

Salmonella pollution of surface waters

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SUMMARY

Surface waters in 14 selected sites were examined for the presence of salmonella using modified Moore's swabs. The sites included an upland impounding reservoir, 3 rivers and 10 streams within Lancashire and Cheshire, selected because of their accessibility to farm livestock. Salmonellas were isolated from 22 out of the 57 swabs examined representing 10 sites. The probable source of pollution was shown to be sewage or farm effluent and an examination of sites over a wider area may be expected to produce similar results.

The significance of these findings is discussed in relation to the epidemiology of salmonella infections and the possible disinfection of effluent discharges.

INTRODUCTION

The examination of surface waters for the presence of salmonella organisms has been widely undertaken by many workers and has been amply reviewed by Williams (1975) and Wray (1975). Whilst the results of many surveys are quoted in the literature it is difficult to compare the prevalence of *Salmonella* in different geographical areas because of the diversity of isolation procedures used. The introduction of standard isolation techniques would allow such comparisons to be made and their adoption may eventually be necessary for purposes of national or international legislation. During studies on the development of standard techniques for the isolation of salmonellas from surface waters a survey was undertaken to determine the prevalence of salmonellas in watercourses in Lancashire and Cheshire.

This paper records the isolation techniques used, and the results of the survey and discusses the significance of salmonellas in surface waters.

Disposal of sewage involves a number of mechanical and biological processes designed to remove organic material. The accumulated sludge is disposed of by

dumping at sea, or tipping or spreading on land as fertilizer, but the final effluent from the treated sewage is allowed to run into rivers or streams. Storm-water overflows and storm tanks operating in wet weather conditions give rise to direct discharge of untreated sewage into watercourses with the consequent release of any pathogens present into the environment.

Where sewerage systems or sewage works are overloaded direct discharge can also occur under drier conditions. Studies in USA have shown raw, partially digested or fully digested sewage to contain *Salmonella* (Kabler, 1959). Popp (1973) studied a waste-water treatment system in Germany and demonstrated that sedimentation did not completely remove salmonellas and biological treatment did not remove any. Similar observations on the ineffectiveness of sewage treatment have also been reported in Germany by Schaaf & Attevald (1965), in South Africa by Coetzee & Fourie (1965), and in Scandinavia by Ojala (1966).

The treatment of sewage is designed to attain acceptable chemical concentrations in the effluents discharged after treatment. These concentrations were originally defined by the Royal Commission on Sewage Disposal (1912) 'so that local authorities shall not be required to purify their sewage more highly than is necessary to obviate the risk of actual nuisance arising from its discharge'.

In practice, the levels of the suspended solids and the Biochemical Oxygen Demand of effluents are defined in relation to the degree of dilution of the effluent after discharge to natural waters.

No bacteriological standards were or have been recommended for sewage effluents, so it is perhaps incorrect to regard sewage treatment as inefficient because of the presence of micro-organisms including pathogenic micro-organisms in effluent. Bacteriological standards have been recently proposed and adopted, however, for member states within the European Economic Community (Council Directive 76/160 EEC 1975) concerning the quality of bathing waters. These include both inland and sea waters where bathing is explicitly authorized or not prohibited and is traditionally practised by a large number of bathers.

Such waters may receive discharges of sewage effluent.

MATERIALS AND METHODS

Sampling sites

A large reservoir, 3 rivers and 10 streams within agricultural areas of Lancashire and Cheshire were sampled at 2-week intervals during the period January to March 1976. Apart from the reservoir and one river which were sampled at sluices, all sites were sampled at points affording access to farm livestock, and the proximity of sewage or farm effluent upstream of sampling points was noted. Two sites (on streams 4 and 6) were in addition sampled above and below the suspected source of pollution.

Swabs

Moore's swabs (Moore, 1948) were prepared by tying a piece of string 2 m in length through the loops of a folded standard sanitary towel.* These were placed in 1 lb honey jars and sterilized in the hot-air oven for 1 h at 180 °C.

The swabs were tied to a convenient support in the centre of the moving water on a Friday and collected on a Monday morning into sterile bottles. They were delivered to the laboratory on the day of collection and examined immediately on receipt.

River sediment

Samples of about 200 g of river sediment were collected into 1 lb honey jars from rivers and streams at the points where swabs were laid.

Water samples

Water was collected in 1 l bottles from streams 4 and 6. Samples were taken above effluent discharge, at point of effluent discharge and 0.5 km downstream 15 min later. This was calculated on water flow rates as the time needed for the original water to have moved downstream.

Bacteriology

At the laboratory the swab was cut in half and one portion was placed in 225 ml buffered peptone water (BPW) prepared by the method of Edel & Kampelmacher (1973). The other half of the swab was discarded. The river sediments were treated by placing 25 g into 225 ml of BPW.

