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Influence of multiple plating from fluid media on salmonella isolation from animal feeding stuffs

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SUMMARY

The influence of multiple plating of fluid cultures on salmonella isolation from animal feeding stuffs was examined. Four platings were made from broth culture after 24 h at 37 °C and four platings from selenite enrichment from 24 h at 43 °C. Selenite enrichment followed broth culture which was used as a pre-enrichment stage. Brilliant green MacConkey agar plates were employed for broth subculture and brilliant green MacConkey and desoxycholate citrate agars for selenite subculture. The eight brilliant green plates subcultured from broth and selenite were examined for salmonellas after incubation for 24 h at 37 °C. The four desoxycholate citrate agars after 24 h at 37 °C were used for motility enrichment. The food sample size was a single 100 g instead of 4 × 25 g cultured in an earlier study. This pooling of samples aimed at technical economy. Quadruple plating played an important part in salmonella isolation from 100 g specimens. The combination of multiple plating with motility enrichment was the most successful technique used.

INTRODUCTION

Harvey & Price (1967) recorded that increasing the sample size of animal feeding stuffs from 25 to 100 g (4 × 25 g sub-samples) doubled the incidence of salmonellas isolated. In the investigation, a selective motility technique applied after selenite enrichment for 24 h was found valuable and was more successful than subculture of the enrichment broth at 24, 48 and 72 h. Since then, selective motility salmonella enrichment has been adopted as an integral part of certain of our isolation methods.

Silliker & Gabis (1973) reported that salmonella-positive lots of low-moisture foods could be detected with equal certainty by analysis of sixty 25 g individual samples, fifteen 100 g or three 500 g lumped samples. The economic advantage of pooling samples is obvious. This approach makes statistical quality control of salmonella contamination economically feasible particularly for high risk foods. Gabis & Silliker (1974) extended this pooling procedure to high moisture foods and demonstrated the value of pre-enrichment of samples in lactose broth. They found the analysis of a single 'lumped' 325 g *pre-enriched* subsample (13 × 25 g) was as efficient for salmonella isolation as analysis of thirteen 25 g pre-enriched subsamples.

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In contrast *directly enriched* pooled subsamples were less efficient in salmonella recovery. Sample pooling could not be effectively used without pre-enrichment. The authors investigated multiple plating from directly enriched cultures as a possible explanation of the poor results obtained and subcultured 13 sets of plates from a 325 g lumped subsample. They could find no evidence that the inferior recovery of salmonellas could be overcome by multiple plating. This result interested us as the multiple plating technique has occasionally been suggested as of value in improving positive salmonella isolations by direct plating of samples (Houston 1914; Gunther & Tuft 1939). The relevance of multiple plating to successful salmonella isolation was discussed many years ago by Loeffler (1906) in relation to recovery of *S. typhi* multiplying in an unselective fluid medium. Personal experience has also indicated that multiple subculture to a series of plates from selenite F inoculated with human faeces and enriched for 24 h at 37 °C can sometimes aid salmonella culture (Harvey 1965).

The current investigation records the influence of multiple plating on three techniques of salmonella isolation.

MATERIALS AND METHODS

The feed samples were provided by the same firm as material used in an earlier study (Harvey & Price 1967). The majority of specimens were meat and bone meals but some samples were submitted without evidence of composition. The investigation period was 24.2.75 to 29.9.79.

In this study, 100 g of each specimen of animal feed was examined in one lot. In the previous project, the same quantity (100 g) was selectively cultured in four subsamples of 25 g. Examination of a single sample in place of four subsamples was an attempt to curtail laboratory work in a similar way to that suggested by Silliker & Gabis (1973).

