

Salmonellas in sewage. A study in latent human infection

BY R. W. S. HARVEY AND T. H. PRICE

*Public Health Laboratory, Institute of Preventive Medicine,
The Parade, Cardiff*

D. W. FOSTER

Medical Officer of Health, Pontypridd Urban District Council

AND W. C. GRIFFITHS

Public Health Inspector, Pontypridd Urban District Council

(Received 18 February 1969)

INTRODUCTION

The salmonella group includes a great many organisms differing in antigenic composition, sometimes in biochemical reactions and often in pathogenicity for man. It is this latter property which probably determines the range of serotypes causing clinical symptoms in human infection. In the present study we have monitored the salmonellas excreted in human sewage irrespective of their ability to cause recognizable illness. In the area selected, general practitioners were asked to look out for and to investigate cases of gastro-enteritis. Those notified during the investigation, however, were not caused by salmonella infection. The current survey was derived from an earlier series of observations on a naturally contaminated river—the river Taff. This study has been briefly described from the technical aspect (Harvey & Price, 1967*a*). The investigation of human sewage was decided on to ascertain the probable source of frequent isolations of salmonellas from river water. The estate chosen for observation was in Pontypridd, about 14 miles north of Cardiff, three miles upstream from the previous sampling site on the river Taff.

THE ESTATE

The area chosen was a residential estate housing 4000 persons containing neither industry nor retail butchers shops. Samples of surface draining water were examined with negative results. Pilot observations on the main sewer revealed that specimens were consistently positive for salmonellas. Sampling points were chosen spreading into the branches of the sewerage system; these points were kept very constant throughout the investigation. A sewer plan with the sampling points used is shown in Fig. 1.

MATERIALS

Moore's swabs (Moore, 1948) were used for the survey. The swab technique has been employed routinely for monitoring salmonellosis in our area since 1955 (Harvey & Phillips, 1955; Harvey, 1957; Harvey & Phillips, 1961; Harvey, Price,

Bate & Allen, 1963; Harvey, Price & Dixon, 1966). It has been applied to bake-houses, abattoirs, knackers yards, retail meat markets and wholesale and retail butchers. By its use, information has been collected on sources of infection in sporadic and epidemic salmonellosis, on the average seasonal degree of abattoir contamination (Harvey, 1965), and on the type of domestic animal excreting salmonellas. Such information would not have been so easily gained in other ways. We now wished to discover the incidence of salmonellas in sewage from a population of 4000 people and to isolate all serotypes present (in so far as this was technically possible).

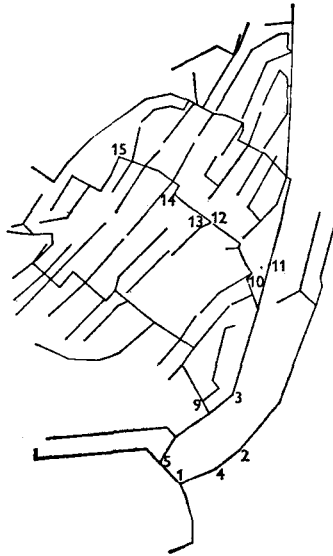


Fig. 1. Sampling points used on sewerage system.

METHODS

The cultural methods used were largely developed in this laboratory. The swabs were brought in weekly, having remained 7 days in the sewers. They were placed, on removal from the sampling point, into 1 lb. screw-capped jars. At the laboratory, selenite F broth was poured into the jars in sufficient volume to cover the swabs. The enrichment broths were incubated at 43° C. for 24 hr. (Harvey & Price, 1968). Subcultures were made to deoxycholate citrate agar and brilliant green MacConkey agar (Harvey, 1956). The plates were incubated at 37° C. and the brilliant green MacConkey plates were examined after 24 hr. incubation. The deoxycholate plates were incubated for 48 hr. to aid differentiation between salmonella and proteus colonies (Harvey & Price, 1968). Suspicious colonies were picked for identification. The deoxycholate citrate agars were used for secondary enrichment by the Craigie tube method of Harvey & Price (1967*b*). All positive samples were finally examined for multiple serotypes by the serological technique of Harvey & Price (1967*a*).

Table 1. Isolation of different salmonella serotypes from a residential estate in Pontypridd

Salmonella serotypes	1967												1968						
	v	vi	vii	viii	ix	x	xi	xii	i	ii	iii	iv	v	vi	vii				
paratyphi B, 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
braunenberg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
dublin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
give	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
anatum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
kraaifontein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
enteritidis, jena	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
paratyphi B, dundee, var 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
typhimurium, untypable	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
typhimurium, U 157	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
typhimurium, 29 oranienburg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
schwarzengrund stanley	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
manhattan newport	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
panama bredeney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
indiana typhimurium, 1 senftenberg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
derby	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
galtema typhimurium, 82	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
kentucky typhimurium, 12a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
livingstone typhimurium, 14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
fischerkietz duisburg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
bleadon uphill	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
paratyphi B, 1 var 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			

Points were sampled as far as possible each week. The columns under each month show the number of samples taken that month.

