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Salmonella isolations in abattoirs in Greece

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SUMMARY

The prevalence of salmonellas in drain swabs from three abattoirs of Athens was studied with the use of conventional methods of *Salmonella* isolation as well as with a new procedure which involves secondary enrichment from the usual selenite broth or Muller–Kauffmann's tetrathionate broth in Rappaport's medium slightly modified.

In all groups studied the secondary enrichment in Rappaport's medium led to an increase in the number of positive swabs, in the number of *Salmonella* serotypes, and in the total number of strains isolated.

The frequency of *Salmonella* isolations was higher in samples from abattoirs killing only pigs and lower in samples from abattoirs killing only cattle or only sheep.

The predominant serotype in abattoirs dealing with cattle was *Salmonella tennessee*, and *S. typhimurium* in abattoirs dealing with sheep. No predominant serotype was found in samples from abattoirs dealing mostly with pigs.

S. abony, *S. drypool*, *S. emek*, *S. indiana*, *S. muenchen* and *S. tennessee* were isolated for the first time in Greece.

INTRODUCTION

The prevalence of salmonellas in abattoirs has been studied by several investigators. In these studies different enrichment and selective media were used but the incubation temperature was usually 37° C. (Jones, Bennet & Ellis, 1961; Harvey & Phillips, 1961; Valette, 1961; Report, 1964; Papadakis, 1965; Papadakis, 1968; and others). Harvey & Thompson (1953), however, who used selenite F broth, reported a better yield of salmonellas, especially from river water and sewage, when the enrichment medium was incubated at 43° C. Since then, this incubation temperature has been used with profit by some authors (Georgala & Boothroyd, 1964; Harvey & Price, 1968; Harvey, Price, Foster & Griffiths, 1969). The advantage of incubating the enrichment media at 43° C. was also evident in a recent comparative study of *Salmonella* isolations from faeces and minced meat in eight European laboratories (Edel & Kampelmacher, 1968).

From a different point of view, several investigators have reported that a secondary enrichment in selenite broth or tetrathionate broth also tends to increase the yield of salmonellas from food or highly contaminated material (Jameson, 1962, 1963; Edel & Kampelmacher, 1968; Martin & Ewing, 1967; Harvey & Price,

1967; and others). In this paper, we report our findings from a comparative evaluation of the isolation of salmonellas from abattoir drains by three different procedures: first, by the use of conventional enrichment media incubated at 37° C.; secondly, by conventional enrichment media incubated at 43° C.; and thirdly, after secondary enrichment in Rappaport's medium. This medium, while allowing a satisfactory growth of salmonellas, is strongly inhibitory to the usual competing organisms (Rappaport, Konforti & Navon, 1956; Vassiliadis, 1968). The frequency of isolation of the various salmonella strains and serotypes in abattoirs in Greece according to species of slaughtered animals is also reported.

MATERIAL AND METHODS

Laboratory technique

The following media have been used: mannitol–selenite F broth (Oxoid), Muller–Kauffmann's tetrathionate broth (MK) containing 1/100,000 of brilliant green and Rappaport's medium (Rappaport *et al.* 1956). In the preparation of the last, only 2·5 ml. of 'solution C' instead of 3 ml. were added to 100 ml. of 'solution A'. We introduced this modification because we have noticed that this amount of malachite green, while still inhibitory to competing organisms, allows a better growth of salmonellas. Enrichment cultures in selenite broth were incubated at 37° C. for 2 days; those in MK broth were incubated at 37° C. and, in a great number of samples, at 43° C. as well, also for 2 days. All secondary enrichments were made in Rappaport's medium, by transferring into it an inoculum, with a 3–4 mm. loop, from the primary enrichment media. Incubation of all the secondary enrichments was made at 37° C. for 1 day. The enrichment and secondary enrichment media were subcultured either on Wilson and Blair's bismuth sulphite agar modification (Mackie & McCartney, 1956) (for 74 drain swabs of groups B and C—see Tables 2 and 3) or on Hynes's deoxycholate-citrate agar (Hynes, 1942) (for 20 drain swabs of group A—see Table 1).

Drain swabs from abattoirs

Abattoir drains were examined by Moore's gauze-swab technique (Moore, 1948). Swabs were placed for a period of 2–4 days in drains of three abattoirs in Athens. In one of these abattoirs there were three different compartments in which cattle, sheep (and very few goats) and pigs were separately killed. Independent drains, however, existed for only the first two compartments, while the drain of the pig compartment received the draining of the second one. In another abattoir, pigs were almost exclusively slaughtered. In a third abattoir cattle and sheep (and very few goats) were killed in one compartment with an independent drain and pigs in another compartment whose drain received the draining of the former. A total of 94 abattoir drain swabs were examined and distributed according to their origin as follows: 14 swabs from abattoir compartments killing only pigs; 38 from compartments killing only cattle; 34 from compartments killing only sheep (and a few goats); and the remaining eight swabs from abattoir compartments in which more than one animal species was slaughtered. No disinfectant was used in any of these abattoirs during the washing.

