APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1982, p. 1111-1115
0099-2240/82/051111-05$02.00/0
Vol. 43, No. 5

Salmonella spp. in the Working Environment of Sewage Treatment Plants in Oslo, Norway

GUNNAR LANGELAND†

Department of Food Hygiene, The Veterinary College of Norway, Dep., Oslo 1, Norway

Received 19 October 1981/Accepted 4 January 1982

The presence of *Salmonella* spp. was investigated at three sewage treatment plants in Oslo, Norway. *Salmonella* bacteria were isolated from floor surfaces and areas with hand contact in the treatment plant, from floor surfaces in the changing rooms, and in one case from floor surfaces in an eating room. The sewage sludge contained from 140 to 140,000 *Salmonella* spp. per 100 g dry weight. Raw and treated sewage contained an average of 130 and 3 of these bacteria per 100 ml, respectively. There was poor correlation between the pattern of serotypes isolated from the sewage works and the patterns of those which were registered among the population of Oslo during the investigation. Neither enteropathogenic bacteria nor parasite eggs were found in fecal samples from employees at the plant. The health significance of the presence of *Salmonella* spp. in the environment of sewage workers is discussed.

Wastewater and sewage sludge contain pathogenic viruses, bacteria, protozoa, and parasite eggs (2, 5, 6, 10, 13, 20). The presence and concentration of pathogens is mainly determined by two factors, namely, the prevalence of pathogens among the population connected to the sewage network and the ability of these organisms to survive the sewage and sludge treatment processes.

The question arises as to the risk employees at sewage works undergo in relation to infectious disease. Pathogens and toxic components may be disseminated through sludge and sewage, as well as through aerosols (7). Exposure risks would seem to be greater for those workers at sewage treatment plants that incorporate a minimum of automated processes.

The purpose of this study was to investigate the presence of *Salmonella* spp. in the working environment and sewage sludge at three sewage works in Oslo.

MATERIALS AND METHODS

Investigations concerning the presence of *Salmonella* spp. in the working environment at three sewage treatment plants in Oslo, Norway were carried out.

Altogether, 10 series of samples were taken at intervals of 2 months from April 1979 to November 1980. The last three series also included samples from plant influent and effluent.

Bekkelaget sewage treatment plant uses mechanical, chemical, and biological purification techniques with simultaneous precipitation (spent pickle liquor containing ferrochloride). The sludge is stabilized anaerobically for approximately 10 days at 30 to 35°C and dewatered by centrifugation after polymer conditioning.

Festningen is a mechanical-chemical plant with secondary precipitation (aluminium sulfate, Boliden's AVR). Sludge from the sedimentation pockets is transported to Bekkelaget treatment plant for anaerobic stabilization and dewatering.

Skarpsno treatment plant is, like Bekkelaget, a mechanical-chemical-biological plant that uses simultaneous precipitation (spent pickle liquor containing ferrochloride). The sludge is dewatered by centrifugation after polymer conditioning.

The plants service populations of 208,400, 153,600, and 63,600, respectively, and together treat wastewater from approximately 90% of the population of Oslo.

Swabs were taken from areas with hand contact and from floor surfaces throughout the plant, including the eating rooms, rest rooms, and changing rooms (see Table 1). Sterile swabs were moistened with sterile 0.9% saline and then wiped over the surface to be investigated, after which they were aseptically transferred to bottles containing selenite broth (Difco 275).

Sewage sludge, grit, and screened material from the plants is trucked to the municipal refuse dump. During each sample series, the inside surfaces of a selection of the gloves used by the lorry drivers were checked for the presence of *Salmonella* spp. The gloves were half filled with selenite broth and shaken by hand, and the broth was transferred to a sterile glass bottle. A total of 65 gloves were investigated.

During the first seven series of samples, 20 g of sewage sludge from each sewage plant was investigated. In the final three series, a quantitative estimation was made of *Salmonella* spp. in the wastewater and sewage sludge by using a most-probable number (MPN) method with 10-fold dilutions and three tubes of enrichment medium at each dilution (1). The ratio of volume of inoculate to selenite broth was approximately 1:10.

† Present address: Asker og Baerum interkommunale naeringsmiddelkontroll, 1371 Asker, Norway.

