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# Comparison of procedures based upon Rappaport–Vassiliadis medium with those using Muller–Kauffmann medium containing Teepol for the isolation of *Salmonella* sp.

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## SUMMARY

A total of 308 samples of different types were examined for the presence of salmonellas by means of three different procedures. The first consisted of pre-enrichment in buffered peptone water followed by enrichment in Rappaport-Vassiliadis medium (P/RV). The second differed only in that 1% Teepol was added to the pre-enrichment medium (PT/RV). In the third, buffered peptone water with 1% Teepol was followed by enrichment in Muller–Kauffmann tetrathionate broth also containing 1% Teepol (PT/MKT). The first of these combinations (P/RV) proved superior to the others both in terms of isolation rates and in the appearance of suspicious colonies.

## INTRODUCTION

In 1985 Skovgaard, Christensen & Gulistani reported that the addition of 1% Teepol 610 (BDH) to both the pre-enrichment medium (P medium) and to laboratory-made Muller-Kauffmann medium (MK medium) yielded more salmonellas from pig faeces than the Rappaport–Vassiliadis medium (RV medium). This finding prompted the undertaking of this study the results of which are recorded here.

## MATERIALS AND METHODS

*Samples.* Between December 1985 and October 1986, 308 samples were examined of which 117 were from pig faeces from the caeca of healthy pigs in the abattoir, 31 were from chicken carcasses and 24 from duck carcasses. The other 136 samples were taken from the sewage polluted waters of two rivers.

*Pre-enrichment media.* A pre-enrichment stage using buffered peptone water

(Edel & Kampelmacher, 1973) was used for each sample throughout this study. The inoculation of pre-enrichment medium was made as reported by Kalapothaki *et al.* (1986). This medium was divided in two equal parts; to one 1% sterile distilled water was added (P medium), while to the other 1% Teepol 610 was introduced (PT medium). Both were incubated at 37 °C for 18–22 h.

*Enrichment and selective media.* The RV medium employed in this study was the RV-tryptone medium prepared as described previously by Vassiliadis (1983) and Vassiliadis *et al.* (1976, 1981*a*, *b*). Throughout this study the RV medium was prepared in the Hellenic Pasteur Institute from the various ingredients.

For the first 86 samples the commercially available dehydrated Muller–Kauffmann tetrathionate broth (MK) (Oxoid CM343) prepared according to the instruction of Edel & Kampelmacher (1969) was used. To this MK medium 1% Teepol was added (MKT Oxoid). This commercial MK medium was employed in part of this work because it is used by most laboratories.

However, for the remaining 222 samples MK prepared from the various ingredients in the Hellenic Pasteur Institute (h.p.i.) according to the ISO formula was used (Anonymous, 1975). To this medium also was added 1% Teepol (MKT, h.p.i.) which made it identical to that used by Skovgaard *et al.* (1985). The selective plating medium was the modified brilliant green agar (Oxoid CM 329) to which 2·5 g of sodium deoxycholate were added per litre of medium before heating (BGDA) (Vassiliadis *et al.* 1979).

*Inoculation methods.* From each sample 0·1 ml of the culture from P medium and 0·1 ml of the culture from PT medium were inoculated to tubes of 10 ml each of RV medium (P/RV and PT/RV). All these paired RV media were incubated at 43 °C for 48 h. From all PT cultures 1 ml was transferred to 10 ml of MKT, Oxoid or 10 ml of MKT, h.p.i. (PT/MKT, Oxoid and PT/MKT, h.p.i.). All PT/MKT tubes were incubated at 37 °C for 48 hn as recommended by Skovgaard *et al.* (1985).

From the tubes containing the enrichment media subcultures were made, after 24 and 48 h incubation, to BGDA plates. From most P/RV and PT/RV enrichments when highly suspicious colonies appeared on BGDA after 24 h no further subcultures were made. The plates were incubated at 37 °C for 24 h and examined for the presence of salmonellae and for the degree of growth of competing organisms. Two suspicious colonies were picked to moist Kligler agar slopes for further identification. The statistical evaluation of the results was made using MacNemar's test for paired samples, except in the comparison between PT/MKT, Oxoid and PT/MKT, h.p.i. in which the unpaired $\chi^2$ was employed.