After overnight incubation at 37 °C, 10 ml of the BPW was transferred to 100 ml Oxoid CM343 Muller Kauffmann tetrathionate broth (TB) and 100 ml Oxoid CM395 selenite broth with the addition of Oxoid L121 sodium biselenite. Both TB and selenite broth were incubated at 43 °C and after 24 h the TB was plated on to single plates of Lab-M bismuth sulphite agar (BSA) and duplicate plates of Oxoid CM329 brilliant green agar (BGA). The selenite was plated onto single plates of BSA and Oxoid CM393 DCLS agar. The plates were incubated at 37 °C overnight and colonies were identified serologically and biochemically.

Counting of salmonellas

A most probable number (MPN) method was used to count salmonellas in the water samples from streams 4 and 6. Volumes of 10, 1 and 0.1 ml of water were each inoculated into three jars of 100 ml BPW which were incubated, passaged into TB and plated onto BGA as described previously.

The MPN/100 ml was calculated from the probability tables of Oblinger & Koburger (1975) according to the number of jars of BPW of each dilution of water which produced positive salmonella isolates.

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RESULTS

Salmonellas were isolated from 22 of the 57 swabs examined (39% of swabs). The positive swabs came from 10 of the 14 sites sampled (71% of sites) of which six sites were positive on more than one occasion. The probable source of contamination was identified for each site (Table 1).

Salmonellas were not isolated from streams 4 and 6 above the point of effluent discharge.

In stream 4, the salmonella MPNs/100 ml were 1100 at the point of discharge of effluent and 23 at a point 0.5 km downstream. In stream 6 the corresponding MPNs were 43 and 3 respectively.

The site on stream 8 which yielded a single positive swab was situated 1 km downstream from effluent discharge from a cottage hospital. Shortly after this isolation a new sewage works was introduced and subsequent resampling failed to reveal salmonella at the original site.

Serotypes were *S. agona* (7), *S. dublin* (1), *S. hadar* (1), *S. heidelberg* (1), *S. indiana* (1), *S. lexington* (1), *S. livingstone* (1), *S. oranienburg* (1), *S. panama* (1), *S. saintpaul* (1), *S. senftenberg* (2), *S. typhimurium* (4) and *S. virchow* (2).

Salmonellas were not isolated from river sediment samples from all 7 sites.

DISCUSSION

Few surface waters are free from pollution. In the present investigation even the upland surface-impounding reservoir was found to be contaminated on 5 out of 6 samplings. As the gathering grounds of such reservoirs are protected from pollution from human sources, the source of pollution was most probably gulls from a large inland colony on the gathering ground. Of the remaining sites, salmonellas were isolated from 6 out of 7 swabs (86%) from 2 sites receiving sewage effluent; from 5 out of 12 swabs (42%) from 3 sites receiving sewage and farm effluent, from 4 out of 16 swabs (25%) from 4 sites receiving farm effluents; and from 2 out of 11 swabs (18%) receiving effluents from other sources (hospital, abattoir, dairy). Salmonellas were not isolated from swabs taken from one river.

In general, the findings suggest that sewage effluent is the source of greater microbial pollution than farm or other effluent.

The MPN counts of salmonella found in this survey at direct point of sewage-effluent discharges are in broad agreement with those found by Cheng, Boyle & Goepfert (1971) and Popp (1973), who studied sewage works effluent, and Hoadley *et al.* (1974), who monitored the environment around a chicken-processing plant.

The concentration of waterborne salmonella necessary to cause disease is unknown, but fairly high numbers of organisms are needed to produce disease under experimental conditions. Taylor (1973) failed to infect calves which were given access to grass sprayed with slurry containing 10^5 *S. dublin*/ml, and Morse & Duncan (1974) considered that doses of 10^8 salmonella are probably necessary to produce infection in healthy swine and other normal livestock. The minimum infective dose of salmonella varies considerably and is influenced, amongst other

Table 1. *Salmonella* isolations from surface waters showing probable source of *Salmonella* upstream of sampling point

