J. Hyg., Camb. (1977), **78**, 439
Printedin Great Britain

Salmonella surveillance with reference to pigs – Cardiff abattoir, 1968–1975

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(*Received* 22 *November* 1976)

SUMMARY

Between 1968 and 1975, well over 3000 samples of pig faeces and 407 samples of pig mesenteric glands were examined for salmonellas. A very wide range of serotypes was isolated. The material from this survey was used to evaluate certain aspects of salmonella isolations. The factors studied were: selective motility enrichment, size of inoculum of faeces, comparison of drain swab with animal sample and comparison of selenite F broth with Muller–Kauffmann tetrathionate broth. The conclusions drawn from these investigations allowed an efficient routine to be developed during the survey.

INTRODUCTION

To examine in detail is a definition of the verb 'to survey'. The qualification is important as the value of a survey is influenced by the effort expended in an examination of factors which may affect the results. The present paper records such a study on salmonella carriage in pigs.

Routine salmonella surveillance in absence of outbreaks of infection started in Cardiff in 1953 when foci of paratyphoid B fever were identified in the City using sewer swabs (Moore, 1948). These foci were plotted on a large-scale map and an attempt was made to associate their geographical position with the occurrence of local paratyphoid B outbreaks. The swab monitoring technique was later extended to detecting salmonellas in bakeries, abattoirs, butcher's shops, markets and human sewage (Harvey & Price, 1970). The drain swab, therefore, became a natural sample to monitor salmonellas in Cardiff.

The current study investigates the importance of the material selected and the bacteriological method used in the selective culture of the samples.

MATERIALS AND METHODS

The materials examined for salmonellas were drain swabs, pig caecal faeces, pig caecal swabs and pig mesenteric glands.

The preparation of the media used has been described elsewhere (Harvey & Price, 1974).

The efficiency of certain salmonella isolation techniques was investigated in a series of comparative studies:

Comparison of direct enrichment in selenite F broth with selective motility enrichment

The material was pig caecal faeces and the inoculum cultured was 80 g. This quantity of faecal matter was expressed through a slit in the sterilized caecal wall into 100 ml of selenite F broth. The enrichment medium and contents were incubated at 43 °C for 24 h. Subcultures were made on deoxycholate citrate agar and brilliant green MacConkey agar (Harvey, 1956). Plates were incubated at 37 °C for 24 h. The brilliant green agars were examined and suspicious colonies picked for further study. Selective motility enrichment (Harvey & Price, 1974) was performed from deoxycholate citrate agar plates by wiping off the surface growth with a short cotton wool tipped swab. The culture coated swab was placed in the inner tube of a modified Craigie tube and incubated at 37 °C for 24 h. Microbial growth progressed down the inner tube and up to the agar surface outside. This surface growth was subcultured on brilliant green MacConkey agar. The brilliant green agar was incubated at 37 °C for 24 h and examined for colonies resembling salmonellas. These were picked for further study.

Culture of caecal faeces and caecal swabs from the same pig

In 1959, Newell *et al.* demonstrated the advantages of culture of pig caecal faeces over culture of caecal swabs for isolating salmonellas. In 1965, Harvey drew attention to a significant increase in the information obtained on excretion of *S. paratyphi B* from examination of large inocula of human faeces compared with that derived from small inocula. It appeared advisable to study salmonella carriage in pigs using inocula of two different sizes. Amounts chosen were 0·5 g and 80 g. The smaller weight was the average quantity of faeces adhering to a caecal swab, the larger was the average weight of faecal matter conveniently squeezed through a slit in the sterilized caecal wall. Swabs were cultured in 10 ml of selenite F and faeces in 100 ml of the same fluid medium. The incubation temperature used was 43 °C. The time of incubation was 24 h and subcultures were then made on brilliant green MacConkey agar. Plates were incubated at 37 °C for 24 h and examined for suspicious colonies, which were picked for further study.