TABLE 1. *Salmonella* spp. in the working environment and sludge at Bekkelaget, Festningen, and Skarpsno sewage treatment plants[a]

Sampling site	*Salmonella* serotypes[b] found at:		
	Bekkelaget	Festningen	Skarpno
Eating rooms, areas with hand contact	–[c]– – – –	– – – – –	– – – – –
	– – – – –	– – – – –	– – – – –
Eating rooms, floors	F– – – –	– – – – –	– – – – –
	– – – – –	– – – – –	– – – – –
Changing rooms, areas with hand contact	– – – – –	– – – – –	– – – – –
	– – – – –	– – – – –	– – – – –
Changing rooms, floors	– – – – –	– – – – –	– – – – –
	– – H I –	– – – – –	– – – – –
Treatment plant, areas with hand contact	– – – – D	– – D – A	– – E – –
	– – – – –	A B D B/R D	E E – – E
Treatment plant, floors	– F – – –	G – – – Q	E E J E –
	– D H I D	– A – A/D D	E E E E U
Sewage sludge	G F N F D	P D D F D	E E E V K
	S T H D/JK B/F	D D A/D D/MI C	E O E/G E G

[a] Ten series of samples were taken with an interval of ca. 2 months between each series.
[b] Serotypes corresponding to the code letters are listed in Table 3.
[c] *Salmonella* spp. were not detected.

The selenite broth was incubated at 41.5°C and plated onto brilliant green agar (Difco 285) after 2 and 3 days of incubation. After 18 to 14 h of incubation at 37°C, lactose- and saccharose-negative colonies resembling *Salmonella* spp. were plated onto brilliant green sorbitol agar (Difco 285, to which 2% sorbitol had been added). Sorbitol-positive colonies were then subjected to further biochemical characterization (9) and slide agglutination with Salmonella Test Sera (Behring). In the case of the first seven test series, one *Salmonella* culture from each positive sample was verified and serotyped at the National Institute of Public Health, Oslo. *Salmonella paratyphi-B* was in addition phage-typed. During the final three series, one *Salmonella* culture from each *Salmonella*-positive MPN tube was also verified and typed at the same institute.

Feces samples from all the employees of the plants (25 workers in all) were examined for the presence of *Salmonella* spp., *Yersinia enterocolitica*, *Campylobacter fetus* subsp. *jejuni*, and *Shigella* spp. by direct plating onto brilliant green agar (Difco 285), *Yersinia* selective agar (Merck 11443), Skirrows agar (16), Drigalski agar, and desoxycholate citrate agar (Oxoid CM 227). The feces samples were further investigated by enrichment in selenite broth (Difco 275) at 22 and 37°C, in modified Rappaport broth with carbenicillin at 22°C (18), and in *Campylobacter* broth at 42°C under microaerophilic conditions (14). After various incubation periods, secondary inoculation onto a range of specific differential media was carried out.

The feces samples were also analyzed for the presence of parasite eggs at the Department of Internal Medicine I, Division of Parasitology, The Veterinary College of Norway.

Finally, serum samples from all seven employees at the Festningen treatment plant were examined with the Widal test for antibodies against salmonella O antigen 4, 5, 12 (*Salmonella paratyphi-B*, *Salmonella typhimurium*, and others).

RESULTS

Salmonella spp. were isolated from all of the samples of sewage sludge and from 30, 60, and 65% of the swabs from the working environment of the treatment areas at Bekkelaget, Festningen, and Skarpsno, respectively. Table 1 shows the frequency of isolation and the serotypes found (with phage types also in the case of *Salmonella paratyphi-B*) in the working environments and sewage sludge.

The wastewater samples showed large variations in the concentration of *Salmonella* spp. On average, the concentration of these bacteria in treatment plant influent and effluent was 130 and 3 bacteria per 100 ml of water, respectively. A correspondingly large variation was also found in the sludge analyzed, with the concentration of *Salmonella* spp. varying between 140 and 140,000 bacteria per 100 g of dry matter (Table 2).

Salmonella bacteria were not isolated from the gloves that were examined.

Neither parasite eggs nor enteropathogenic bacteria were found in the feces samples. One of these samples was from a new employee with acute gastrointestinal symptoms. The clinical picture in this case correlated well with that described for intoxication after uptake of airborne endotoxins (sewage worker syndrome; 15).