Site	Probable source of pollution	Samples						River sediment	Serotypes
		1	2	3	4	5	6		
Reservoir									
River									
1	Possibly gulls	+	-	+	+	+	0	0	<i>S. hadar</i> 1, <i>S. senftenberg</i> 1, <i>S. typhimurium</i> 3
2	Sewage effluent	+	+	-	0	0	0	0	<i>S. senftenberg</i> 1, <i>S. heidelberg</i> 1
3	Unknown	-	-	-	-	0	0	-	-
	Sewage and farm effluent 1 km	+	-	+	-	0	0	-	<i>S. agona</i> 1, <i>S. typhimurium</i> 1
Stream									
1	Farm effluent $\frac{1}{4}$ km	-	-	+	-	0	0	0	<i>S. virchow</i> 1
2	Farm effluent $\frac{1}{4}$ km	-	-	-	-	0	0	-	-
3	Farm effluent $\frac{1}{4}$ km	-	-	-	-	0	0	0	-
4	Sewage effluent $\frac{1}{2}$ km	+	+	+	+	0	0	0	<i>S. agona</i> 3, <i>S. oranienburg</i> 1, <i>S. livingstone</i> 1
5	Farm effluent $\frac{1}{4}$ km	+	-	+	+	0	0	0	<i>S. agona</i> 1, <i>S. saintpaul</i> 1, <i>S. levington</i> 1
6	Sewage and farm effluent $\frac{1}{2}$ km	-	+	+	-	0	0	-	<i>S. agona</i> 1, <i>S. indiana</i> 1, <i>S. virchow</i> 1
7	Sewage and farm effluent $\frac{1}{4}$ km	-	-	-	+	0	0	-	<i>S. agona</i> 1
8	Hospital effluent $\frac{1}{4}$ km	-	-	+	-	0	0	0	<i>S. panama</i> 1
9	Abattoir effluent $\frac{1}{4}$ km	-	-	-	-	0	0	-	-
10	Dairy effluent $\frac{1}{2}$ km	+	-	-	-	0	0	-	<i>S. dublin</i> 1

0 = Not tested. + = Positive *salmonella* isolation.

factors, by serotypes, animal species, age and immunological status, feeding and husbandry methods and the presence of intercurrent disease.

The significance of salmonella-contaminated waters in terms of the potential hazard to livestock may lie not only in the number of organisms present, which appears to be below the infective dose, but in the possibility that animals ingesting organisms may later excrete them in large enough numbers to infect animals in contact. Similarly the repeated ingestion of low numbers of organisms may produce clinical disease in some animals only as a result of a secondary influence such as intercurrent disease or stress. In these ways animals help to perpetuate the salmonella cycle by infecting other animals and man and by directly or indirectly contributing salmonellas to the effluent from farm premises.

The reduction of salmonella numbers found in streams 4 and 6 below the entry of sewage effluent is to be expected and can be explained by dilution, temperature, settling of particulate matter with which the organisms are associated and natural die-off due to bactericidal factors in the water. Similar observations were made by Kampelmacher & Jansen (1976). The possibility of continued survival of salmonellas in the environment was studied by examination of river sediments. All the 7 sites sampled were negative. Hendricks (1971) reported that isolations from river sediments in the USA were eight times greater than from water and that this may have been due to sedimentation and adsorption of the organism on the sands and clay of the sediments. The salmonellas were recovered from sites nearer the outfall probably owing to inadequate mixing and dispersal. This may explain failure to isolate salmonellas from sediments in the present survey.

In order to reduce the number of pathogenic organisms released into the environment it may be necessary to consider disinfection of effluents discharged. Disinfection of wastewater is practised in many countries, chlorine being the most widely used agent. Sellick & Collins (1975), describing the development of disinfection processes, indicated that although chlorination could achieve the necessary bactericidal and virucidal effect it was also highly toxic to aquatic life. Dechlorination procedures should be included where the effects on aquatic life are to be minimized but this adds considerably to cost of wastewater disposal.

In general, the processes of wastewater disinfection are poorly understood and the holding times necessary in chlorination are affected by many factors, including pH and the ammonia nitrogen/chlorine ratio. The chemical constituents in the water can also influence the effectiveness of the process. Final concentrations of chlorine in effluent are difficult to control and may necessitate costly plant modifications.

Fears have been expressed that the treatment of tertiary effluent with chlorine may lead to the production of carcinogenic compounds and that wastewater disinfection should be avoided unless it is shown to be necessary to protect public health (Sellick & Collins, 1975). It is further claimed that coliform organisms can recover and regrow after chlorination, indicating that the process is not totally effective (Shuval, 1975). It is not known if salmonellas are capable of recovery.

Special consideration needs to be given to effluent discharge from farm premises. Disposal of farm effluent containing salmonellas into a watercourse may lead to

recycling of the organism within farm livestock – in particular of species-related serotypes such as *S. dublin*. Although treatment of farm effluents may be advantageous the high cost of introducing satisfactory treatment processes would create problems for many farmers.

In view of obvious difficulties in producing effluents which are free from pathogenic organisms it is likely that the discharge of treated and untreated effluent into rivers and streams will continue to present a hazard to humans and livestock. Consideration must be given to developing more effective methods of treating effluent both from farms and sewage works. Disinfection processes may not be entirely satisfactory but selective chlorination followed by dechlorination where necessary may be indicated in areas where the risk to livestock is to be minimized.

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