## RESULTS

It can be seen from Table 1 that, of the 308 samples examined 141 were positive for salmonellas with simple pre-enrichment in buffered peptone water followed by enrichment in RV medium (P/RV), whereas only 125 were positive after pre-enrichment in buffered peptone water containing 1% Teepol (PT/RV) and only 83 after pre-enrichment in buffered peptone water containing 1% Teepol followed by enrichment in MK medium with 1% Teepol (PT/MKT). These differences are

Table 1. *Isolation of salmonella with two pre-enrichment media followed by enrichment in RV broth and in Muller–Kauffmann tetrathionate broth*

| Nature of sample | No. examined | No. positive* | Pre-enrichment and enrichment procedures | | | No. of serotypes isolated |
|---|---|---|---|---|---|---|
| | | | P/RV† | PT/RV‡ | PT/MKT§ | |
| Pig faeces | 117 | 53 | 38 | 44 | 26 | 14 |
| Chicken and duck carcasses | 55 | 36 | 35 | 32 | 19 | 6 |
| Sewage-polluted river water | 136 | 75 | 68 | 49 | 38 | 24 |
| Total | 308 | 164 | 141 | 125 | 83 | 29 |
| No. of serotypes isolated | — | — | 27 | 22 | 20 | 29 |

* Total positive with all three methods (P/RV, PT/RV, PT/MKT) combined.

† P/RV Pre-enrichment in buffered peptone water (P) followed by enrichment in RV medium.

‡ PT/RV, Pre-enrichment in P containing 1% Teepol 610 (BDH) followed by enrichment in RV medium.

§ PT/MKT, Pre-enrichment in P containing 1% Teepol followed by enrichment in Muller–Kauffmann broth containing 1% Teepol.

Table 2. *Statistical comparison of the utilized procedures of enrichment (P/RV; PT/RV; PT/MKT, Oxoid; PT/MKT, h.p.i.)**

| | | Paired $\chi^2$ | $P$ |
|---|---|---|---|
| P/RV/ve, PT/RV−ve | 37 | 4·4 | $< 0{\cdot}05$ |
| P/RV−ve, PT/RV+ve | 21 | | |
| P/RV+ve, PT/MKT (Oxoid)−ve | 28 | 28·0 | $< 10^{-6}$ |
| P/RV−ve, PT/MKT (Oxoid)+ve | 0 | | |
| P/RV+ve, PT/MKT (h.p.i.)−ve | 39 | 18·8 | $< 10^{-4}$ |
| P/RV−ve, PT/MKT (h.p.i.)+ve | 9 | | |
| PT/RV+ve, PT/MKT (Oxoid)−ve | 25 | 22·2 | $< 10^{-5}$ |
| PT/RV−ve, PT/MKT (Oxoid)+ve | 1 | | |
| PT/RV+ve, PT/MKT (h.p.i.)−ve | 36 | 6·0 | $< 0{\cdot}05$ |
| PT/RV−ve, PT/MKT (h.p.i.)+ve | 18 | | |

* For symbols see footnote on Table 1; h.p.i. = MK made in the Hellenic Pasteur Institute according to ISO formula.

statistically significant (Table 2). Furthermore 162 samples overall were positive with P/RV and PT/RV combined; 6 samples were positive only after 48 h incubation in the P/RV procedure, and 9 only after 48 h incubation in the PT/RV method. The comparison between the commercially available and the laboratory prepared MK medium has shown that the former (PT/MKT, Oxoid) was inferior to the latter (PT/MKT, h.p.i.) (18·6% and 30·2% positive samples respectively, unpaired $\chi^2 = 4{\cdot}2$ $P < 0{\cdot}05$). The number of serotypes isolated from each type of sample and with each procedure used is recorded in Table 1. A total of 29 serotypes were isolated from the samples examined.

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## DISCUSSION

Recently Skovgaard *et al.* (1985) compared the efficiency of the Rappaport–Vassiliadis medium (RV) with Muller–Kauffmann tetrathionate broth (MK) to which 1% of Teepol 610 (BDH) was added (MKT). Both these enrichment media were inoculated with a pre-enrichment culture in buffered peptone water (P) to which, also, 1% Teepol was added (PT/RV and PT/MKT pre-enrichment methods). These authors found that PT/MKT method was more efficient in the isolation of salmonellas from pig faeces than the PT/RV procedure. In their study they did not use pre-enrichment in buffered peptone water without addition of Teepol. In this study we found that, from 308 samples of different sorts including pig faeces, *Salmonella* sp. were detected in 141 using the simple pre-enrichment in peptone water followed by enrichment in RV medium (P/RV method), in 125 with the PT/RV procedure and in only 83 when the PT/MKT method was used (Table 1). These differences are statistically significant. Furthermore, the P/RV and PT/RV procedures were more successful in this study than the PT/MKT procedure, in which MK medium was either commercially (Oxoid) or laboratory prepared (h.p.i.) (Table 2).

The discrepancies between our results and those of Skovgaard *et al.* (1985) are difficult to explain. They may be due in part to the fact that they did not use the P/RV procedure but only the PT/RV method. Another factor may be that in their study they found only 28 positive samples and only 3 serotypes while in our comparable investigation, 164 samples contained *Salmonella* sp. of 29 different serotypes (Table 1). Finally, the P/RV procedure is far more specific than the PT/MKT one in as much as among suspicious colonies 94 and 22% respectively were subsequently confirmed as *Salmonella* sp.

The P/RV procedure is recommended as the method of choice for the isolation of *Salmonella* sp. from environmental and clinical specimens.

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