TABLE 2. MPN of *Salmonella* spp. per 100 ml of sewage and 100 g of sewage sludge, dry weight, from the final three sample series

Sampling site	MPN and *Salmonella* serotypes[a] from		
	Bekkelaget	Festningen	Skarpsno
Raw sewage	15 FH	930 D	3 E
	–[b]	–	–
	9 J	–	210 L
Treated sewage	–	–	–
	–	7 DM	4 E
	–	9 D	7 L
Sewage sludge	140 H	18,000 AD	45,000 EG
	1,100 DJK	140,000 DIM	65,000 E
	1,700 BF	1,700 C	23,000 G

[a] Serotypes corresponding to the code letters are listed in Table 3.
[b] *Salmonella* spp. were not detected.

The serum samples did not contain antibodies against *Salmonella* O antigen 4, 5, 12.

DISCUSSION

The results show that *Salmonella* spp. were present in the working environment at the sewage treatment plants. Pathogens are likely to contaminate clothes, shoes, and hands. Bekkelaget sewage works is the most automated of the three works, which may explain why the registered frequency of *Salmonella* spp. from areas with hand contact was lower there than in the other two plants. The one positive sample recorded at Bekkelaget (*Salmonella newport*) contained, among other things, material from a wastewater pump that was under repair because of leakage.

Salmonella spp. and other pathogens may be transferred to the mouth directly or indirectly, e.g., by smoking without prior hand washing or eating without washing and changing work clothes. Certain pathogenic bacteria (e.g., *Salmonella typhi* and *Shigella dysenteriae*), viruses (e.g., hepatitis A), and parasite eggs (*Ascaris lumbricoides* and *Trichuris trichiura*) have such a low infectious dose that oral uptake of even a few organisms can result in infection (8, 19, 20).

Blood samples had been taken previously from the employees and analyzed for antibodies to hepatitis A virus. These results did not indicate any significant difference in incidence from normal levels (J. C. Siebke, The National Institute of Public Health, Oslo, personal communication).

With the exception of *Salmonella paratyphi-B*, the *Salmonella* strains that I detected were unlikely to cause disease after oral intake, assuming the quantitative data is reasonably accurate. If contamination of a suitable foodstuff occurs, multiplication of these *Salmonella* strains could reach the level required for an infective dose.

The MPN concentrations of *Salmonella* spp. in Table 2 are probably lower than the true values because two of the conditions required by the MPN method were not met (3). First, some attenuated bacteria may succumb during enrichment in selenite broth, and secondly, the bacteria are unlikely to be randomly distributed in the samples.

Investigations from other countries, such as England, Holland, and Denmark, have also shown that the concentrations of *Salmonella* spp. in samples of wastewater can vary considerably with time (11, 21; and K. Grunnet, Ph.D. thesis, University of Århus, Århus, Denmark, 1975). On the other hand, the contents of fecal coli and sulfite-reducing *Clostridium* spp. in wastewater from sewage treatment plants in Oslo are relatively constant (I. Hellesnes and B. Maere, personal communication). Table 3 shows that there is a poor correlation between the pattern of *Salmonella* serotypes isolated from the treatment plants and the pattern of those which were registered among the population of Oslo from 2 weeks before the first sample series until 2 weeks after the last series.

Salmonella saint-paul, monophasic variant 9-12:lv:-, and 12 other *Salmonella* types that together accounted for 73% of the isolates from the treatment plants, were not isolated from the human population (The National Institute of Public Health, Oslo).

Salmonella parathyphi-B phage type Taunton, which was isolated from sludge, floors, and areas with hand contact in the treatment plant, has not been registered in Oslo since 1967. *Salmonella paratyphi-B* phage type I, which was isolated from two of the works, has likewise not been registered since 1970.