The drain swabs were divided, in the laboratory, into two equal parts, each of which was introduced into a screw-capped jar containing the enrichment medium.

Our examinations were carried out between March 1969 and February 1970, but they were interrupted for about 2½ months during the summer. The approximate number of animals slaughtered during the days the swabs were left in position was as follows: 1900 pigs, 3800 cattle, 30,000 sheep, and 300 goats. All the cattle, pigs and goats were raised in various parts of Greece, while the great majority of the slaughtered sheep were imported from Hungary.

RESULTS

Isolations in relation to the procedure employed

The 94 abattoir drain swabs were classified into three groups, A, B and C.

Group A drain swabs

In this group, 20 drain swabs were examined by enrichment in selenite broth and in tetrathionate broth incubated at 37° C., followed by secondary enrichment in the modified Rappaport's medium. During the 2-month period that the swabs of group A were left in position in the abattoirs, 350 cattle, 4000 sheep (and goats)

Table 1. Salmonella *isolations from 'group A' abattoir drain swabs*

(Among the 20 large swabs of 'group A' 11 were negative in all procedures.)

No. of isolated strains by serotype	Enrichment medium*			
	S 37° C.	MK 37° C.	S 37° C./R	MK 37° C./R
S. abony	0	0	1	1
S. braenderup	0	0	0	1
S. indiana	0	0	1	1
S. senftenberg	0	0	0	1
S. tennessee	0	0	1	2
S. typhimurium	0	0	1	5
Total no. of isolated strains	0	0	4	11
Total no. of serotypes	0	0	4	6
Total no. of positive swabs	0	0	4	9
Swabs positive as percentage of total	0	0	20·0	45·0

* S 37° C. or MK 37° C. = enrichment in selenite broth or Muller–Kauffmann's tetrathionate broth, incubated at 37° C. for 48 hr.; S 37° C./R or MK 37° C./R = secondary enrichment in Rappaport's broth from selenite broth or MK broth respectively. The growths from the enrichment and secondary enrichment media were subcultured on Hynes's modification of deoxycholate citrate agar.

and 300 pigs were killed. The results of the isolations in this group are shown in Table 1. The secondary enrichment in Rappaport's medium led to a significant number of salmonella isolations particularly when combined with primary enrichment in MK tetrathionate broth. It should be noted that no salmonellas were isolated when only selenite or tetrathionate broth was used as enrichment medium.

This complete failure may be attributed to the use, in this particular group of examinations (group A), of large swabs which, when added to the enrichment media, covered more than 45 % of the total volume. As a result, an abundance of competing bacteria were present in the subcultures on the Hynes's agar, while these germs were inhibited to a great extent by the secondary enrichment in Rappaport's broth.

Two different serotypes from a single swab were isolated twice, while in seven other instances, only one serotype was isolated from every positive swab.

Table 2. Salmonella *isolations from 'group B' abattoir drain swabs*

(Among the 21 regular swabs of 'group B' 11 were negative in all procedures.)

No. of isolated strains by serotype	Enrichment medium*			
	S 37° C.	MK 37° C.	S 37° C./R	MK 37° C./R
S. braenderup	0	0	2	1
S. derby	2	0	0	0
S. senftenberg	1	0	1	0
S. tennessee	0	3	1	4
S. typhimurium	0	1	2	2
Total no. of isolated strains	3	4	6	7
Total no. of serotypes	2	2	4	3
Total no. of positive swabs	3	4	6	7
Swabs positive as percentage of total	14·3	19·0	28·6	33·3

* See footnote on Table 1.

The growths from the enrichment and secondary enrichment media were subcultured on Wilson and Blair's bismuth sulphite agar.

Group B and group C drain swabs

In these groups the volume of the swab added to the enrichment media did not exceed 20 % of the total volume and all subcultures from enrichment and secondary enrichment media were made on bismuth sulphite agar. During the 2-month period that the swabs of group B were left in position in the abattoirs, the approximate number of killed animals was 700 cattle, 11,400 sheep (and goats) and 300 pigs while, during the 4½ month period that the swabs of group C were left in position, the number of slaughtered animals was approximately 1300 pigs, 2750 cattle and 14,900 sheep (and goats). The results of the examinations are summarized in Tables 2–4. It can be seen from these tables that secondary enrichment in Rappaport's medium increases the frequency of *Salmonella* isolations from abattoir drain swabs to a considerable extent. In addition, our results show the superiority of 43° C. over 37° C. as the incubation temperature for enrichment in Muller–Kauffmann's tetrathionate broth.

