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COMPARATIVE STUDIES ON ENRICHMENT AND SELECTIVE MEDIA FOR ISOLATION OF SALMONELLAE FROM FAECAL SPECIMENS

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ROBERTSSON, J. Å. and O. SÖDERLIND: *Comparative studies on enrichment and selective media for isolation of salmonellae from faecal specimens.* Acta vet. scand. 1977, *18*, 300—307. — Enrichment media (tetrathionate, selenite and Rappaport broths) and selective media (desoxycholate citrate agar and brilliant green agar) were tested in different combinations to ascertain their capacity for isolation of salmonella bacteria. The material consisted of 299 samples of cattle faeces from two herds infected with salmonella (Table 1), and of 111 artificially contaminated samples of pig faeces (Table 3). The tetrathionate and selenite broths were equally useful for the material as a whole, whereas the results varied between different species of salmonella which is of great practical interest. The number of salmonella isolations was much lower when enrichment with Rappaport broth was used. The rate of salmonella isolations can often be increased by parallel enrichments with two different media. Of the selective agar media tested, brilliant green agar was superior to desoxycholate citrate agar.

salmonellae; isolation; enrichment broths; selective media; faecal specimens.

For isolation of salmonellae, special media have long been used which favour the growth of the salmonellae at the expense of the rest of the flora. Such enrichment and selective methods generally yield a higher rate of salmonella isolations than direct culture on solid media (*Wokatsch & Rohde* 1976). The composition of the liquid and solid media used for the isolation of salmonellae is subject to change as new reports become available (*Dunn & Martin* 1971, *Grau & Smith* 1972, *Edel & Kampelmacher* 1974, *Pietzsch et al.* 1975).

It is of importance to have some knowledge of the suitability of the current media when using various enrichment broths, solid selective media, or different combinations of them. The literature

gives no definite guidance in this respect, since materials and methods vary greatly in the various investigations.

We have compared some of the media that are most commonly used in Sweden, the object being to find out which combinations of these media are most suitable for routine use in the isolation of salmonellae in veterinary bacteriological work, and to what extent some of the combinations should be used in parallel tests.

MATERIAL AND METHODS

The material comprised 299 faecal specimens from cattle of two naturally infected herds with Salmonella typhimurium (S. typhimurium) and 111 artificially contaminated faecal specimens from pigs. The faecal samples from the pigs were contaminated with broth cultures from 111 different salmonella strains representing 11 serotypes.

The salmonella strains used for artificial contamination of pig faeces were isolated at the National Veterinary Institute during the period 1969—1976 and were stored freeze-dried. The following serotypes were used: S. agona (10 strains), S. cholerae suis (10 strains), S. enteritidis (10 strains), S. infantis (10 strains), S. montevideo (10 strains), S. senftenberg (10 strains), S. tennessee (10 strains), S. thompson (10 strains), S. typhimurium (10 strains), S. derby (11 strains) and S. dublin (10 strains).

Broth cultures of the salmonella strains were incubated for 18 hrs. at 37°C and then diluted with physiologic saline. Twenty g of fresh pig faeces were thoroughly mixed with 1 ml of broth culture containing 10^6 viable salmonellae calculated after plating on agar.

The samples of contaminated pig faeces were enriched in different fluid selective media in proportions of 1:5 for 18 hrs. at 37°C and subcultured on solid selective media which were incubated for 18 hrs. at 37°C.

Specimens of cattle faeces (about 20 g) from spontaneously infected herds were treated in the same way as the artificially contaminated pig faeces samples.

The following enrichment and selective media were used. Fluid media: 1) Tetrathionate broth (*Kauffmann* 1930), TB; 2) Selenite broth (Oxoid CM 395), SE; 3) Rappaport broth (*Rappaport et al.* 1956), RA. Solid media: 1) Desoxycholate citrate

agar (Oxoid CM 35), DC; 2) Brilliant Green agar (Difco B 285), BG. The media were examined in the following combinations: 1) SE-BG; 2) SE-DC; 3) TB-BG; 4) TB-DC; 5) RA-BG; 6) RA-DC.

Salmonella-like colonies were tested with salmonella 0—1 phage (*Thal & Kallings* 1955, *Gunnarsson et al.* 1977) and for urease activity.

