13.1
livingstone	Tg 5 (2), 4; Sm 18.0
manhattan	Fl 13.1, 13.3, 13.5
newport	Cs 23.0
oranienburg	Tg 3, 4, 5, 6
C_1 (not identified)	Tg 5
oslo	Sm 18.17
paratyphi B	Sm 18.15
poona	Tb 14.1
redlands	Sm 18.16, 18.2
rubislaw	Tg 3 (2), 3.1, 3.2, 8, 4
siegburg	Tb 14.1 (2)
saint-paul	CL 21.7
tennessee	Sm 18.11
thomasville	Cs 23.0
thompson	Tg 3, 4, 6, 6.1; Fl 13.1, 13.3; CL 21.4
typhimurium	Tg, 16 stations (64); Fl, 8 stations (9); Sm, 8 stations (10); Cs, 23.0; CL 21.7 (2)
typhimuirum (H_2S negative)	Tg 6, 7, 8, 10

[a] Sm, Sixmile; Fl, Fall; Tb, Trumansburg; Tg, Taughannock; and Cs, Cascadilla Creeks. Rn, Renwick Brook. CL, Cayuga Lake. Numbers in parentheses following station numbers indicate the number of separate isolations when there was more than one.

vicinity, such as dwellings, water fowl, and stream discharges, could not be excluded. Near the sewage treatment plant on Trumansburg Creek, *S. holcomb* was found once downstream, but this unusual type was also present well upstream toward the other side of the village. *S. poona* and *S. siegburg* were found upstream but not below the plant. *S. typhimurium* was not isolated from Trumansburg Creek.

On one occasion, 18 to 20 white-tailed deer (*Odocoileus virginianus*) were encountered near Taughannock Creek. Seventeen freshly voided fecal samples from the herd were negative for *Salmonella*.

Salmonellae were never found upstream of the cattle feedlot (station 2). The uppermost detected entry of *S. typhimurium* was where overland drainage from watering trough overflow entered the stream (station 2.1). Further downstream, cattle numbering several hundred at times were penned on the steep banks and frequently permitted to enter the stream. Manure, soil, and stained liquid runoff intermittently entered the stream from the pens, especially during wet periods. At an intermediate point, septic seepage from a household associated with the feedlot drained down the slope. A swab from this seepage yielded *S. typhimurium*.

Many other stations not affected by the feedlot yielded a variety of *Salmonella* serotypes in addition to *S. typhimurium*, some of them repeatedly but more frequently not. At station 3 on Reynoldsville Creek, *Salmonella* serotypes *derby, enteritidis, eppendorf, livingstone, oranienburg, thompson, typhimurium,* and *rubislaw* were found. On one day during high water, an H_2S-negative biotype of *S. typhimurium* was found at stations about 8 km apart (stations 8 and 6) and at two intermediate stations, but was not detected over an equal distance further downstream to the lake. A relatively short tributary on Taughannock Creek yielded five different serotypes at station 5.

On Fall Creek, immediately above, below, and within New York potable waters, *S. typhimurium, S. thompson,* and *S. anatum* were present.

Data (2.5-year averages) from chemical and microbiological analyses of Taughannock water flowing from the feedlot to the lake suggest the probable fate of the salmonellae in the water. The densities of the different types of bacteria appeared to subside in the same way as the inanimate soluble and particulate chemical substances (Fig. 3 and 4). Subsequent to the initial dilution by Reynoldsville Creek between stations 1 and 8, both the bacteria and the chemical entities associated with the soil particulate matter (ammonia, nitrogen, and phosphates) decreased downstream in similar fashions or remained at low levels. The solubles (nitrates and chlorides) gradually increased somewhat downstream, probably because of the influx of ground water.