One hundred grammes of the material was placed in a single large container and 200 ml of nutrient broth was added. The mixture was stirred with a sterile swab and incubated at 37 °C for 24 h. Subculture was then made to 4 plates of brilliant green MacConkey agar (Harvey 1956), using a 3 mm diameter loop. The loop was sterilized by heat between each plating. Two hundred millilitres of double strength selenite F (0·8 % sodium hydrogen selenite) were added to the broth/feed mixture and further incubation continued at 43 °C for 24 h (Harvey & Thomson 1953). The material was subcultured to 4 plates of billiant green MacConkey agar and 4 plates of desoxycholate citrate agar using a 3 mm diameter loop. Media were prepared according to Harvey & Price (1974). Selective agars, subcultured from both broth and selenite, were incubated at 37 °C for 24 h. The brilliant green MacConkey plates were examined for salmonella colonies with a hand lens in day light. Colony differentiation is better under day light conditions than it is under artificial lighting. Salmonella-like colonies were confirmed by direct slide agglutination and biochemical tests if necessary. In Cardiff, we prepare selective agars with as low a concentration of agar as is compatible with stability. This permits slide agglutination as a screening procedure with corresponding time saving (Harvey & Price 1974). Colonies which agglutinated with H and O salmonella agglutinating sera were picked for further examination.

The incubated desoxycholate citrate agar plates were taken for the motility enrichment technique. In a short previous study (unpublished), *S. dublin* was best recovered by motility enrichment using desoxycholate citrate agar to prepare the inoculum introduced into the Craigie tube. Since then we have used desoxycholate plates for this purpose as a routine. In the current study, no attempt was made to examine them for salmonella colonies. All the growth on each plate was removed with a short sterile swab and the growth coated swab was placed in the inner tube of a modified Craigie tube (Craigie 1931; Harvey & Price 1967; 1974). From each 100 g sample of animal feed, four Craigie tubes were, therefore, derived. The Craigie tubes were incubated at 37 °C for 24 h. Growth migrated from the soiled swabs down the inner tubes and up to the agar surface outside. Subcultures were made from this surface to brilliant green MacConkey agar, each Craigie tube being plated on a separate plate. These were incubated at 37 °C for 24 h and examined for salmonella colonies as before.

RESULTS

A total of 1008 samples of animal feed were examined. One hundred and forty nine of these (14.8 %) contained salmonellas. Contamination incidence varied from year to year and was highest in 1975 and lowest in 1979. One hundred and twenty four of the 149 positive samples were meat and bone meals. Twenty-two were unidentified and three were herring meals. Results are recorded in Table 1.

The 149 samples found contaminated with salmonellas represent the combined

Table 1. *Incidence of salmonella positive samples in animal feed from a single compounder* 1975–1979

Year	Total samples examined	Samples positive for salmonellas
1975	297	102 (34·3)
1976	180	14 (7·8)
1977	136	13 (9·6)
1978	164	13 (7·9)
1979	231	7 (3·0)
Total	1008	149 (14·8)

Figures in parentheses are percentages.

Table 2. *Cumulative number of salmonella isolations obtained with* 1, 2, 3 *and* 4 *platings using three enrichment techniques*

	Cumulative number of salmonella isolations obtained from		
No. of Platings	Broth	Selenite F	Motility enrichment
1	45	94	83
2	63	102	106
3	71	108	122
4	76	118	139
No. of failures	73	31	10

Failures for each method are calculated on the basis of a maximum number of 149 isolations.

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Table 3. *Comparison of salmonella isolations from selenite enrichment and motility enrichment*

Selenite positive Motility enrichment positive	346
Selenite positive Motility enrichment negative	75
Selenite negative Motility enrichment positive	104
Selenite negative Motility enrichment negative	71

Total plates examined from each enrichment = 596 (4 × 149).
Total positive samples examined = 149.

Table 4. *Serotype isolations from quadruple plating in combination with three enrichment techniques*

	No. of isolations with:		
Serotype	Broth	Selenite F	Motility enrichment
S. agama	1	1	1
S. agona	24	36	47
S. anatum	5	6	3
S. bredeney	2	5	7
S. colindale	0	1	0
S. cubana	16	21	22
S. derby	1	1	1
S. drypool	0	0	1
S. eimsbuettel	9	17	16
S. give	3	3	4
S. heidelberg	1	1	1
S. indiana	0	1	1
S. kedougou	0	0	3
S. lexington	0	1	1
S. livingstone	13	16	15
S. london	1	1	1
S. montevideo	2	3	3
S. new-brunswick	1	2	4
S. portsmouth	1	1	1
S. saint-paul	10	10	13
S. schwarzengrund	1	0	1
S. senftenberg	9	16	19
S. taksony	0	2	2
S. tennessee	2	1	0
S. thomasville	0	1	1
S. typhimurium 12a*	0	0	1
S. typhimurium 135*	0	1	1
S. worthington	1	1	0
S.4, 12:d:-	1	0	1
Total strains isolated	104	149	171

* phage type

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results of broth culture, selenite enrichment and motility enrichment. The efficiency of isolation varied with the number of platings (1–4) from broth and selenite and the number of plates subcultured from selenite broth and subjected to motility enrichment (1–4). Failures with each technique are calculated on the basis of a maximum number of 149 positive specimens. Table 2 records the results.