RESULTS

Table 1 shows the results from the examination of 299 faecal specimens collected in two herds of cattle infected with S. typhimurium. The highest number of isolations (Salmonella carriers) was obtained with the combinations TB-BG and SE-BG, namely 53 and 52 isolations respectively. The other combinations yielded a definitely lower number of positive reactions.

Table 1. Isolation of Salmonella typhimurium from cattle faeces.

Number of faecal specimens investigated	Number of samples positive for salmonellae					
	SE		TB		RA	
	BG	DC	BG	DC	BG	DC
299	52	41	53	27	44	36

SE = selenite broth.
TB = tetrathionate broth.
RA = Rappaport broth.
BG = brilliant green agar.
DC = desoxycholate citrate agar.

It will be seen from Table 2 that the number of re-isolations from a total of 30 specimens of swine faeces artificially contaminated with S. typhimurium, S. dublin and S. senftenberg,

Table 2. Isolation of salmonella bacteria from artificially contaminated pig faeces.

Number of samples and strains	Number of samples positive for salmonella						Salmonella type
	SE		TB		RA		
	BG	DC	BG	DC	BG	DC	
10	8	8	9	7	9	6	S. typhimurium
10	10	10	5	4	0	1	S. dublin
10	10	6	9	3	9	4	S. senftenberg
30	28	24	23	14	18	11	

For abbreviations see Table 1.

respectively, was highest with the combinations SE-BG (28 positive isolations), SE-DC (24 positive isolations), and TB-BG (23 positive isolations).

The results presented in Tables 1 and 2 show that the number of salmonella-positive specimens was much lower after enrichment with Rappaport broth. This medium was therefore omitted in the subsequent study.

Table 3 also includes the S. typhimurium, S. dublin and S. senftenberg strains presented in Table 2. The total material comprises 111 strains and refers to enrichment with TB and SE. The combination SE-BG, with the highest number of re-isolations (78) shows the lowest number of investigated colonies (102), whereas the combination TB-DC with the lowest number of re-isolations (27) has the highest number of investigated colonies.

Table 3. Isolation of salmonella bacteria from artificially contaminated pig faeces.

Number of samples and strains	Number of samples positive for salmonella				Salmonella type
	SE		TB		
	BG	DC	BG	DC	
10	8	8	9	7	S. typhimurium
10	10	10	5	4	S. dublin
10	10	6	9	3	S. senftenberg
10	9	10	7	1	S. agona
10	10	10	9	4	S. montevideo
10	10	8	7	2	S. thompson
10	3	1	2	0	S. enteritidis
10	0	2	1	1	S. cholerae suis
11	4	3	8	2	S. derby
10	6	6	9	2	S, tennessee
10	8	4	10	1	S. infantis
111	78/ 102	68/ 196	76/ 128	27/ 224	Number of pos. samples/investigated salmonella-like colonies

For abbreviations see Table 1.

Table 4 shows the incidence of recovered salmonella strains from the 111 contaminated specimens of pig faeces using different combinations of media. The incidence varied between 24 %, with TB-DC, and 87 % when all four combinations were used. SE-BG gave 78 isolations, and among these, 13 strains were

Table 4. Comparison of media combinations used for isolation of salmonellae from pig faeces.

Number of samples contaminated with salmonellae (serotypes given in Table 3)	Media combination	Number (and incidence) of samples positive for salmonellae	
		for individual combinations	in total
111	SE — BG	78 (70.3 %)	96 (86.5 %)
	TB — BG	76 (68.5 %)	
	SE — DC	68 (61.3 %)	
	TB — DC	27 (24.3 %)	

For abbreviations see Table 1.

not isolated with TB-BG. Of the 76 strains isolated with TB-BG, 15 were missed with SE-BG. Accordingly, the use of both SE-BG and TB-BG will increase the number of isolations from 78 and 76 strains, respectively, to 91.

The variations in the yield for the different serotypes will also be seen from Table 3. As regards S. dublin, S. senftenberg, S. agona, S. montevideo and S. thompson, enrichment with SE gave a slightly higher number of isolations, while TB gav a higher yield of S. derby, S. tennessee and S. infantis. For S. cholerae suis and S. enteritidis, the yield was poor with all the combinations, whereas for S. typhimurium the recovery rate was high throughout.