The large deviations of the actual weekly bacterial counts from the smoothed trends shown by the 2.5-year means did not permit conclusions about the "aftergrowth" or death of bacteria coming from any particular source; however, results showed that the concentrations of fecal organisms became progressively lower, on the average, downstream from the feedlot. At times of high stream velocities, greater concentrations occurred in the lower

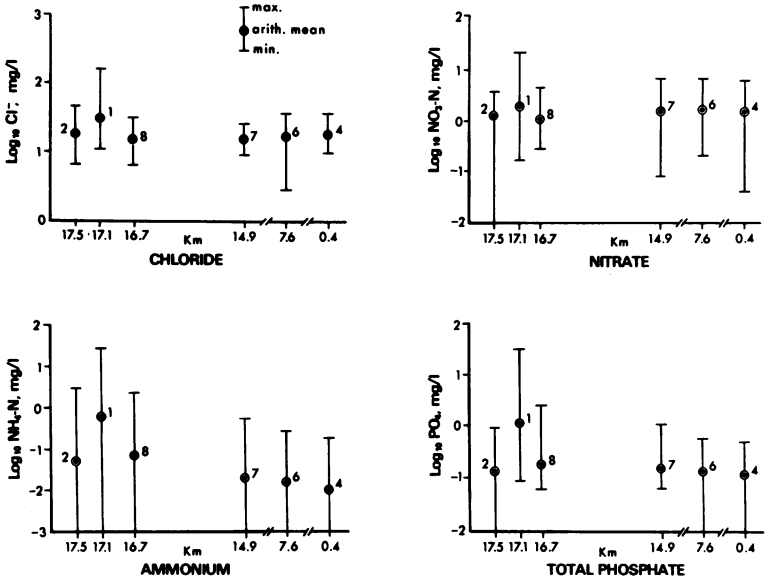

FIG. 3. *Concentrations of chemical substances along the course of Taughannock Creek from just upstream of the cattle feedlot (station 2) to the mouth of the stream at Cayuga Lake (station 4). The direction of flow is from left to right. Kilometers (Km) on the abscissa are distances from Cayuga Lake. Data points were obtained from 74 to 91 observations obtained over 2.5 years, during the entire year. Chlorides and nitrates represent soluble materials; ammonia and total phosphate represent chemicals associated with particulates. Numerals next to mean values are sampling station designations. Between stations 1 and 8 there was a relatively large influx of water from a major branch of the stream. Minimal ranges extending to the abscissa represent 0 concentration. Data from reference 17.*

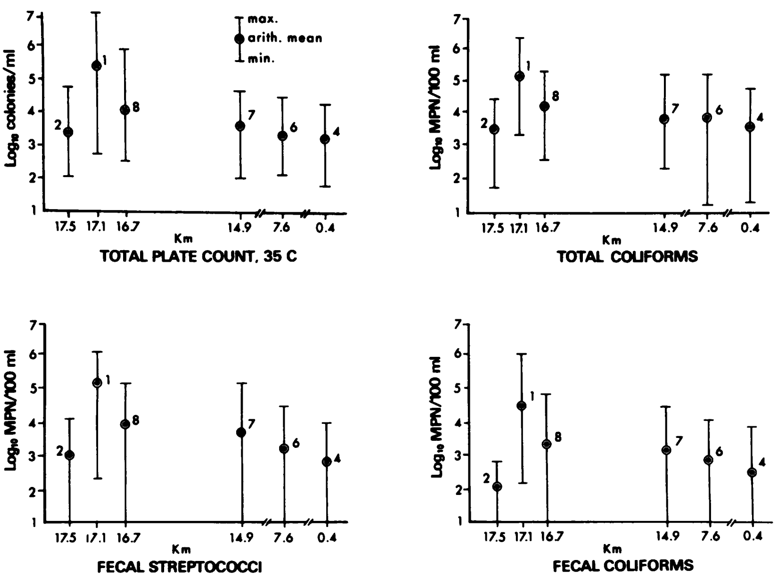

FIG. 4. *Concentrations of bacteria along Taughannock Creek. Explanation of the graphs as for Fig. 3. Data from reference 17.*

reaches. There is little reason to believe that *Salmonella* would behave in a quantitatively different fashion from the other gram-negative enteric organisms.

Soil runoff and disturbance of the sediments by high velocities were undoubtedly factors in the transport, frequency of isolation, and possibly survival of the salmonellae (14, 37). During immersion, the swabs became heavily coated with particulate matter transported by the water.