The lack of correlation may be explained by the fact that salmonellosis can give such mild symptoms that medical help is not sought. Consequently, fecal samples are not sent for bacteri-

TABLE 3. *Salmonella* spp. isolated from three sewage treatment plants in Oslo and *Salmonella* spp. detected from the human population during the same period[a]

	Salmonella spp.	No. of isolates in: Treatment plants	Humans
	S. typhi	0	8
	S. paratyphi-A	0	1
	S. paratyphi-B phage type Dundee	0	2
	S. paratyphi-B phage type 3 a I	0	1
A	*S. paratyphi-B* phage type Taunton	5	0
B	*S. paratyphi-B* phage type I	3	0
C	*S. parathyphi-B* not typable by phages	1	0
D	Monophasic *Salmonella* (9–12:lv:–)	20	0
E	*S. saint-paul*	19	0
F	*S. newport*	7	2
G	*S. enteritidis*	4	12
H	*S. virchow*	4	2
I	*S. heidelberg*	3	10
J	*S. brandenburg*	3	0
K	*S. typhimurium*	2	27
L	*S. reading*	2	0
M	Monophasic *Salmonella* (4–12:d:–)	2	0
N	*S. infantis*	1	3
O	*S. java*	1	2
P	*S. derby*	1	1
Q	*S. adelaide*	1	0
R	*S. kapemba*	1	0
S	*S. krefeld*	1	0
T	*S. livingstone*	1	0
U	*S. newington*	1	0
V	*S. san-diego*	1	0
	S. agona	0	5
	S. braenderup	0	2
	S. bredeney	0	2
	S. bovis-morbificans	0	2
	S. haifa	0	2
	S. montevideo	0	2
	S. ohio	0	2
	S. oslo	0	2
	S. anatum	0	1
	S. blockley	0	1
	S. cerro	0	1
	S. chester	0	1
	S. emek	0	1
	S. hadar	0	1
	S. indiana	0	1
	S. mbandaka	0	1
	S. oranienburg	0	1
	S. richmond	0	1
	S. senftenberg	0	1
	S. tennessee	0	1
	S. thompson	0	1
	S. weltevreden	0	1
	Not specified	0	3

[a] Data from the National Institute of Public Health, Oslo, Norway.

ological investigation. Furthermore, there will also always be clinically healthy carriers in a large population.

Whether *Salmonella* spp. can multiply in wastewater has been discussed. Grunnet (Ph.D. thesis) has shown that the four most common serotypes in sludge from Århus (*Salmonella typhimurium, Salmonella paratyphi-B, Salmonella senftenberg*, and *Salmonella baildon*) are able to grow in wastewater at temperatures between 10 and 42°C.

Wild and domestic animals (including pets) may also provide a reservoir for *Salmonella* spp. These bacteria were not, however, isolated at the Oslo abattoir during the investigation.

Among the population of Oslo, almost half of the isolates (46%) consisted of *Salmonella typhimurium, Salmonella enteritidis*, and *Salmonella heidelberg*. These serotypes were also found at the sewage treatment plants but accounted for only 10% of the isolates.

Of those *Salmonella* types which were registered in the population of Oslo, 27 were not recovered at the sewage works. This may be because they were not present in the sludge, wastewater, and working environment at the time of sampling or because the patients belonged to the 10% of the population connected to other sewage works. The method of isolation used in this investigation is not suited for *Salmonella typhi*, and it is possible that the method favors certain other *Salmonella* serotypes. In addition, different types may have different abilities to survive in wastewater and sludge, and when the samples contain different serotypes, some types can be overlooked.

In the investigation of Grunnet (Ph.D. thesis) in Denmark and in a corresponding work from Sweden (4), similarly poor correlations have been found between the serological pattern of *Salmonella* spp. from sewage works and those isolated directly from the population. Likewise, *Salmonella paratyphi-B* has been isolated strikingly often in relation to the registered incidence of the population.

In a Dutch investigation in 1980, the serotypes found in wastewater and feces from the inhabitant and dog population proved to be identical (11). This investigation was unusual, however, in that both wastewater and feces from household pets and 76% of the 540 inhabitants in the area were analyzed during a 3-week period. None of the human carriers had sought medical help or received treatment for salmonellosis.

It is debatable whether sewage workers suffer from an increased incidence of infection or other diseases (12, 17). However, the risk of exposure to pathogens from the working environment should be maintained at an acceptable low level. It is important for these workers to have suitable protective clothing, shoes, and gloves. Ventilation should be satisfactory, and treatment processes should be automated to the fullest extent possible. Perhaps the most important single factor is to make sure that sewage workers know how to avoid infection and that they are aware of and use protective measures in their daily work.