Comparison of information obtained from examination of abattoir drain swabs and pig samples over the same time period

As indicated in the Introduction, drain swabs had been used successfully for many years to monitor salmonellosis in Cardiff. The current survey provided an opportunity to compare information gained from drain swabs and pig samples over the same period of time. Between October 1968 and May 1969, 24 abattoir drain swabs were examined in parallel with 24 batches of pig caeca. There were approximately 30 caeca in each batch. Drain swabs were covered with selenite F broth and 80 g of faeces from each caecum were added to 100 ml of the same medium. Screw-capped jars of 8 fl. oz. capacity (227 ml) were used for both types of specimen. Samples were incubated at 43 °C for 24 h and subcultured on brilliant green MacConkey agar. These plates were incubated at 37 °C for 24 h and examined for salmonellas.

Comparison of selenite F broth and Muller–Kauffmann tetrathionate broth for isolation of salmonellas from pig faeces

The decision to make this comparison stemmed from the development of elevated temperature enrichment for isolating salmonellas (Rodet, 1889; Vincent, 1890; Harvey & Thomson, 1953; Harvey & Price, 1968).

In 1968, Edel & Kampelmacher produced evidence suggesting that elevated temperature incubation was suitable for salmonella enrichment using Muller–Kauffmann tetrathionate broth and recommended a standard technique based on this principle (Edel & Kampelmacher, 1969). These workers used commercial Muller–Kauffmann tetrathionate: first a modified Oxoid CM 29 and later Oxoid CM 343.

In Cardiff, our experience with Oxoid CM 29 was disappointing (unpublished) and our testing of Oxoid CM 343 against an alternative Muller–Kauffmann tetrathionate (Harvey & Price, 1974) indicated that the commercial medium was inhibitory. It would not allow multiplication of small numbers of salmonellas (Harvey, Price & Crone, 1975). The Cardiff tetrathionate broth, incubated at 43 °C would, however, permit growth of minimal numbers of salmonellas as would our seitz-filtered selenite F broth incubated at the same temperature. These two media were, therefore, chosen for a prolonged comparative study in the isolation of salmonellas from pig faeces. An earlier investigation of a similar nature using sewage-polluted natural water as test material has been published (Harvey & Price, 1976).

In the current study equal weights of pig faeces (80 g) were introduced into selenite F broth and Muller–Kauffmann tetrathionate broth in 8 fl. oz. (227 ml) screw-capped jars. The amount of enrichment media used was 100 ml. Incubation was carried out at 43 °C for 24 h. Subcultures were made on deoxycholate citrate agar. Plates were incubated at 37 °C for 24 h and were subjected to the selective motility technique described in study No. 1. After passage of the growth from deoxycholate plates through a Craigie tube, subcultures were made on brilliant green MacConkey. The brilliant green plates were incubated at 37 °C for 24 h and were examined for salmonellas.

Comparison of selenite F broth and Muller–Kauffmann tetrathionate broth for the isolation of salmonellas from pig mesenteric lymph glands

A similar comparison to the faecal study was made using 10 g of mesenteric lymph glands as inoculum. As in the previous investigation, samples were paired. The same two enrichment media were used in 100 ml quantities. Incubation was carried out at 43 °C for 24 h and subcultures were made on deoxycholate citrate agar. After these plates were incubated at 37 °C for 24 h, the selective motility technique was used as before. Craigie tubes were incubated at 37 °C for 24 h and were subcultured on brilliant green MacConkey agar. The brilliant green agars were incubated at 37 °C for 24 h and examined for salmonella colonies.

Table 1. *Comparison of direct enrichment in selenite F with selective motility enrichment*

Direct enrichment	Selective motility enrichment	
+	+	53
+	−	2
−	+	23
−	−	736

Material examined: 814 caecal faeces from pigs.

Table 2. *Comparison of caecal faeces* (80 g *inoculum*) *with caecal swabs* (0·5 g *inoculum*). *Enrichment medium, selenite F*

80 g inoculum	0·5 g inoculum	
+	+	30
+	−	48
−	+	10
−	−	726

Material examined: 814 caecal faeces and caecal swabs. Samples were paired, and each pair came from one pig.

RESULTS

The results are presented in Tables 1–7.