Isolations of several serotypes from individual samples have been reported previously (39). In this earlier investigation 18 samples contained at least two sero- or biotypes. These included the less commonly occurring *Salmonella* serotypes *oslo, eppendorf, rubislaw,* and *agona*.

The virulence of 144 *Salmonella* isolates was low or essentially nonexistent as judged by animal inoculation. The numbers of viable cells required to cause the death by intraperitoneal injection of 80% of the mice in 7 days ranged from approximately 10^6 (3% of cultures tested) to 10^8 per mouse. One culture of *S. typhimurium* killed at about 2×10^5 cells. Among the cultures which were fatal at 10^6 cells were *S. agona*, *S. enteritidis*, a few *S. typhimurium* isolates, and *S. saint-paul*. However, an isolate of *Citrobacter* was also lethal at the 10^6 level. None of the cultures caused gastroenteritis or was lethal by oral gavage at the highest dose of 2×10^8 viable cells. All of the serotypes were tested except *S. drypool*. Isolates of paracolon and an unidentified nonsalmonella from the surface waters had the same low levels of virulence. In contrast, virulent strains from other sources killed mice at doses ranging from 10 to 100 cells intraperitoneally (G. Fukui, unpublished data). Five of the *Salmonella* isolates were tested with iron for enhancement of virulence. When ferric ammonium citrate was injected with the cells, the LD_{80} was reduced by 99%.

DISCUSSION

Frequent isolations of salmonellae from the surface waters of an area give rise to questions as to the origins of the organisms, their survival or persistence, and their relevance to the public health.

In this report, the cattle feedlot was the only definitely identified source and yielded only *S. typhimurium*. Apart from the urban and smaller residential areas, there were no other obvious or definitely identified contaminating sources. The lower reaches of Fall, Sixmile, and Cascadilla Creeks were exposed to storm drainage of the city of Ithaca and to runoff from housing areas on the urban fringes. Urban drainage can be heavily contaminated (38) and has been known to contain *Salmonella* (6). In view of this, the villages and hamlets on some of the upper tributaries deserved closer scrutiny. Salmonellae were also found in areas not subject to such drainage, however. Rural dwellings scattered throughout the watersheds could have been point sources, particularly since the soils of the area are poorly absorptive for septic effluents, or as foci for dissemination by accessory means. Other possibilities must also be included. Both domestic (10, 12) and feral animals (3, 28) have been found to harbor *Salmonella* at times; however, the animals may have become infected by contact with the environment of humans (10). Gulls have been known to excrete *Salmonella* (2). *S. gallinarum* was not found in these studies, nor was there a notable incidence of other animal-adapted serotypes. The variety of serotypes recovered was not known to exist among the cattle of the region (D. W. Bruner, personal communication). In the fresh feces of the deer herd, no salmonellae were found. Deer often feed in fields of fodder crops, in pastures, or in fields where manure has been spread. It was not possible in this study to test animals or animal feces systematically.

The densities of fecal streptococci and fecal coliforms in Taughannock Creek were such as to indicate the constant presence of some fecal contamination, with substantial levels occurring at times (Fig. 4). The densities of *Salmonella* were estimated to have been of the same order of magnitude as found by Smith and Twedt in tributaries to Lake Erie near Detroit, Mich. (31).

Because of the geographical distribution of the isolates, our results indicate that multiple sources existed. The variety of serotypes was also consistent with multiple sources.

There have been surveys to determine the occurrence of *Salmonella* in streams and lakes. Some have covered long reaches of streams (e.g., 7, 9, 31, 32), placing emphasis, for the most part, on large pollution sources as contributors of *Salmonella*. In contrast, this study dealt with short streams and closely spaced sampling sites. Except for the urban areas and the feedlot, there were no conspicuous or definite point sources of fecal contamination. Since similar conditions exist on larger streams, the apparent transport of viable *Salmonella* over long distances may be confounded by myriad intermediate introductions, especially when such common serotypes as *S. typhimurium* are present. Although salmonellae have been known to survive in contaminated water for up to 4 months (10, 26), such data establish only potentials for survival and are not directly applicable to a stream. Streeter (33), in analyzing the disappearance of bacteria from polluted streams, considered several factors affecting the presence or the removal which were not related to the intrinsic viability of the cells. The inferences to be drawn from the statistical trends of bacterial and chemical parameters in Taughannock Creek are limited. The absence of the respective microorganisms in significant numbers in the waters from above the feedlot or from above station 3 on Reynoldsville Creek indicates that the feedlot was the source of many bacteria, fecal organisms, and also of some salmonellae.