Table 2. *Salmonella* isolations from different sampling points

Salmonella serotypes	Sampling point														Other isolations in Glamorgan	
	1	2	3	4	5	9	10	11	12	13	14	15				
<i>paratyphi B</i> , 1	46	2	.	.	38	2	2	.	1	.	1	.				
<i>brandenburg</i>	10	2	.	1	8	2	8	1	2	3	1	.				Drains, Central Market, Cardiff, 16. v. 57
<i>dublin</i>	.	.	1	Pig & cattle drains, Cardiff abattoir, 1. v. 67
<i>give</i>	.	.	.	1	
<i>anatum</i>	3	.	.	.	5	1	2	3	Cattle drain, Cardiff abattoir, 18. iv. 67 Knackers yard 24. xi. 67
<i>kraaifontein</i>	1	3	
<i>enteritidis</i> , jena	1	.	.	1	
<i>paratyphi B</i> , dundee, var. 1	1	
<i>typhimurium</i> , untypable	1	
<i>typhimurium</i> , U 157	1	1	1	
<i>typhimurium</i> , 29	1	
<i>oranienburg</i>	1	
<i>schwarzengrund</i>	1	
<i>stanley</i>	6	1	.	.	4	.	5	2	.	4	1	
<i>manhattan</i>	1	.	.	.	1	.	1	1	Drains, Central Market Cardiff, 25. ix. 67
<i>newport</i>	.	1	.	.	1	.	1	3	
<i>panama</i>	5	1	.	.	3	.	3	4	2	.	4	1	.	.	.	Drains, Central Market, Cardiff, 14. xi. 67
<i>bredeney</i>	3	.	.	.	3	1	.	4	Cardiff abattoir, 8. vii. 68
<i>indiana</i>	4	.	.	.	2	.	2	7	Pontypridd abattoir, 26. vii. 67; Cardiff abattoir, 27. xi. 67
<i>typhimurium</i> , 1	1	
<i>senftenberg</i>	1	Drains, Central Market, Cardiff, 9. i. 68
<i>derby</i>	1	.	.	.	1	
<i>galiema</i>	1	.	1	
<i>typhimurium</i> , 32	1	1	
<i>kentucky</i>	1	
<i>typhimurium</i> , 12a	2	.	2	1	.	
<i>livingstone</i>	1	.	.	Pig drains, Pontypridd abattoir, 25. iv. 68; 2. v. 68; 9. v. 68
<i>typhimurium</i> , 14	1	.	.	
<i>fischerkietz</i>	2	.	1	.	1	.	1	
<i>duisburg</i>	1	.	.	.	
<i>bleadon</i>	1	
<i>uphill</i>	1	1	
<i>paratyphi B</i> , 1, var. 1	1	

RESULTS

The results are given in Tables 1 and 2. Table 1 records the serotype isolations in terms of time and Table 2 in terms of sampling point. The tables are self-explanatory.

DISCUSSION

The main object of this investigation was to provide evidence that man is frequently infected with a wide range of salmonella serotypes. The term infection used here does not distinguish between latent and overt salmonellosis and epidemiologically it would be difficult to justify such a distinction. Serotypes producing latent infection in one area may produce, by chance, overt infection in another. We believe that we have succeeded in our object and that salmonellosis has been shown to occur regularly in a population of 4000 persons. We can indeed take this a step further if we consider the information derived from Point 11 (Table 2). The population sampled at this point was estimated at 1000 and no shops of *any* sort were situated in this section of the estate. At this sampling point 48% of specimens were positive for salmonellas. Some serotypes in Table 1 are frequently isolated (*S. stanley*, *S. panama*, *S. indiana*, *S. brandenburg*). These probably represent local 'prevalences' (Parker, 1954). *S. stanley* and *S. panama* have recently caused considerable illness in France as well as in the United Kingdom (Le Minor *et al.* 1967; Vernon, 1967) and the local isolations may well reflect a wider geographical distribution. In this series *S. typhimurium* does not dominate the pattern which contrasts with the usual finding in overt salmonellosis in man. Other serotypes less frequently recorded in Table 1 suggest an exotic origin (*S. kraaifontein*, *S. galiema*, *S. fischerkietz*, *S. duisburg*, *S. bleedon*, *S. uphill*).

While overt salmonella infection was not noted in the estate, some of the serotypes isolated caused clinical infection elsewhere in Glamorgan, e.g. *S. stanley*, *S. panama*, *S. indiana* and *S. fischerkietz*.

