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Secondary Selective Enrichment of Salmonellae from Naturally Contaminated Specimens by Using a Selective Motility System

T. I. SMELTZER* AND F. DUNCALFE

Animal Research Institute, Queensland Department of Primary Industries, Yeerongpilly, Queensland 4105 Australia

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A selective motility medium was used as a secondary selective enrichment medium to examine specimens naturally contaminated with salmonellae. The medium, incubated at 37°C, was inoculated from either selenite brilliant green sulfa enrichment broth or Müller-Kauffman tetrathionate broth, both of which had been incubated at 42°C. The use of the selective motility medium resulted in an increase in the number of positive specimens from 65 and 74% to 80 and 82%, when inoculated at 24 and 48 h, respectively, from tetrathionate broth. Tetrathionate broth, when used singly, was significantly better than selenite brilliant green sulfa broth, which detected 55% of positive specimens at both 24 and 48 h. The use of the selective motility medium of Harper and Shortridge (J. Hyg. 67: 181-186, 1969) for the further examination of specimens culturally negative on primary selective enrichment is advocated.

Numerous cultural procedures have been advocated for the isolation of salmonellae. Some of these procedures have found little use, whereas others, such as the selenite and tetrathionate broths, are widely accepted. Various other modifications in technique, such as pre-enrichment in lactose broth (10) or buffered peptone water (2, 3, 13, 15) and incubation at elevated temperatures (1, 4, 9, 11), are used to increase the isolation rate of salmonellae.

Secondary selective enrichment is advocated by some workers (6, 8, 14, 15, 16), whereas others (9) report little benefit from its use. Motility media may be used both for primary enrichment (5, 12) and for secondary enrichment (6, 7).

This paper describes the use of the selective motility medium (SMM) of Harper and Shortridge (5) as a means of increasing the isolation rate of salmonellae by its use as a secondary selective enrichment medium. Pre-enrichment was in buffered peptone water, and primary selective enrichment was carried out at 42°C in selenite brilliant green sulfa enrichment broth (SBGS) and in Müller-Kauffman tetrathionate broth (MKT).

MATERIALS AND METHODS

All salmonella specimens were pre-enriched in buffered peptone water at 37°C for 18 to 24 h. Selective enrichment was carried out in SBGS (Difco) and MKT (Oxoid), which were incubated at 42°C in a thermostatically controlled water bath. At 24 and 48 h, both selective enrichment broths were subcultured onto brilliant green sulphadiazine agar (BGSA). At the same time, 0.2 ml was also inoculated into the center tube of the SMM of Harper and Shortridge (5). The SMM tubes were incubated at 37°C and inspected daily until the growth, detected as either turbidity or blackening due to H_2S production, reached the top of the outer portion of the tube. Two drops of the medium from the outer portion of the tube were then streaked onto a BGSA plate.

All BGSA plates were incubated at 37°C and examined at 24 and 48 h. Three suspect colonies from each plate were screened biochemically by using Kohns two-tube medium (Oxoid). Colonies having the correct salmonella reaction were checked using salmonella polyvalent O and H antisera. Confirmed cultures were sent to the Salmonella Reference Laboratory for serotyping. The results of the three colonies from each of the eight plates from each specimen were recorded.

Only specimens found to contain salmonellae were included in the analysis of the results. These specimens included bovine manures (37 specimens); avian litters (6 specimens); egg pulp (2 specimens); avian fluff (3 specimens); soil (3 specimens); rinses from bovine livers (37 specimens); abattoir knives (3 specimens); water and sewage (67 specimens); meat meal (17 specimens); and mashes and pelleted animal feeds (36 specimens). The performance of each procedure was then assessed by using the following two criteria: (i) the number of the specimens found to be positive; and (ii) the average number of serotypes isolated per sample by each method.

The results of a number of combinations of procedures were also analyzed. The selection of these com-

binations was based, first, on those methods which showed the most promise and, second, on the ease of performance of the method for routine examinations (see Table 1 for a list of methods).

RESULTS

Inoculation of the SMM from the 24-h MKT broth resulted in an increase in the number of specimens positive from 65% after primary enrichment to 80% (Table 1). The average number of serotypes isolated also increased, but the increase was not significant. Similarly, inoculation of the SMM from the 48 h MKT broth resulted in an increase in the percentage of the specimens found to be positive from 74% after primary enrichment to 82%. The average number of serotypes isolated also increased. This procedure was the most successful of any single plating procedure.

The SMM, when used as a secondary selective enrichment after SBGS broth, failed to achieve any increase in either the percentage of specimens found to be positive or in the average number of serotypes isolated. When the SMM was inoculated after primary enrichment in SBGS for 48 h, there was a significant decrease in the average number of serotypes isolated ($P < 0.01$).

