Factors Influencing Detection of Salmonellae in Rendered Animal By-Products

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Detection of salmonellae in animal by-products is influenced by the enrichment and plating media and by quantity of product tested, and is related to the total plate count. A linear relationship exists between detection of salmonellae and total plate counts from $10^4$ through $10^7$ per gram.

Detection of salmonellae in contaminated food and feed ingredients is dependent upon many factors. No single method has been developed that satisfactorily recovers all Salmonella serotypes from all types of foods (1).

The purpose of this study was to develop a better understanding of the factors influencing the detection of salmonellae in naturally contaminated animal by-products. The influence of media and the relationship between total counts and isolation of salmonellae were examined.

MATERIALS AND METHODS

During 1967 and 1968 samples were collected from plants throughout the industry for analysis in our laboratory. Total plate counts were determined by using plate count agar (Difco) at 37 C for 48 h. Two 20-g samples were analyzed for Salmonella. One 20-g sample was added to 80 ml of tetrahydroxyl-Brilliant Green-iodine (TB) broth and the other 20-g was added to 80 ml of selenite-cystine broth (SCB). Both broths contained 0.6% Tergitol no. 7 (Union Carbide Chemical Co.) and were incubated 24 h at 37 C. The enrichment broths were streaked onto plates of Brilliant Green-sulfa agar (BGS; Difco) and Salmonella-Shigella agar (SS; Difco) plates and incubated 24 h at 37 C. Plates showing less than three suspect colonies were incubated an additional 24 h at room temperature and reexamined. Three Salmonella-like colonies were picked from each plating medium to triple sugar iron (TSI) agar slants. Cultures with Salmonella-like reactions on TSI after 18 to 24 h were tested serologically by using polyvalent O and H antisera. Cultures reacting positively in either or both serological tests were confirmed biochemically.

RESULTS

Of 183 Salmonella-positive samples, 139 and 148 samples were found positive in TB and SCB, respectively (Table 1). The combination of SCB and SS agar plates gave the highest number of positive samples (127). The combination of SCB with BGS agar plates gave the lowest number of positive samples (92). From 3 to 9% of the samples were positive in only one of the four possible media combinations.

Table 2 shows a grouping of 405 samples according to total plate count results. The rate of salmonellae isolations increased from 12.7 to 70.6% as the total plate counts increased from less than $10^4$/g to $10^7$/g. Samples with total plate counts in excess of $10^7$/g were associated with a decreasing rate of salmonellae isolations.

Linear regression analysis confirmed a positive relationship between the total plate count and detection of salmonellae ($P = 0.05$). However, examination of the regression line by chi-square analysis revealed a lack of fit, for samples having total plate counts below $10^4$ and at $10^4$/g and above. This supports the conclusion that the relationship is linear only for samples having total plate counts from $10^4$ through $10^7$/g.

The probability of detecting salmonellae in animal by-products was developed from further statistical analysis of the data. For example, if the product has a total plate count in the range of $10^4$ to $10^7$/g, then the probability of detecting salmonellae in a single sample would be 0.40 (Table 3) by using the methods described.

Comparing the data for samples positive from only one enrichment (20 g) versus both enrichments (40 g), we found that 40 g significantly ($P = 0.05$) increased the detection of salmonellae. This conclusion was dependent upon the level of contamination and the assumption that the concentration of salmonellae would be higher in samples having higher total plate counts. The data in Table 2 support this...
assumption. For example, a higher percentage (64%) of the positive samples was found positive in only one enrichment (20 g of product) when the total counts were lower (10^4/g). At 10^4 total counts per g, 25% of the positive samples were positive in one enrichment.

**DISCUSSION**

No practical difference was found between TB and SCB in terms of the number of samples found positive. Both TB and SCB failed to detect salmonellae in approximately one out of five of the total 183 positive samples (Table 1). The best combination of media (SCB-SS) found only 69% of the 183 to be positive. This failure to detect salmonellae could be due to inhibition of certain serotypes by the enrichment media, a low level of contamination, or uneven distribution of salmonellae through the product, or all three (5, 9).

Using both TB and SCB results in a higher rate of salmonellae isolations from animal feed ingredients (2, 5, 9). However, using both enrichments also doubles the quantity of product analyzed. The degree to which sample size influences the isolation rate of salmonellae in animal by-products deserves more consideration.