ACKNOWLEDGMENTS

I am grateful to Ellen Fuglerud for technical assistance, Jorunn Sundar of the National Institute of Public Health for typing of the *Salmonella* bacteria and for carrying out the Widal test, Asbjørg Husdal for the parasitological analyses, and to the sewage workers for their cooperation.

This work was supported by a grant from the Agricultural Research Council of Norway.

LITERATURE CITED

1. **American Public Health Association, American Water Works Association, and Water Pollution Control Federation.** 1976. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
2. **Bryan, F. L.** 1977. Diseases transmitted by foods contaminated by wastewater. J. Food Prot. **40**:45–56.
3. **Cochran, W. G.** 1950. Estimation of bacterial densities by means of the "most probable number." Biometrics **6**:105–116.
4. **Danielsson, M. L.** 1977. *Salmonella* in sewage and sludge. Serological profiles of isolates, their removal and/or survival in relation to potential health hazards to man and animals. Acta Vet. Scand. Suppl. **65**:1–126.
5. **Dixon, F. R., and L. J. McCabe.** 1964. Health aspects of wastewater treatment. J. Water Pollut. Control Fed. **36**:984–989.
6. **Dudley, D. J., M. N. Guentzel, M. J. Ibarra, B. E. Moore, and B. P. Sagik.** 1980. Enumeration of potentially pathogenic bacteria from sewage sludges. Appl. Environ. Microbiol. **39**:118–126.
7. **Hickey, J. L. S., and P. C. Reist.** 1975. Health significance of airborne microorganisms from wastewater treatment processes. J. Water Pollut. Control Fed. **47**:2741–2757.
8. **Jones, A. W.** 1967. Introduction to parasitology. Addison-Wesley Publishing Co., Inc., Reading, Mass.
9. **Lassen, J.** 1975. Rapid identification of Gram-negative rods using a three-tube method combined with a dichotomic key. Acta Pathol. Microbiol. Scand. Sect. B **83**:525–533.
10. **Leclerc, H., A. Perchet, C. Savage, S. Andrieu, and R. Nguematcha.** 1971. Microbiological aspects of sewage treatment, Paper I-23/1-I-23/9. 5th International Conference on Water Pollution Research 1970, Proceedings, San Francisco.
11. **Oosterom, J., J. C. de Wit, M. van Schothorst, F. M. van Leusden, and E. H. Kampelmacher.** 1980. Epidemiological studies on Salmonella in a certain area. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe A **248**:190–201.
12. **Pahren, H., and W. Jakubowski (ed.).** 1980. Wastewater aerosols and disease. U.S. Environmental Protection Agency, Cincinnati, Ohio.
13. **Roneus, O., and G. Dalborg.** 1977. The occurrence of parasitic eggs in sludge and effluent from wastewater treatment plants, SNV PM 812. National Swedish Environment Protection Board, Solna, Sweden.
14. **Rosef, O.** 1981. Isolation of *Campylobacter fetus* subsp. *jejuni* from the gallbladder of normal slaughter pigs, using an enrichment procedure. Acta Vet. Scand. **22**:149–151.
15. **Rylander, R., and M. Lundholm.** 1980. Responses to wastewater exposure with reference to endotoxin, p. 90–98. *In* H. Pahren and W. Jakubowski (ed.), Wastewater aerosols and disease. U.S. Environmental Protection Agency, Cincinnati, Ohio.
16. **Skirrow, M. B.** 1977. Campylobacter enteritis: a "new" disease. Br. Med. J. **2**:9–11.
17. **Swedish Water and Waste Water Works Association.** 1978. Health risks in sewerage systems. Swedish Water and Waste Water Works, Association, Stockholm.
18. **Wauters, G.** 1973. Improved methods for the isolation and the recognition of *Yersinia enterocolitica*. Contrib. Microbiol. Immunol. **2**:68–70.
19. **Wilson, G. S., and A. A. Miles.** 1975. Topley's and Wilson's principles of bacteriology, virology and immunity. Edward Arnold Ltd., London.
20. **World Health Organization.** 1979. Human viruses in water, wastewater and soil. Technical report series no. 639. World Health Organization, Geneva.
21. **Yaziz, M. I., and B. J. Lloyd.** 1979. The removal of salmonellas in conventional sewage treatment processes. J. Appl. Bacteriol. **46**:131–142.