Among the 10 positive swabs of group B, seven yielded one serotype each and the remaining three two serotypes each. Among the 47 positive swabs of group C, 27 yielded one serotype, 16 two serotypes, 3 three serotypes and the last one four serotypes.

Salmonella isolations in relation to the species of slaughtered animal

The frequency distribution of the serotypes and strains of salmonellas isolated from all drain swabs in relation to the species of killed animals is shown in Table 5. The frequency of positive swabs was higher in abattoir compartments killing only pigs and lower in abattoir compartments in which only cattle or sheep were killed.

Table 3. Salmonella *isolations from 'group C' abattoir drain swabs*

(Among the 53 regular swabs of 'Group C' 6 were negative in all procedures.)

No. of isolated strains by serotype	Enrichment medium*			
	MK 37° C.	MK 43° C.	MK 37° C./R	MK 43° C./R
S. abony	7	7	7	7
S. braenderup	3	2	1	0
S. bredeney	0	2	1	1
S. derby	2	1	5	2
S. drypool	2	2	1	1
S. emek	2	2	6	8
S. infantis	1	2	3	3
S. kottbus	1	1	1	2
S. meleagridis	0	0	1	0
S. muenchen	2	0	3	2
S. oranienburg	0	2	0	1
S. richmond	0	1	1	1
S. senftenberg	1	1	2	3
S. tennessee	8	10	7	9
S. typhimurium	4	4	7	7
S. westerstede	0	1	0	0
*S.*O-6, 7: unidentified	0	1	0	0
*S.*O-8: unidentified	0	0	1	1
Total no. of isolated strains	33	39	47	48
Total no. of serotypes	11	15	15	14
Total no. of positive swabs	32	39	43	45
Swabs positive as percentage of total	60·4	73·6	81·1	84·9

* MK 37° C. or MK 43° C. = enrichment in Muller–Kauffmann's tetrathionate broth incubated at 37°C. or 43°C. respectively; MK 37°C./R or MK 43°C./R = secondary enrichment in Rappaport's broth from MK enrichment incubated at 37 °C. or 43° C. respectively.

The growths from the enrichment and secondary enrichment media were subcultured on Wilson and Blair's bismuth sulphite agar.

It may also be added that *Salmonella* isolations were in general more frequent in group C than in group B. Although an explanation of this fact may be found in the correspondingly larger number of pigs and cattle killed, further exploration of the effect of the number of slaughtered animals by individual species on the frequency of salmonella isolations has not been possible because of the diversity of the conditions and the different laboratory techniques.

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Table 4. *Comparative evaluation of four procedures for* Salmonella *isolation from abattoir drain swabs*

I. MK 43° C. versus MK 37° C.	
Total number of swabs (group C)	53
Swabs positive on MK 43° C.	39
Swabs positive on MK 37° C.	32
Swabs positive on MK 43° C. and negative on MK 37° C.	9
Swabs positive on MK 37° C. and negative on MK 43° C.	2
χ^2 for paired samples with Yates correction	3·27 $P < 0·10$
II. MK 37° C./R versus MK 37° C.	
Total number of swabs (groups B and C)	74
Swabs positive on MK 37° C./R	50
Swabs positive on MK 37° C.	36
Swabs positive on MK 37° C./R and negative on MK 37° C.	14
Swabs positive on MK 37° C. and negative on MK 37° C./R	0
χ^2 for paired samples with Yates correction	12·07 $P < 0·001$
III. MK 43° C./R versus MK 43° C.	
Total number of swabs (group C)	53
Swabs positive on MK 43° C./R	45
Swabs positive on MK 43° C.	39
Swabs positive on MK 43° C./R and negative on MK 43° C.	6
Swabs positive on MK 43° C. and negative on MK 43 °C./R	0
χ^2 for paired samples with Yates correction	4·17 $P < 0·05$

Relative efficiency of the four compared procedures for *Salmonella* isolation from abattoir drain swabs group C, assuming efficiency of MK 37° C. = 100.
MK 37° C. = 100. MK 43° C. = 122. MK 37° C./R = 134 MK 43° C./R = 141.

DISCUSSION

It can be seen from Tables 1 to 4 that a secondary enrichment in Rappaport's medium, from either selenite broth incubated at 37° C. or Muller–Kauffmann's tetrathionate broth incubated at 37° C. or 43° C., led to a significant increase in the number of positive swabs as well as in the number of *Salmonella* strains isolated. The advantages of the secondary enrichment in Rappaport's broth were not substantially affected by the conditions and media of the primary enrichment. It may also be noted that the superiority of the procedures involving secondary enrichment in Rappaport's medium was apparent in all three groups of abattoir drain swabs (groups A, B and C), although the frequency of isolations was generally lowest in group A, presumably because of the excessive size of the swabs, and highest in group C, possibly because of the correspondingly larger number of pigs and cattle. The secondary enrichment in Rappaport's medium does not seem to alter the pattern of serotypes isolated. Although the data in Table 2 suggest that secondary enrichment favours the isolation of *S. braenderup* and does not favour the isolation of *S. derby*, the data in Table 3 point in the opposite direction and indicate the importance of chance factors.

