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Comparison of selenite F, Muller-Kauffmann tetrathionate and Rappaport's medium for salmonella isolation from chicken giblets after pre-enrichment in buffered peptone water

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SUMMARY

Six hundred and eighty three samples of chicken giblets were examined for salmonellas. Three hundred and forty nine of these were neck and crop specimens and 334 were combined liver and heart samples. Two hundred and ten, in all, contained salmonellas.

The technique of examination included pre-enrichment in buffered peptone water at 37 °C for 18 h and subculture to three enrichment media: Muller-Kauffmann tetrathionate, selenite F and Rappaport's magnesium chloride malachite green broth. Inocula from buffered peptone water to 10 ml of tetrathionate and selenite were 1 ml in each case. The inoculum from the pre-enrichment medium to 10 ml of Rappaport was 0·005 ml. Tetrathionate and selenite were incubated at 43 °C for 48 h. Rappaport's medium was incubated at 37 °C for 48 h. Subcultures from all three enrichment broths were made at 24 h and 48 h to brilliant green MacConkey agar. Selective agars were incubated at 37 °C for 24 h.

The most successful technique for salmonella isolation used Rappaport's medium, which was significantly more efficient than either tetrathionate or selenite. This finding reinforces results obtained using sewage polluted natural water as test material and it is suggested that routine examination of environment samples for salmonellas could be based on Rappaport's medium alone.

If *S. typhi*, *S. dublin* or subgenus III salmonellas were likely to be present in the sample, the technique described here would require modification.

INTRODUCTION

Harvey, Price & Xirouchaki (1979*a*) found Rappaport's magnesium chloride, malachite green medium to be more efficient than either Muller-Kauffman tetrathionate or selenite F for isolating salmonellas from sewage polluted natural water. Pre-enrichment in buffered peptone water was used in the study. We wished to determine, in the current investigation, the relative success of the same three media using naturally contaminated poultry samples as test material. The object of the trial was to develop a rational method for routine salmonella isolation from environmental samples.

MATERIALS AND METHODS

The samples examined were chicken giblets collected weekly from the University Hospital of Wales kitchen. We have commented elsewhere on this area of potential salmonella contamination (Harvey, Price & Joynson, 1979*b*). Giblets included liver, heart, neck and crop. The investigation was carried out from 11.12.78–8.10.79.

The enrichment media used were selenite F broth (Leifson, 1936; Hobbs & Allison, 1945; Harvey & Price, 1974), Muller-Kauffmann tetrathionate broth (Anon, 1975) and Rappaport's magnesium chloride, malachite green broth (Rappaport, Konforti & Navon, 1956; Vassiliadis *et al.* 1970).

Harvey *et al.* (1979*a*) produced evidence for avoiding commercial media in critical trials of salmonella isolation. All media in the current study were prepared from individual ingredients.

The plating medium employed was brilliant green MacConkey agar. This selective agar has proved its value in our laboratory for many years (Harvey, 1956) and has recently received favourable comment from an area with considerable experience of salmonella isolation (Chau & Leung, 1978).

Crops and necks were processed together in a stomacher with 100 ml of buffered peptone water (van Schothorst & van Leusden, 1972; Anon, 1975). Livers and hearts were also combined and treated in the same way. The fluids were poured off the two types of sample into separate containers and were incubated at 37 °C for 18 h. After incubation, 1 ml of each peptone water culture was introduced separately into 10 ml of selenite F and 10 ml of Muller-Kauffmann tetrathionate. The Rappaport's media (10 ml) were inoculated with 0·005 ml (using a graduated loop)* of the buffered peptone water culture. Selenite and tetrathionate broths were incubated at 43 °C for 48 h and were subcultured to brilliant green MacConkey agar at 24 and 48 h. Rappaport's enrichment medium was incubated at 37 °C for 48 h and was also subcultured to brilliant green MacConkey agar at 24 and 48 h.

RESULTS

The results are recorded in Tables 1–4 which are largely self-explanatory. Two hundred and ten (31 %) of 683 samples of chicken giblets were contaminated with salmonellas. One hundred and twenty nine isolations were made from the crop and neck (37 %) while 81 (24 %) were found in the combined liver and heart samples (Table 1).

Timing of subculture from enrichment media is an important part of salmonella isolation and in certain studies multiple subculture is an integral part of technique (Harvey & Phillips, 1955). Two subcultures only were possible in the present investigation to conclude the examination within the working week. The data are recorded in Table 2.

Table 3 presents the results in terms of their statistical significance using MacNemar's test for paired samples. In each of the three comparisons recorded one medium was significantly superior to the other member of the pair.

Table 4 lists the number of salmonella serotypes isolated by each of the three enrichment media.

* Graduated loop obtained from Medical Wire & Equipment Co. (Bath) Ltd, Potley, Corsham, Wiltshire.

Table 1. *Salmonella isolation from chicken giblets*

Sample	Number positive (percentage)	Number examined
Crop + neck	129 (37)	349
Liver + heart	81 (24)	334
Total	210 (31)	683

Table 2. *Timing of positive subcultures from the three enrichment media*

(Percentages of combined positive results in parenthesis.)