It has been reported that the survival of *Salmonella* in sediment closely followed survival of fecal coliforms (37). The weight of evidence, with a few exceptions (30), indicates that neither group competes well with the natural biota of soils, streams, or sewage (16, 27, 34), although they may be able to survive for prolonged periods on the nutrients found in waters (16), in stream sediments (13), or in biologically treated sewage (4) in the absence of the natural biota.

Inquiry at the county hospital revealed that there was not a high incidence of human salmonellosis. Two or three isolations per year from stool cultures were a normal yield. This information was consistent with the low concentrations of *Salmonella* in the waters and with the low virulence.

For lack of better means, mice were used for virulence estimates. The nonsalmonella isolates, which can be assumed to be nonpathogens in the ordinary sense, had the same potency as the *Salmonella* isolates. High-temperature isolation procedures do not necessarily attenuate the virulence of the strains (J. Timoney, unpublished data), although attenuation cannot be entirely excluded.

The concentrations of *Salmonella* were so low as to preclude, except as a rarity, the ingestion of an immediately infectious dose either by cattle or by humans directly from the water. McCullough and Eisele (20, 21), who fed adult human volunteers with several *Salmonella* serotypes isolated from commercially available spray-dried eggs, determined that doses on the order of 10^6 to 10^8 cells were necessary to produce clinical symptoms. The organisms were shed for variable periods in the feces of the humans who received subclinical doses. The most likely possibility of establishing a line of infection or intoxication from such low-virulence sources would require an intermediate niche to provide the opportunity for massive growth, thereby enhancing endotoxin production or genetic opportunities for increased infectivity. Evidence to support such possibilities can be cited. *S. gallinarum*, being adapted to the avian host, has extremely low virulence for humans, and recorded cases of human infection or gastroenteritis are rare. About 10^9 to 10^{10} cells taken orally have been found necessary to cause brief illness in humans (22); however, an outbreak of gastroenteritis attributed to *S. gallinarum* occurred from rice pudding served in a military mess (24). Of 423 persons involved, 173 required hospitalization. Reversion to increased virulence in *S. typhimurium* clones has also been reported (35, 40) as the result of the host or cultural conditions selecting for the resistant and virulent cells. Possibly, strains of intrinsically low virulence have been recovered in our studies, or prolonged survival apart from the host may have favored lower virulence.

The surface waters of the area studied do not seem to have been an important vehicle for the transmission of clinically significant salmonellosis, either under normal conditions or during several incidents of severe flooding.

From both Fall and Sixmile Creeks, salmonellae were isolated from above and below the intakes of potable water supplies. The waters of the lake are used in some of the lakeside dwellings without any treatment, and in some communities the water is drawn from lakeside wells for treatment. In rural areas, water is obtained from individual wells.

An item of interest with regard to dissemination should be noted. The rare type *S. holcomb* (6,8:1v-enx) was first reported by the Rochester, N.Y., Health Laboratory, where it was isolated from stools from human enteric infection (29). The second reported isolation was from the Gananoque River in Ontario, Canada (7). To our knowledge, the two isolations from Trumansburg reported here would be the third incident. Trumansburg, N.Y., is approximately 73 road miles from Holcomb, N.Y., 98 miles from Rochester, and 220 miles from Gananoque. A circumstantial connection between the areas may be inferred from the popularity of the Gananoque-Rideau Lakes region as a summer recreation and sport fishing area for residents of central New York State.

If the salmonellae isolated during this study were as low in virulence for humans and other animals as for mice, such a condition would be congruous with the situation in which little or no salmonellosis either required medical attention or was diagnosed as such.

There is no question that salmonellae have been involved in water-borne disease of humans and animals. The potential for persistence of virulence or possibly its recovery after loss is always present; however, individual strains tend to be stable in their characteristics on isolation (26) unless special conditions exist. The position is somewhat enigmatic; however, it is not inconceivable that the *Salmonella* genus is analogous to other enteric genera such as *Escherichia, Klebsiella,* or *Vibrio* in which nonpathogenic strains are commonplace.

