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A comparison of enrichment media for the isolation of salmonellae from seagull cloacal swabs

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SUMMARY

The relative efficiency of three selective enrichment broths (Muller–Kauffmann tetrathionate, Rappaport's and selenite F) was investigated for the isolation of salmonellae from seagull cloacal swabs. Pre-enrichment in buffered peptone water was employed throughout the study, which involved the examination of 560 gulls, sixty (10·7 %) of which were found to be carrying salmonellae. Rappaport's broth as modified by Vassiliadis for incubation at 43 °C (Vassiliadis *et al.* 1976) yielded the highest number of positive swabs (57) and the widest range of serotypes. It was significantly more efficient that either selenite F or tetrathionate broth, although the results obtained with Rappaport's broth incubated at 37 and 43 °C did not differ significantly ($P > 0{\cdot}5$). Eleven serotypes were isolated during the study, the most prevalent being *Salmonella virchow*.

INTRODUCTION

Much work has been reported on the use of different selective enrichment media for the isolation of salmonellae from a variety of sources. Harvey, Price & Xirouchaki (1979) found Rappaport's broth incubated at 37 °C to be more efficient than either Muller–Kauffman tetrathionate (MKTB) or selenite F (SFB) broths for recovering salmonellae from sewage-polluted water, and Harvey & Price (1981) using the same incubation temperature demonstrated its superiority in isolating salmonellae from chicken giblets.

Vassiliadis and his co-workers (Vassiliadis *et al.* 1976) showed that a modified form of Rappaport's broth, suitable for incubation at 43 °C, allowed the growth of a wide range of salmonella serotypes, whilst having a much stronger inhibitory action on competing bacteria than other broths. This modification has been used to good effect in the isolation of salmonellae from a wide range of samples (Vassiliadis *et al.* 1977, 1978*a*, *b*, 1979).

Seagulls have been implicated as having a causative role in the infection of farm animals with salmonellae (Williams *et al.* 1977; Johnston, Maclachlan & Hopkins, 1979; Johnston *et al.* 1981) and a variety of workers have reported salmonella carrier rates in gulls of 7–31 % (Fennell, James & Morris, 1974; Williams, Richards & Lewis, 1976; Fenlon, 1981; Fricker, Girdwood & Munro in prep.). The differences

in the reported carrier rates may be attributable to a number of factors pertaining to the behaviour and feeding habits of the gulls, and also to the differing isolation procedures used by different groups of workers. The purpose of this study was to determine the most successful enrichment procedure for the isolation of salmonellae from seagull cloacal swabs.

MATERIALS AND METHODS

A total of 560 birds, consisting of herring (*Largus argentatus*), lesser black-backed (*L. fuscus*) and great black-backed (*L. marinus*) gulls, were captured by cannon netting, between March and May 1982. Each bird was cloacally swabbed using a plain cotton-wood swab, and the swab broken off into 10 ml buffered peptone water, before being transported back to the laboratory. After incubation at 37 °C for 20–24 h, each pre-enrichment culture was subcultured, using sterile Pasteur pipettes, into duplicate 10 ml volumes of selenite F and tetrathionate broths at an inoculation ratio of one in ten and to one bottle of each of two formulations of Rappaport's broth, that described by Vassiliadis *et al.* (1970) for incubation at 37 °C (RB 25) and a modification (RB 10) for use at 43 °C (Vassiliadis *et al.* 1976), at an inoculation ratio of one in two hundred. MKTB and SFB were prepared using a commercially available dehydrated base (Oxoid CM 343 and CM 395), whereas the two formulations of Rappaport's broth were prepared in the laboratory from individual constituents (Rappaport, Konforti & Navon, 1959; Vassiliadis *et al.* 1970, 1976).

MKTB and SFB were incubated at 37 and 43 °C, RB 25 at 37 °C and RB 10 at 43 °C. Each enrichment broth was subcultured at 24 and 48 h onto xylose lysine desoxycholate and desoxycholate citrate agars (Oxoid CM 469 and CM 227). The plating media were incubated at 37 °C for 20–24 h and up to four suspicious colonies from each plate were identified by standard biochemical and serological techniques. No attempt was made to enumerate the salmonella colonies yielded by the different procedures. The isolation procedure used is shown in Fig. 1.