DISCUSSION

At the beginning of the study we tried enrichment with Rappaport broth, which has been used by some workers for faecal cultures, but we soon found that this yielded a lower number of isolations than did the other enrichment media.

The combinations TB-BG and SE-BG gave an equal number of isolations and were better than the other combinations in the investigated cattle faeces from herds infected with S. typhimurium.

Tetrathionate broth and selenite broth were equally useful regarding the material as a whole, whereas the isolation results for the various serotypes showed great differences (Table 3). Thus a careful selection of enrichment medium is often necessary when a certain type of salmonella infection is suspected. The results also show that it is an advantage to use, in parallel, two different enrichment media. *Thal et al.* (1960) and *Harvey & Price* (1976) came to the same conclusion.

In studies of the individual nutritional requirements of salmonella strains, great divergencies have been noted between different serotypes and occasionally between different strains belonging to the same serotype. Some serotypes are for instance unable to synthesize essential growth factors, which must be added to the medium (*Lederberg* 1947, *Stokes & Bayne* 1958). However, most salmonella types can grow in basal media that contain ammonium nitrogen, salts and glucose (*Kauffmann* 1954).

S. typhimurium has the ability to grow in basal media, whereas S. dublin requires addition of nicotinic acid as growth factor, since it cannot synthesize this essential vitamin in a basal medium (*Bayne & Thal* 1962). According to the present study, the advantages of enrichment with SE for the isolation of S. dublin that have been demonstrated for cattle faeces (*Harvey & Price* 1975) are also valid for pig faeces.

In the examination of the artificially contaminated pig faeces, the number of recovered strains was much higher for S. typhimurium than for S. cholerae suis. All the combinations of media gave poor isolation of S. cholerae suis. Other comparable studies have also demonstrated the good growth ability of S. typhimurium and have shown that only a relatively small number of bacteria are needed in the original sample to make a re-isolation possible after enrichment. A hundredfold increase of the number of S. cholerae suis bacteria, compared with S. typhimurium, is needed in the enrichment sample for re-isolation of a comparable number of the two serotypes (*Krogstad* 1974). The explanation of this is that some strains of S. cholerae suis have special requirements with respect to the contents of vitamins and amino acids in the medium (*Lederberg*). According to *Jones & Hall* (1975), enrichment with McConkey Brilliant Green medium gives a better growth of S. cholerae suis from pig faeces.

Of the solid selective plating media tested in this study brilliant green agar proved superior to desoxycholate citrate agar. The rate of salmonella isolations was higher from BG plates, despite the fact that a much larger number of subcultures of salmonella-like colonies were made from the DC plates. The suitability of brilliant green agar for isolation of salmonella types other than S. typhi has been demonstrated earlier by *Dunn & Martin* (1971).

Plating from enrichment broths on selective solid media is

usually carried out after incubation for 24 hrs. at 37°—43°C. Another plating from broth after 48 hrs. of incubation increases the possibility of demonstrating salmonella bacteria from infected specimens according to *Edel & Kampelmacher* (1973) and *Reusse et al.* (1975).

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SAMMANFATTNING

Jämförande studier av anriknings- och selektiva medier för isolering av salmonella från träckprov.

Anrikningsbuljongerna tetrathionat, selenit och Rappaport samt de selektiva substraten desoxycholatcitratagar och brilliantgröntagar undersöktes i olika kombinationer beträffande lämpligheten för isolering av salmonellabakterier.

Undersökningsmaterialet utgjordes av 299 nötträckprov från två salmonellainfekterade besättningar (tabell 1) samt 111 artificiellt kontaminerade svinträckprov (tabell 3).

Tetrathionat- och selenitbuljong var likvärdiga för undersökningsmaterialet i dess helhet, medan resultaten varierar beträffande olika species av salmonella, vilket är av betydande praktiskt intresse. En förhöjning av antalet salmonellaisoleringar kan dock ofta erhållas genom parallell anrikning i två olika medier. Antalet salmonellaisoleringar blev avsevärt lägre vid anrikning i Rappaportbuljong.

Av de prövade fasta selektiva spridningsmedierna framstod brilliantgröntagarn som överlägsen desoxycholatcitratagarn.

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