From the technical aspect there are some points worth noting. The amount of information recorded in Table 1 would have been scanty if the serological method for separating serotypes had not been used (Harvey & Price, 1967*a*). The nearer the sampling point was to the actual focus of excretion in the sewerage system, the better the chance of an isolation. If we had confined our investigations to Point 1 (draining the whole estate), less than half the total number of serotypes would have been found. Without multiple sampling points, the number of phage-types of *S. typhimurium* discovered would have been greatly reduced. The regular finding of *S. paratyphi B*, phage-type 1, in Points 1 and 5 (Table 2) probably was due to the existence of a chronic carrier nearby. It was noticed that other serotypes were more readily isolated when points above 1 and 5 were examined. In such an investigation the best results are obviously obtained by using as many sampling sites as is conveniently possible.

The relationship between the isolations from human sewage and the isolation of identical serotypes from other local sources is given in Table 2. This table is merely an extension of work published previously (Harvey, 1957; Harvey & Phillips, 1961). The natural inference is that animal and human salmonellosis are closely inter-

related. We are not going to indulge in any speculation in this paper as to the ultimate source of salmonellosis. Neither shall we, at this stage, express an opinion as to vehicles of spread of the disease from animals to man. Further work on those points is obviously necessary.

SUMMARY

In a residential estate of 4000 persons, containing neither industry nor retail butchers shops, salmonellas were regularly found in the sewerage system. They were frequently found in the sewage of a portion of the estate housing 1000 persons. The range of serotypes found was wide and some types suggested an exotic origin. No overt salmonella infection in the estate was reported during the period of survey, although local general practitioners had been previously alerted. Overt infection due to serotypes found in the survey were, however, reported in other areas of Glamorgan. Multiple sampling points in the sewerage system and a serological technique for examining samples contaminated with multiple salmonella serotypes were essential for the technical success of the survey.

We should like to thank Prof. Scott Thomson for his advice in the preparation of this paper; Dr E. S. Anderson of the Central Enteric Reference Laboratory and Bureau, Colindale, for phage-typing the strains of *S. typhimurium* and *S. paratyphi B*; and Dr G. J. G. King of the Public Health Laboratory, Bournemouth, for identifying the serotypes isolated. We should also like to thank Mr T. R. Liddington and Mr J. H. Price for their technical assistance.

REFERENCES

- HARVEY, R. W. S. (1956). Choice of a selective medium for the routine isolation of members of the salmonella group. *Mon. Bull. Minist. Hlth* **15**, 118.
- HARVEY, R. W. S. (1957). The epidemiological significance of sewage bacteriology. *Br. J. clin. Pract.* **11**, 751.
- HARVEY, R. W. S. (1965). A study of the factors governing the isolation of salmonellae from infected materials and the application of improved techniques to epidemiological problems. M.D. Thesis, University of Edinburgh.
- HARVEY, R. W. S. & PHILLIPS, W. P. (1955). Survival of *Salmonella paratyphi B* in sewers: its significance in investigation of paratyphoid outbreaks. *Lancet* *ii*, 137.
- HARVEY, R. W. S. & PHILLIPS, W. P. (1961). An environmental survey of bakehouses and abattoirs for salmonellae. *J. Hyg. Camb.*, **59**, 93.
- HARVEY, R. W. S. & PRICE, T. H. (1967*a*). The examination of samples infected with multiple serotypes. *J. Hyg., Camb.* **65**, 423.
- HARVEY, R. W. S. & PRICE, T. H. (1967*b*). The isolation of salmonellas from animal feeding stuffs. *J. Hyg., Camb.* **65**, 237.
- HARVEY, R. W. S. & PRICE, T. H. (1968). Elevated temperature incubation of enrichment media for the isolation of salmonellas from heavily contaminated materials. *J. Hyg., Camb.* **66**, 377.
- HARVEY, R. W. S., PRICE, T. H., BATE, W. & ALLEN, D. R. (1963). An outbreak of food poisoning caused by *Salmonella typhi-murium*, phage-type 12, probably spread by infected meat. *J. Hyg., Camb.* **61**, 419.
- HARVEY, R. W. S., PRICE, T. H. & DIXON, J. M. S. (1966). Salmonellas of subgenus III (Arizona) isolated from abattoirs in England and Wales. *J. Hyg., Camb.* **64**, 271.
- LE MINOR, L., LE MINOR, S., BARBE, E., COLARD, N. & LERAT, M.-TH. (1967). Activités du Centre français des *Salmonella* de l'Institut Pasteur (5^e rapport 1964-1966). *Revue Hyg. Méd. soc.* **15**, 221.

- MOORE, B. (1948). The detection of paratyphoid carriers in towns by means of sewage examination. *Mon. Bull. Minist. Hlth* **7**, 241.
- PARKER, M. T. (1954). Symposium on human and animal sources of gastro-intestinal infection. (a) The spread of some bowel infections from human sources. *Roy. Soc. Hlth J.* **74**, 847.
- VERNON, E. (1967). Food poisoning in England and Wales, 1966. *Mon. Bull. Minist. Hlth* **26**, 235.