Huhtanen et al. (3) analyzed 16 samples of meat and bone meal by using ten 3-g samples and ten 30-g samples for each. They found only 38 individual 3-g samples positive as compared to 86 to 89 individual 10-g samples positive. Laramore and Moritz (4) analyzed 73 samples of meat meal by subdividing each into two 30-g samples. They found results from the samples to agree only 86.2% of the time.

Adding 20 g of meat and bone meal to both tetrathionate and selenite broths, Leistner et al. (5) found 12 samples positive. By using 10 g from the same samples, they found only seven samples positive. They then reported 37 samples positive from tetrathionate or selenite broths, or both. However, 13 (35%) of the 37 samples were positive in only one or the other enrichment broths. They state that this could be due to a low or nonuniform level of contamination (or both) as well as inhibition of certain serotypes.

We did not conduct quantitative determinations to learn the concentration of salmonellae. However, assuming the concentration of salmonellae to increase relative to the total plate counts, it was possible to statistically determine that quantity of product (20 versus 40 g) is a significant factor.

We conclude that the difference we observed between enrichment media for detecting sal-

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**TABLE 1. Comparison of media for detecting salmonellae in 183 positive samples of animal by-products**

| Media combination          | No. of samples positive out of 183 | Samples positive (%) | No. of samples positive in only 1 medium | Samples positive in only 1 medium (%) |
|----------------------------|-----------------------------------|----------------------|----------------------------------------|--------------------------------------|
| Tetrathionate broth        | 139                               | 76                   | 35                                     | 19                                   |
| Selenite-cystine broth     | 148                               | 81                   | 44                                     | 24                                   |
| TB + Brilliant Green-sulfa agar | 105                           | 57                   | 5                                      | 3                                    |
| TB + Salmonella-Shigella agar | 111                           | 61                   | 7                                      | 4                                    |
| SCB + Brilliant Green-sulfa agar | 92                            | 50                   | 11                                     | 6                                    |
| SCB + Salmonella-Shigella agar | 127                           | 69                   | 16                                     | 9                                    |

*183 ~ 100%.

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**TABLE 2. Relationship between total plate counts and salmonellae isolations from animal by-products**

| Total plate count/g (log_{10}) | No. of samples examined | Samples positive (%) |
|---------------------------------|-------------------------|----------------------|
| <4                              | 55                      | 12.7                 |
| 4-5                             | 65                      | 30.8                 |
| 5-6                             | 89                      | 47.2                 |
| 6-7                             | 90                      | 64.4                 |
| 7-8                             | 68                      | 70.6                 |
| 8-9                             | 19                      | 52.6                 |
| >9                              | 19                      | 31.6                 |

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**TABLE 3. Estimated number of samples required to detect Salmonella in animal by-products when the total plate count is known**

| Total plate count/g (log_{10}) | Probability of detecting salmonellae if only one sample is analyzed | Minimum no. of samples to ensure detection of at least 1 positive sample |
|--------------------------------|---------------------------------------------------------------------|------------------------------------------------------------------------|
| 4-5                            | .40                                                                 | 10                                                                     |
| 5-6                            | .47                                                                 | 8                                                                      |
| 6-7                            | .55                                                                 | 6                                                                      |
| 7-8                            | .63                                                                 | 5                                                                      |

*P = 0.99.
monellae is more apparent than real. Others have clearly established that an inhibitory effect exists due to the failure of certain serotypes to grow in the enrichment media. The data which is becoming available for naturally contaminated animal by-products suggests that the quantity of product being tested, at least in the range of 3 to 40 g, may be the more important factor.

Our data confirms earlier reports (6, 7) that a relationship exists between total plate counts and the detection of salmonellae in animal by-products. However, it was learned that the linearity of the relationship exists only in the total count range of $10^4$ through $10^7$/g. There is a decrease in the percentage of Salmonella-positive product having total plate counts greater than $10^7$/g. This is probably due to overgrowth of salmonellae by other bacterial species during enrichment or plating, or both. It is less likely that the product is actually less contaminated with salmonellae.

The use of total counts as a measure of the microbiological safety of foods must be assessed in terms of the particular situation presented (8). Animal by-products appear to be one of the few materials where a linear relationship exists between total counts and the incidence of salmonellae. This is of practical value for in-plant control purposes and for evaluating improvements in manufacturing and sanitation. It is important that the limitations of the total plate count be recognized and it is suggested that total counts be used to supplement salmonellae testing programs.

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