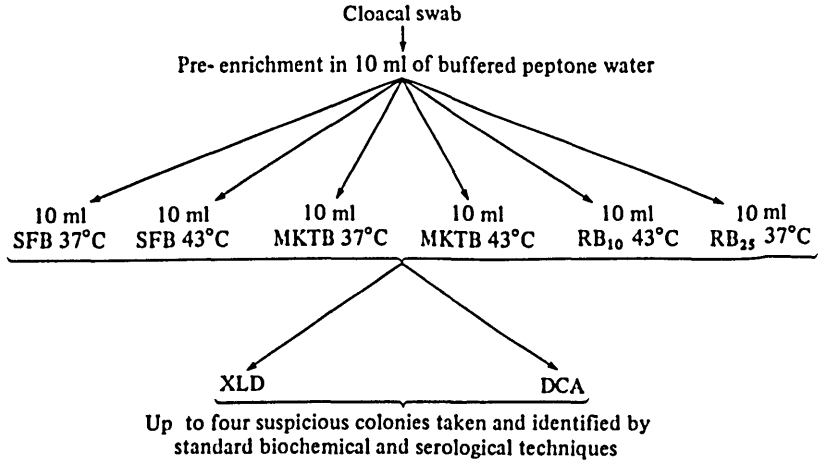

Fig. 1. Procedure for the isolation of salmonellae from seagull cloacal swabs.

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RESULTS

Sixty (10·7 %) of the 560 gulls examined were found to be carrying salmonellae by at least one of the procedures used. Table 1 shows the number of salmonella isolations from each of the six procedures compared and the time of subculture yielding a salmonella isolate.

Table 2 lists the salmonella serotypes and the number of strains isolated by each procedure. RB 10 yielded the greatest number of salmonella isolates. In addition, a greater number of different serotypes was recovered using this procedure.

Table 3 presents the data in terms of its statistical significance using MacNemar's test for paired samples. The results obtained with Rappaport's broth incubated at 37 and 43 °C, selenite F at 37 °C and tetrathionate at 43 °C are compared. The number of isolations using the different formulations of Rappaport's broth are not statistically significant ($P > 0{\cdot}5$) although both forms were significantly better than selenite or tetrathionate broths. Tetrathionate broth was superior to selenite.

Table 1. *Number of salmonella isolations from each of the six methods of enrichment*

	RB 10	RB 25	MKTB 37	MKTB 43	SFB 37	SFB 43
+ve 24 h, +ve 48 h	53	46	22	29	17	10
+ve 24 h, −ve 48 h	4	2	3	7	3	1
−ve 24 h, +ve 48 h	0	7	11	5	8	4
Total +ve	57	55	36	41	28	15
Percentage +ve	10·1	9·8	6·4	7·3	5·0	2·7

RB 10 – Rappaport's broth (modified) at 43 °C
RB 25 – Rappaport's broth at 37 °C
MKTB 37 – Muller–Kauffmann tetrathionate broth at 37 °C
MKTB 43 – Muller–Kauffmann tetrathionate broth at 43 °C
SFB 37 – Selenite F broth at 37 °C
SFB 43 – Selenite F broth at 43 °C

Table 2. *Number of strains of individual salmonella serotypes isolated using six different procedures of enrichment*

	RB 10	RB 25	MKTB 37	MKTB 43	SFB 37	SFB 43
S. derby	1	1	1	0	0	0
S. haardt	1	0	0	0	0	0
S. hadar	1	1	1	1	1	0
S. java	1	1	0	0	0	0
S. montevideo	5	5	3	3	2	2
S. saint-paul	1	2	1	1	0	0
S. schwarzengrund	1	0	0	0	0	0
S. stanley	5	6	4	5	4	1
S. typhimurium	9	8	7	7	5	2
S. virchow	31	30	19	24	16	10
S. 4, 12: d: −	1	1	0	0	0	0
Total	57	55	36	41	28	15

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Table 3. *Statistical comparison of four methods of enrichment using MacNemar's test for paired samples*

(The results compared are RB 10 (43°), RB 25 (37°), MKTB (43°) and SFB (37°).)