When the results of the plates from the two primary selective media were compared, it was found that the MKT broth performed significantly better than the SBGS broth (Table 1). The SBGS broth, subcultured at 24 and 48 h, resulted in 55% of the specimens being positive at each subculture, whereas the MKT broth resulted in 65 and 74% of specimens being positive, respectively. The average number of serotypes isolated from the 48-h MKT broth was significantly higher ($P < 0.01$) than that of the SBGS at 24 or 48 h.

The use of two plates inoculated from different selective enrichment media or at different times did not always result in an increase in the isolation rate of salmonella. A significant improvement ($P < 0.01$) in the average number of serotypes per sample was evident in methods 2, 3, 4, and 5. The use of the SBGS broth was indicated in only one of these combinations. Methods 9, 11, and 12 involving SBGS resulted in fewer specimens found to be positive than some single plating procedures.

No combination of subculturings was able to

TABLE 1. *Performance of methods and selected combinations of methods used for the examination of 211 naturally contaminated samples for salmonellae*

Method No.	MKT				SBGS				No. of Spec. positive (%)	Av. no. of serotypes per sample*
	24 hour incubation		48 hour incubation		24 hour incubation		48 hour incubation			
	BGSA	SMM	BGSA	SMM	BGSA	SMM	BGSA	SMM		
1	▨	▨	▨	▨	▨	▨	▨	▨	211 (100)	1.8
2			▨	▨					194 (92)	1.26
3	▨	▨							181 (86)	1.18
4	▨		▨						175 (83)	1.13
5			▨		▨				174 (83)	1.16
6				▨					173 (82)	0.88
7		▨							168 (80)	0.87
8			▨						157 (74)	0.87
9					▨	▨			140 (67)	0.91
10	▨								137 (65)	0.77
11					▨		▨		135 (64)	0.81
12							▨	▨	135 (64)	0.76
13					▨				117 (55)	0.64
14							▨		116 (55)	0.59
15						▨			113 (54)	0.56
16								▨	91 (43)	0.40

* Least Significant Difference = 0.14 ($P < 0.01$).

▨ Denotes procedures used for each method for which performance was calculated.

isolate all the salmonellae isolated by the complete procedure. The closest was the use of the SMM with both the 24- and 48-h MKT-broth subcultures which resulted in the isolation of salmonellae from 98% of the specimens. Subculturing both the MKT broth and the SBGS broth at 24 and 48 h without the use of the SMM resulted in the isolation of salmonellae from only 87% of the positive specimens.

Analysis of the results of the various specimen types revealed that the performance of each of the methods was largely independent of specimen type. The SMM performed well in all instances. The most successful methods for various specimens are listed in Table 2.

Forty-one serotypes of salmonellae were isolated during this trial together with four serotypes of *Salmonella arizonae*. There was no evidence that any of the 41 serotypes encountered were isolated preferentially by any of the methods used, except that 10 isolations of nonmotile strains were made after primary selective enrichment, but not from the SMM.

DISCUSSION

The use of the SMM of Harper and Shortridge (5) as a secondary selective enrichment medium was effective in increasing the isolation rate of salmonella from a variety of naturally contaminated specimens. Overall, 13%, or 27 of the positive specimens, would have been reported as negative if the SMM had not been used.

When used in conjunction with MKT, which may initially provide a more rapid result, the SMM provides an effective system for the further examination of specimens found to be culturally negative after primary selective enrichment. This procedure resulted in the isolation of salmonellae from 11% more of the positive specimens than did the more usual procedure of using two primary selective enrichment media (SBGS and MKT).

TABLE 2. *Performance of various methods compared to the complete procedure for the examination of five types of specimens for salmonellae*

Specimen	Most successful methods[a]
Bovine livers	2[b], 3, 4, 5
Meat meal and mash	2, 3, 4, 5, 9
Pellets	2, 3, 7
Avian	2, 3, 4, 5, 8
Bovine manure	2, 6

[a] All other methods were significantly less effective ($P < 0.01$).

[b] Numbers refer to methods listed in Table I.

The time factor for inoculation is minimal; it requires less time to inoculate than to streak an agar plate, and the medium is less costly than the use of a second primary selective enrichment medium such as SBGS. The incubation period was variable. Specimens positive in the primary enrichment media were usually ready for subculture from the SMM at 24 to 48 h. Specimens culturally negative for salmonellae from the primary enrichment media generally required longer incubation periods of up to 1 week. Occasionally this period was longer, as with one manure specimen in this trial from which salmonellae were isolated from the SMM after 21 days. Salmonellae were not isolated from this specimen by any other method.

The use of the SMM was shown to be an effective alternative to the use of dual primary selective enrichment media, especially in laboratories which require a sensitive method and where an early result is not essential.

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