Tables 1–6 record the findings in the comparative trials. Table 7 lists the salmonella serotypes isolated and records infection in man caused by the serotypes during the course of the survey. The tables are self-explanatory.

DISCUSSION

Table 1 demonstrates the efficiency of selective motility enrichment in the isolation of salmonellas from pig faeces. The principle of the method is well attested and documented (Carnot & Garnier, 1902; Friedburger, 1919; Wassén, 1930; Pijper, 1952; Stuart & Pivnick, 1965). There is, however, some disagreement on the explanation of the phenomenon. We initially employed this technique for isolating salmonellas from drain swabs, sewage-polluted natural water and animal feed (Harvey, Mahabir & Price, 1966; Harvey & Price, 1967).

The current study was the first occasion on which it had been used in an animal survey in our laboratory. The success of the technique ensured that selective motility was used throughout most of the entire period studied. It is probable, however, in this investigation that some isolations of salmonellas were missed by the selective motility technique when we were dealing with non-motile strains or strains with poorly developed motility. Our more recent experience with human and animal food emphasizes that direct subculture from enrichment media and selective motility enrichment are complementary techniques, and that neither method should be used exclusively.

Table 2 confirms the advantages of adequate inoculation of the enrichment

Table 3. *Serotypes isolated from caecal faeces and caecal swabs*

Serotype	No. isolated from faeces	No. isolated from swabs
S. bredeney	10	9
S. chester	38	17
S. dublin	7	3
S. goettingen	1	3
S. hvittingfoss	1	0
S. indiana	5	1
S. minnesota	1	0
S. muenchen	1	0
S. reading	1	0
S. stanley	1	0
S. typhimurium, 1 var. 5	1	0
S. typhimurium, 1*a*	1	1
S. typhimurium, U157	1	0
S. typhimurium, U19	1	0
S. typhimurium, 29	5	2
S. typhimurium, 18	1	0
S. typhimurium, 1*b*	1	1
S. typhimurium, 4	1	1
S. –4, 12:d:–	5	3
No. of serotypes isolated	19	10

Figures after *S. typhimurium* are phage types.

medium. It is interesting that 10 samples were found positive with the 0·5 g inoculation and negative with the 80 g inoculation. Other authors have noted negative results associated with over-inoculation of enrichment broths (Rappaport, Konforti & Navon, 1956). In general, we favour a heavy inoculation in salmonella isolation techniques, particularly if elevated temperature enrichment is used. The optimum time of subculture from selenite F broth is also dependent on the size of inoculum (Leifson, 1936), and some of the 10 negative results in Table 2 might have been found positive if a 48 h subculture time from the selenite broth had been used. Table 3 emphasizes the increased information obtained for the pig survey by use of large inocula of faeces. Nineteen serotypes were isolated from caecal faeces and only 10 from the corresponding swabs.

Table 4 compares information drawn from culture of abattoir drain swabs and that derived from examination of caecal faeces from pigs. In Cardiff, we have used drain swabs for surveys for many years and this type of sample was extremely valuable in the study of bakehouses where diplomacy was necessary and undue interference with staff and bakehouse routine was to be avoided (Harvey, 1957). It was natural to extend the technique to abattoirs and other premises (Harvey & Price, 1970). Nevertheless, in the current study we though it necessary to examine information gained from the swab sample and the animal sample over the same time period.

The comparison is of course unfair as it contrasts data from a single weekly specimen with approximately thirty weekly specimens. Even if the number of the two contrasted samples taken in a week were the same, we doubt if drain swabs could provide equal survey information to that obtained from animal faeces. In

Table 4. *Comparison of information obtained from abattoir drain swabs and pig caecal faeces*, 12. x. 68–24. v. 69