As motility enrichment isolations were derived from selenite enrichment subcultures it is instructive to note the relation between selenite isolations and motility enrichment isolations. One hundred and seventy five plates would have been recorded as negative by selenite enrichment. One hundred and four 'negative' plates from selenite enrichment became salmonella positive by subsequent motility enrichment. Results are presented in Table 3.

Table 4 records the serotype isolations by the three techniques using four platings for each method. Tables 2 and 4 indicate that the combination of motility enrichment with quadruple plating was the most successful method used in this study.

DISCUSSION

Table 1 records changes in the contamination rate of animal feed samples from a single firm over the years 1975–1979. From conversations with compounders we think this dramatic change may be due to careful selection of raw materials from suppliers capable of producing consistently salmonella free samples.

This study was devised to observe the association between multiple plating (4 plates) and three salmonella recovery techniques – broth culture, selenite enrichment and motility enrichment. Quadruple plating was a factor contributing to salmonella isolation with all three methods. It was most efficient in the motility technique. By the selective motility method using quadruple plating 139/149 samples (93%) were identified as salmonella positive.

The explanation of the success of quadruple plating is probably twofold. In the first place, in direct as opposed to enrichment culture, it is associated with lack of homogeneity of salmonella distribution throughout the test material (Kroger 1951). Secondly, it is possible that enrichment cultures, at certain stages in their periods of incubation, have a scanty number or an inhomogeneous distribution of salmonellas. Under such circumstances a single subculture of a loopful (possibly 0·0005 ml using a standard loop) would be less likely to result in a positive salmonella isolation while four loopfuls ($4 \times 0{\cdot}0005$ ml = 0·002 ml) might succeed. Thus multiple plating might be an alternative to incubation at 43 °C for 6 h to obtain rapid diagnosis of salmonella infection in man (Dixon 1961). The association of incubation time and multiple plating was discussed by Loeffler (1906) in relation to a broth culture of *S. typhi*. The discussion was made on theoretical and not on practical grounds.

Motility enrichment has been successfully used by several microbiologists (Carnot & Garnier 1902; Carnot & Weill-Halle 1915; Friedberger & Putter 1920; Ino & Graber 1955; Stuart & Pivnick 1965; Mohit, Aly & Bourgeois 1975; Chau & Huang 1974, 1976). Our own version of the technique differs from those cited in that we prepare the inoculum for motility enrichment from plates subcultured from enrichment broths (Harvey, Mahabir & Price 1966; Harvey & Price 1967). In Table 3, the relation between selenite enrichment and subsequent motility

enrichment is presented. The two techniques are complementary to each other and failure to isolate salmonellas would occur if either method was omitted. This modifies our original belief that motility enrichment could be used exclusively to supplant conventional selenite enrichment (Harvey & Price 1967). Table 2 emphasizes the value of quadruple plating in the motility enrichment technique and 56/139 samples (40%) required more than one subculture plating to reveal salmonellas.

Table 4 records the serotypes isolated by the three different procedures. The combination of quadruple plating with motility enrichment was the most successful technique.

It would appear that examination of 100 g samples of animal feed should incorporate multiple plating as part of the procedure if the maximum number of salmonella isolations is to be obtained.

Multiple plating has also been used to isolate a different enteropathogen – *Shigella flexneri*. A specimen (number 193 of 1976) was received from the P.H.L.S. Quality Control Laboratory. The sample was a simulated faeces accompanied by a history of intestinal symptoms. We plated the specimen on desoxycholate citrate agar 22 times in an attempt to obtain a result. Only five platings were successful. All the selective agars belonged to the same batch of medium.

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