ACKNOWLEDGMENTS

We are grateful to D. W. Bruner for confirming identities of some of the isolates and for advice and comments. J. C. White provided assistance and additional laboratory facilities. The technical assistance of Ming Chen and Christine Flood is appreciated. The following graciously permitted access to sampling sites on their properties: Supreme Cattle Co., Finger Lakes State Parks Commission, and Frank Edminster. The funds for this research were provided by the Office of Water Resources Research of the U.S. Department of the Interior through the Water Resources and Marine Sciences Center of Cornell University and by the Agricultural Experiment Station.

The generous cooperation of Ray T. Oglesby, Department of Natural Resources, Cornell University, is appreciated for making available the researach vessel *James G. Needham* for taking samples from Cayuga Lake.

LITERATURE CITED

1. **American Public Health Association.** 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Inc., Washington, D.C.
2. **Berg, R., and A. Anderson.** 1972. Salmonellae and *Edwardsiella tarda* in gull feces: a source of contamination in fish processing plants. Appl. Microbiol. 24:501-503.
3. **Boycott, J.** 1962. *Salmonella* species in turtles. Science 137:761-762.
4. **Butler, C., and P. Ludovici.** 1969. Survival and recovery of *Salmonella* in Tucson's wastewater reclamation program. J. Water Pollut. Control Fed. 41:738-744.
5. **Child, D., R. Oglesby, and L. Raymond.** 1971. Land use data for the Finger Lakes Region of New York State. Publication no. 33, Cornell University Water Resources and Marine Sciences Center, Ithaca, N.Y.
6. **Claudon, D., D. Thompson, E. Christenson, G. Lawton, and E. Dick.** 1971. Prolonged *Salmonella* contamination of a recreational lake by runoff waters. Appl. Microbiol. 21:875-877.
7. **Dutka, B., and J. Bell.** 1973. Isolation of salmonellae from moderately polluted waters. J. Water Pollut. Control Fed. 45:316-324.
8. **Edwards, P., and W. Ewing.** 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis, Minn.
9. **Gallagher, T., and D. Spino.** 1968. The significance of numbers of coliform bacteria as an indicator of enteric pathogens. Water Res. 2:169-175.
10. **Gibson, E.** 1965. Diseases of dairy cattle. *Salmonella* infection in cattle. J. Dairy Res. 32:97-134.
11. **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. 18:989-990.
12. **Guinee, P., E. Kampelmacher, A. Van Keulen, and K. Hofstra.** 1964. Salmonellae in healthy cows and calves in the Netherlands. Zentralbl. Veterinaermed. Reihe B 11:728-740.
13. **Hendricks, C.** 1971. Enteric bacterial metabolism of stream sediment eluates. Can. J. Microbiol. 17:551-556.
14. **Hendricks, C.** 1971. Increased recovery rate of salmonellae from stream bottom sediments versus surface waters. Appl. Microbiol. 21:379-380.
15. **Henson, E., A. Bradshaw, and D. Chandler.** 1961. The physical limnology of Cayuga Lake, New York. Memoir 378, Cornell University Agricultural Experiment Station, Ithaca, N.Y.
16. **Kabler, P., H. Clark, and N. Clarke.** 1961. Pathogenic microorganisms and waterborne disease, p. 9-56. *In* Public health hazards of microbial pollution of water. Proc. Rudolfs Res. Conf. Rutgers Univ., New Brunswick, N.J.
17. **Kampelmacher, E., and L. Van Noorle Jansen.** 1970. *Salmonella*—its presence in and removal from a wastewater system. J. Water Pollut. Control Fed. 42:2069-2073.
18. **Khare, M., C. Thomas, and N. Dondero.** 1975. The effects of a cattle feedlot on the chemical and microbiological qualities of a tributary to Cayuga Lake, N.Y. Tech. Rep. no. 99, Cornell University Water Resources and Marine Sciences Center, Ithaca, N.Y.
19. **Kohl, W.** 1969. Vorkommen und Nachweis von Salmonellen in Oberflachengewassern Österreichs. Wien. Tieraerztl. Monatsschr. 56:379-381.
20. **McCullough, N., and C. Eisele.** 1951. Experimental human salmonellosis. I. Pathogenicity of *Salmonella meleagridis* and *Salmonella anatum* obtained from spray-dried whole egg. J. Infect. Dis. 88:278-289.
21. **McCullough, N., and C. Eisele.** 1951. Experimental human salmonellosis. III. Pathogenicity of strains of *Salmonella newport, Salmonella derby,* and *Salmonella bareilly* obtained from spray-dried whole egg. J. Infect. Dis. 89:209-213.
22. **McCullough, N., and C. Eisele.** 1951. Experimental human salmonellosis. IV. Pathogenicity of strains of *Salmonella pullorum* obtained from spray-dried whole egg. J. Infect. Dis. 89:259-265.
23. **Miner, J., L. Fina, and C. Piatt.** 1967. *Salmonella infantis* in cattle feedlot runoff. Appl. Microbiol. 15:627-628.
24. **Mitchell, R., F. Garlock, and R. Broh-Kahn.** 1946. An outbreak of gastroenteritis presumably caused by *Salmonella pullorum*. J. Infect. Dis. 79:57-62.
25. **Moore, B., E. Perry, and S. Chard.** 1952. A survey by the sewage swab method of latent enteric infection in an urban area. J. Hyg. 50:137-156.
26. **National Academy of Sciences.** 1969. An evaluation of the *Salmonella* problem. National Academy of Sciences, Washington, D.C.
27. **Prescott, S., C. E. Winslow, and M. McCrady.** 1946. Water bacteriology, 6th ed. John Wiley & Sons, Inc., New York.
28. **Presnell, M., and J. Miescier.** 1971. Coliforms and fecal coliforms in an oyster-growing area. J. Water Pollut. Control Fed. 43:407-416.
29. **Ramsey, C., and P. Edwards.** 1959. Two new *Salmonella* types: *Salmonella holcomb* and *Salmonella newrochelle*. Int. Bull. Bacteriol. Nomencl. Taxon. 9:111-112.
30. **Seligman, R., and R. Reitler.** 1965. Enteropathogens in water with low *Esch. coli* titer. J. Am. Water Works Assoc. 57:1572-1574.
31. **Smith, R., and R. Twedt.** 1971. Natural relationships of