Week ending	Drain swab isolations	Caecal faeces isolations
12. x. 68	—	*S. infantis; S. typhimurium*, untypable
19. x. 68	*S.* unidentified	*S. dublin*
26. x. 68	*S. indiana*	*S. typhimurium*, 1 var. 5; *S. dublin; S. indiana*
2. xi. 68	*S. dublin*	*S. typhimurium*, 1*a*; *S. stanley; S. hvittingfoss*
16. xi. 68	—	*S.* –4, 12:d:–; *S. minnesota; S. goettingen; S. dublin*
23. xi. 68	*S. dublin*	*S. indiana*
30. xi. 68	*S. dublin*	*S. bredeney*
7. xii. 68	*S. dublin*	*S. typhimurium*, U157
14. xii. 68	*S. dublin*	*S. dublin*
4. i. 69	—	*S. typhimurium*, U157, 29
11. i. 69	*S. dublin*	—
18. i. 69	*S. dublin*	*S. dublin*
25. i. 69	*S. chester*	*S. chester; S. muenchen*
1. ii. 69	*S. chester*	*S. chester*
8. ii. 69	*S. chester; S. dublin*	*S. typhimurium*, 18; *S. chester*
15. ii. 69	*S. chester; S. dublin*	*S. typhimurium*, U163
1. iii. 69	—	*S. typhimurium*, U163, 4; *S.* –4, 12:d:–; *S. senftenberg; S. livingstone*
8. iii. 69	*S. dublin*	—
5. iv. 69	*S. dublin*	*S. reading*
19. iv. 69	—	*S. typhimurium*, 4; *S. dublin*
26. iv. 69	*S. dublin*	*S. dublin*
10. v. 69	*S. dublin*	—
19. v. 69	*S. dublin*	—
24. v. 69	—	*S. typhimurium*, 1*a*
Serotypes isolated	4	24

Table 5. *Isolation of salmonellas from pig caecal faeces using selenite F broth and Muller–Kauffmann tetrathionate broth*, 8. vii. 70–19. xii. 73

Selenite F broth	Tetrathionate broth	
+	+	172
+	–	140
–	+	82
–	–	3036

Total paired samples, 3430.
Positive samples, 394 (11·5 %).
Number of strains of *S. typhimurium* isolated from selenite F only, 52.
Number of strains of *S. typhimurium* isolated from tetrathionate only, 29 ($P < 0{\cdot}02$).
Number of strains of *S. dublin* isolated from selenite F only, 24.
Number of strains of *S. dublin* isolated from tetrathionate only 4 ($P < 0{\cdot}01$).
In absolute numbers of salmonella isolations from pig faeces selenite F broth is more efficient than tetrathionate broth ($P < 0{\cdot}01$) (cf. Table 6).

Table 6. *Isolation of salmonellas from pig mesenteric glands using selenite F broth and Muller–Kauffmann tetrathionate broth*, 8. i. 71–29. vii. 75

Selenite F	Tetrathionate	
+	+	36
+	−	9
−	+	33
−	−	329
Total paired samples		407

Muller–Kauffmann tetrathionate broth is more efficient than selenite F broth for salmonella isolation from pig mesenteric glands (cf. Table 5). $P < 0{\cdot}01$.

Table 7. *Salmonella serotypes in pigs, Cardiff abattoir* 1968–1975

(Each asterisk indicates isolation of a serotype in the same year from man and pigs.)