As mentioned in the Introduction, other investigators have already shown that secondary enrichment in selenite broth or tetrathionate broth increases the relative efficiency of salmonella isolations. Although there is no strict comparability between the results of the various investigations and our examinations were so far

restricted to abattoir drain swabs, our results suggest that secondary enrichment in the slightly modified Rappaport medium may improve the efficiency of *Salmonella* isolations at least as much as secondary enrichment in other more conventional media.

Table 5. Salmonella *isolations from abattoir drain swabs according to the species of the slaughtered animals*

No. of isolated strains by serotype	Abattoirs killing only pigs	Abattoirs killing only cattle	Abattoirs killing sheep and goats	Abattoirs killing various species	All abattoirs
S. abony	1	3	4	0	8
S. braenderup	3	1	0	2	6
S. bredeney	0	0	1	1	2
S. derby	3	1	1	3	8
S. drypool	2	1	0	1	4
S. emek	2	2	1	3	8
S. indiana	1	0	0	0	1
S. infantis	1	0	0	2	3
S. kottbus	0	2	0	0	2
S. meleagridis	0	0	1	0	1
S. muenchen	1	0	0	3	4
S. oranienburg	1	0	0	1	2
S. richmond	0	0	1	0	1
S. senftenberg	3	2	0	1	6
S. tennessee	1	17	2	1	21
S. typhimurium	1	5	10	0	16
S. westerstede	1	0	0	0	1
*S.*O-6, 7: unidentified	0	0	0	1	1
*S.*O-8: unidentified	0	1	0	0	1
Total no. of isolated strains	21	35	21	19	96
Total no. of serotypes	13	10	8	11	19
Total no. of positive swabs	14	24	21	7	66
Total no. of swabs	14	38	34	8	94
Swabs positive as percentage of total	100·0	63·2	61·8	87·5	70·2
Isolated strains per positive swab	1·5	1·5	1·0	2·7	1·5

The favourable influence of incubation at 43° C. rather than at 37° C. upon the primary enrichment medium has been stressed in other publications already mentioned. The results of our series of examinations in group C of abattoir drain swabs confirm this superiority and suggest that this fact holds equally well for samples from abattoir drains enriched in MK medium.

The frequency of *Salmonella* isolations was highest in abattoir compartments in which almost exclusively pigs were killed, as compared to abattoir compartments in which only cattle or only sheep and goats were killed (Table 5). The proportion of positive swabs from abattoirs killing only pigs (14/14 or 100 %) differed significantly from the corresponding proportion in abattoirs killing only cattle (24/38 or 63·2 %; $\chi^2 = 5{\cdot}30$ $P < 0{\cdot}05$) and from that in abattoirs killing only sheep and

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goats (21/34 or 61·8%; $\chi^2 = 5{\cdot}54$ $P < 0{\cdot}05$). The importance of the findings is emphasized by the fact that the total number of slaughtered pigs was considerably smaller (1900) than the corresponding number for the cattle (3800) and the sheep and goats (30,300).

In the Report of the British Working Party of the Public Health Laboratory Service (Report, 1964), a different relation was found between the frequency of salmonella isolations and the species of animals killed. Thus, it was observed that salmonellas were isolated more often from abattoirs killing a large proportion of cattle and less often from abattoirs in which more sheep were killed, while the influence of the proportion of pigs killed proved more difficult to assess. This difference may be accounted for by the results of earlier studies in Greece which show that a high proportion of pigs, but a very low proportion of cattle, sheep and goats, were contaminated with salmonellas (Patéraki, Politi & Vassiliadis, 1966; Vassiliadis, Patéraki & Politi, 1969). It should be noted, however, that although the proportion of positive swabs was practically the same in abattoirs killing either only cattle or only sheep and goats, the number of strains isolated was considerably higher amongst the former (strains isolated per positive abattoir drain swab: cattle = 1·5; sheep = 1·0).

The highest number of different serotypes (13) was found among the 21 strains isolated from abattoir compartments killing only pigs. No predominance of any single serotype was noted in this group. By contrast, the serotype most frequently isolated (10 times) from abattoirs dealing with sheep was *S. typhimurium*, and *S. tennessee* the most frequently isolated (17 times) from those dealing with cattle.

The following serotypes were isolated for the first time in Greece: *S. abony*, *S. drypool*, *S. emek*, *S. indiana*, *S. muenchen* and *S. tennessee*.

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