Subculture positive or negative	Medium		
	Tetrathionate	Selenite	Rappaport
24 h and 48 h +	113	118	180
24 h + and 48 h −	16	11	10
24 h − and 48 h +	51	36	7
Total 24 h +	129 (61)	129 (61)	190 (90)
Total 48 h +	164 (78)	154 (73)	187 (89)
Combined positive results using both subcultures	180 (86)	165 (79)	197 (94)
Combined positive results with three media		210 (100)	

Table 3. *Statistical significance of comparisons between the three media*

Comparison	Medium positive or negative		χ^2	P
1.	Tetrathionate and Rappaport positive	171		
	Tetrathionate positive Rappaport negative	9	7·3	< 0·01
	Tetrathionate negative Rappaport positive	26		
2.	Selenite and Rappaport positive	155		
	Selenite positive Rappaport negative	10	18·5	< 0·01
	Selenite negative Rappaport positive	42		
3.	Tetrathionate and Selenite positive	149		
	Tetrathionate positive Selenite negative	31	4·17	< 0·05
	Tetrathionate negative Selenite positive	16		

DISCUSSION

In an investigation of the salmonella content of chicken giblets Gould & Rhodes (1969) suggested that positive neck samples might reflect surface contamination from cross infection and positive liver samples a possible bacteraemia. In their series, as in ours, neck specimens were more often contaminated with salmonellas than livers (Table 1).

The magnesium chloride, malachite green medium was the most successful of the three enrichment broths for the isolation of salmonellas in this study (Table 3). It was significantly superior to selenite F and Muller-Kauffmann tetrathionate. This reinforces findings of an earlier investigation of the same three media (Harvey

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Table 4. *Number of serotypes isolated from the three enrichment media*

Serotype	Number of isolations from Tetrathionate	Selenite	Rappaport	Phage types
S. anatum	2	2	3	
S. braenderup	1	1	4	
S. bredeney	67	49	68	
S. enteritidis	2	3	3	8, 13
S. hadar	1	0	2	
S. heidelberg	35	32	29	
S. indiana	14	18	19	
S. infantis	23	25	34	
S. kentucky	4	4	1	
S. livingstone	2	5	6	
S. senftenberg	1	1	1	
S. typhimurium	7	6	11	12a, 49, 99, 168, 204
S. virchow	21	19	19	
No. of strains isolated	180	165	200	

et al. 1979*a*) and enhances the value of Rappaport's medium as a basis for standardizing salmonella isolation from environmental samples.

Rappaport's medium *must* be used with a high inoculum ratio (Jameson, 1963; Harvey & Price, 1980). This term denotes the ratio of the volume of inoculum to the volume of the medium into which it is introduced. Harvey *et al.* (1979*a*) found Rappaport's broth to be the best of the three enrichment media investigated when an inoculum ratio of 1 : 2000 was used. It was, however, the worst of the three media when an inoculum ratio of 1 : 10 was employed. This property can be an advantage if pre-enrichment is used. Small inocula from the pre-enrichment culture are likely to contain salmonellas capable of multiplying in the enrichment broth (Loeffler, 1906). A high inoculum ratio can, however, be a liability with direct enrichment when large inocula may be necessary, as in the study of pig faeces using 80 g inocula (Harvey, Price & Morgan, 1977). Under these circumstances we would prefer selenite and/or tetrathionate which can accept large amounts of potentially infected material. The use of two enrichment media has certain advantages as each may have a bias towards isolation of certain serotypes (Harvey & Price, 1976). In the current series of observations recorded in Table 4, no useful comment on serotype bias can be made.

The comparison between selenite and tetrathionate in Table 3 favours the latter and the advantage of tetrathionate over selenite is significant. We have compared these media on several occasions. Not all results have been in agreement with each other (Harvey, Price & Morgan, 1977). We believe that comparisons between selenite and tetrathionate are influenced by the prevalent serotypes in the material (Harvey & Price, 1975), by the material examined, the detailed composition and preparation of the media and whether pre-enrichment or direct enrichment is used.

Table 2 records that 90% of the possible isolations were obtained with Rappaport's medium at the 24 h subculture. This figure was raised to 94% when subcultures were made at 24 h and 48 h. Recovery of salmonellas at an early stage in the examination saves media, time and effort. While rapid salmonella isolation

from environmental samples is seldom as urgent as it is from clinical specimens (Dixon, 1961) it is nevertheless a point in favour of Rappaport's medium. The magnesium chloride malachite green broth is also cheap to prepare and keeps well in the refrigerator. We have used one batch kept for 6 months with good results. For food, water and animal feed we now base our isolation technique on Rappaport's enrichment alone incubated at 37 °C and subcultured to brilliant green MacConkey. Adjustments of method must, of course, be made if *S. typhi*, *S. dublin* or lactose fermenting salmonella are thought to be present in the sample. (Harvey & Price, 1975; Harvey, Price & Hall, 1973).

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