		χ^2	P
RB 10 +ve, RB 25 +ve	53		
RB 10 +ve, RB 25 −ve	4	0·16	> 0·5
RB 10 −ve, RB 25 +ve	2		
RB 10 +ve, SFB +ve	28		
RB 10 +ve, SFB −ve	29	26·9	< 0·001
RB 10 −ve, SFB +ve	0		
RB 10 +ve, MKTB +ve	39		
RB 10 +ve, MKTB −ve	18	5·6	< 0·025
RB 10 −ve, MKTB +ve	2		
RB 25 +ve, SFB +ve	28		
RB 25 +ve, SFB −ve	27	25·0	< 0·001
RB 25 −ve, SFB +ve	0		
RB 25 +ve, MKTB +ve	38		
RB 25 +ve, MKTB −ve	17	4·2	< 0·05
RB 25 −ve, MKTB +ve	3		
MKTB +ve, SFB +ve	24		
MKTB +ve, SFB −ve	17	6·9	< 0·01
MKTB −ve, SFB +ve	4		

DISCUSSION

Previous reports on the isolation of salmonellae from a number of sources, including pork sausages, minced meat, sewage, pig faeces (Vassiliadis *et al.* 1977, 1978*a*, *b*; 1979), sewage-polluted water (Harvey *et al.* 1979) and chicken giblets (Harvey & Price, 1981) have all recommended the use of Rappaport's broth. In support of these findings this study has shown that when used to recover salmonellae from seagull cloacal swabs, Rappaport's broth is significantly superior to either selenite F or Muller–Kauffmann tetrathionate broth, both in terms of the number of salmonella isolates and in the variety of serotypes obtained.

The difference between the two forms of Rappaport's broth, whilst not being statistically significant, may be important if multiple subculture of the enrichment broth is not practical. Although the timing of subculture from enrichment media has a role in salmonella isolation and multiple subculture may be an important part of technique in some circumstances (Harvey & Phillips, 1955; Grunnet, 1975; Harvey & Price, 1982), in this study we found that incubation of the RB 10 medium beyond 24 h yielded no further salmonella isolations. This is inconsistent with the findings of other workers (Vassiliadis *et al.* 1978; Harvey *et al.* 1979; Harvey & Price, 1981). All of the other procedures used yielded further isolations on subculture at 48 h. This must be considered as a point in favour of the use of Rappaport's broth at 43 °C, since early isolation markedly reduces the cost of examination of specimens. It should be noted that if the enrichment media had been subcultured at 48 h only, a number of isolates would have been missed.

The growth of competing organisms was not recorded during this study, but

Vassiliadis *et al.* (1976) have recorded a much greater inhibition of lactose- and sucrose-negative bacteria using RRB 10 at 43 °C whilst allowing a luxuriant growth of salmonellae. The effect of this must be to increase the probability of selecting a salmonella colony when examining the solid media.

The carriage of *S. typhi* in European gulls (Steiniger, 1970) and of subgenus III salmonellae in Australian silver gulls (Western Australia Public Health Department, 1977) has been reported. Although these serotypes are relatively infrequently experienced in the United Kingdom and to our knowledge have not been isolated from seagulls in Scotland, if these serotypes, or indeed *S. dublin* were being sought from gulls, an alternative enrichment medium such as selenite or tetrathionate broth should be employed, as Rappaport's medium is not suitable for the isolation of these particular serotypes (Rappaport, Konforti & Navon, 1959; Harvey, Price & Hall, 1973; Harvey & Price, 1975).

In spite of these reservations, Rappaport's broth incubated at 43 °C allowed the recovery of a wide range of salmonella serotypes in this study, and we would recommend its use for the isolation of salmonellae from seagull cloacal swabs.

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