indicator and pathogenic bacteria in stream waters. J. Water Pollut. Control Fed. 43:2200-2209.

32. Spino, D. 1966. Elevated-temperature technique for the isolation of *Salmonella* from streams. Appl. Microbiol. 14:591-596.
33. Streeter, H. 1934. A formulation of bacterial changes occurring in polluted water. Sewage Works J. 6:208-233.
34. Taylor, J., and J. McCoy. 1969. *Salmonella* and *Arizona* infections, p. 3-72. *In* H. Reimann (ed.), Food-borne infections and intoxications. Academic Press Inc., New York.
35. Thomas, C., and J. Wilson. 1960. Association of resistance to bile salts and virulence in *Salmonella typhimurium* strains. Proc. Soc. Exp. Biol. Med. 103:292-294.
36. U.S. Environmental Protection Agency. 1975. A compendium of lake and reservoir data collected by the National Eutrophication Survey in the northeast and north-central United States. Working Paper no. 474, Corvallis Environmental Research Laboratory, Corvallis, Ore.
37. Van Donsel, D., and E. Geldreich. 1971. Relationships of salmonellae to faecal coliforms in bottom sediments. Water Res. 5:1079-1087.
38. Weibel, S., R. Anderson, and R. Woodward. 1974. Urban land run-off as a factor in stream pollution. J. Water Pollut. Control Fed. 36:914-924.
39. Yoshpe-Purer, Y., S. Ricklis, and M. Paist. 1971. A convenient method for isolation of salmonellae from sewage and contaminated sea water. Water Res. 4:113-120.
40. Zelle, M. 1942. Genetic contributions of host and pathogen in mouse typhoid. J. Infect. Dis. 71:131-152.