Serotype	Faeces	Glands	Serotype	Faeces	Glands
S. agama	2	0	*S. reading*	1	0
S. abony	1	0	*S. saintpaul***	14	2
*S. agona****	12	5	*S. senftenberg*	4	1
S. altona	3	2	*S. stanley***	5	0
*S. anatum**	8	0	*S. stanleyville**	7	1
S. aragua	1	0	*S. tennessee*	1	0
Arizona 26:29:30	1	0	*S. typhimurium*, 1*	0	2
*S. brandenburg**	1	0	*S. typhimurium*, 1*a****	50	6
*S. bredeney***	19	2	*S. typhimurium*, 1*b*	2	0
S. chester	39	0	*S. typhimurium*, 3*a**	4	0
S. cubana	4	1	*S. typhimurium*, 4*	9	1
*S. derby****	47	13	*S. typhimurium*, 9*	4	1
*S. dublin*****	49	6	*S. typhimurium*, 12	0	1
S. eimsbuettel	1	0	*S. typhimurium*, 18	1	0
S. enteritidis, 1*	4	1	*S. typhimurium*, 29	4	0
S. enteritidis, 8***	3	3	*S. typhimurium*, 1 var. 5**	4	1
S. enteritidis, 9	1	0			
S. enteritidis, 11	1	0	*S. typhimurium*, U19	1	0
S. enteritidis, untyped	0	1	*S. typhimurium*, U20**	2	3
S. fischerkietz	4	0	*S. typhimurium*, U40	17	1
S. give	14	1	*S. typhimurium*, U65	1	1
S. goettingen	3	0	*S. typhimurium*, U71	3	0
*S. heidelberg****	59	5	*S. typhimurium*, U121**	47	5
S. hvittingfoss	1	0	*S. typhimurium*, U157	3	0
*S. indiana****	18	2	*S. typhimurium*, U163**	3	0
*S. infantis****	10	3	*S. typhimurium*, U165	4	0
S. johannesburg	7	0	*S. typhimurium*, U193	1	0
S. kapemba	11	2	*S. typhimurium*, U234	0	0
*S. livingstone**	3	0	*S. typhimurium*, U239*	15	7
*S. meleagridis**	1	0	*S. typhimurium*, U258*	1	0
S. minnesota	1	0	*S. typhimurium*, U267*	0	1
*S. montevideo**	4	5	*S. typhimurium* RDNC(1)**	4	1
S. muenchen	1	0	*S. typhimurium*, untyped	18	1
S. newington	2	1	*S.* unidentified (rough)	1	1
*S. newport**	1	1	*S.* 4, 12:b: −	1	0
*S. ohio**	2	1	*S.* −4, 12:d: −**	21	2
*S. panama****	28	2			

https://doi.org/10.1017/S0022172400056333 Published online by Cambridge University Press

South Wales, *S. dublin* infection is endemic in cattle and this serotype dominates the isolations from drain swabs. The geographical position of drains in abattoirs is not planned for the convenience of microbiologists wishing to sample faeces from a particular animal species. Since the advantages of the animal sample over the drain swab were demonstrated we have ceased to use swabs to survey abattoirs.

Table 5 records the significant advantage selenite F has over Muller–Kauffmann tetrathionate broth in absolute numbers of salmonella isolations of all serotypes and in the number of isolations of *S. typhimurium* and *S. dublin*. A bias favouring isolation of *S. typhimurium* has recently been demonstrated for selenite F (Harvey & Price, 1976). A similar bias has also been published for *S. dublin* (Harvey & Price, 1975). It must be emphasized that the current comparison was made without a pre-enrichment stage. Pre-enrichment techniques in standard salmonella isolation procedures are now accepted as valuable (ISO 3565, 1975) but the current survey was started before they began to be recommended for 'wet' samples and we did not wish to study their effect in this particular investigation. We have some evidence that the relative efficiency of selenite and tetrathionate would be reversed if pre-enrichment were used. This is the subject of a further study.

The results recorded in Table 6 are in sharp contrast to Table 5 in that the tetrathionate broth produced significantly better results than selenite F. As the media used were identical with those employed for faecal samples this finding cannot be explained by media variation. We have made the point previously that using two complementary enrichment broths is better than using only one (Harvey & Price, 1976).

Table 7 demonstrates the very wide variety of serotypes isolated from pigs. A representative from each strain was sent for confirmation to the Salmonella and Shigella Reference Laboratory, London. The serotype range isolated in Cardiff is in contrast to that recorded in a recent paper where the pigs examined were clinically ill (Osborne, 1976). We had no reason to believe that the animals which we were investigating in Cardiff were other than healthy.

The association between salmonellosis in pigs and animal feed has been the subject of an earlier publication involving several laboratories (P.H.L.S. Working Group, Skovgaard & Neilson, 1972). In that paper the role of the pig as a potential source of human salmonellosis was briefly discussed. The current study is an extension of that investigation and seeks to clarify the importance of certain technical factors in abattoir surveillance.

We should like to thank the Directors and staff of the Salmonella and Shigella Reference Laboratory and the Enteric Reference Laboratory for serotyping and phage typing the salmonellas isolated.

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