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Fluorescent-Antibody Technique in Detection of Salmonellae in Animal Feed and Feed Ingredients

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Comparative studies of fluorescent antibody procedure and a cultural method for the detection of *Salmonella* were made on 1,013 feed and feed-ingredient samples. The agreement between the two methods was 92.1%. There were more false positives (5.7%) than false negatives (2.2%). Of the 22 false negatives, 15 (68%) were obtained on meat meal. Of the total number of samples, 37% were meat meal. An additional study of 73 samples of meat meal indicated that correlation between methods was better than correlation between samples.

In recent years, considerable attention has been given to the possibilities of using the fluorescent-antibody (FA) technique as a rapid method for detecting *Salmonella* in food products (1). Some investigators have reported good correlation between FA and cultural methods (3, 4, 5), whereas others have reported it less satisfactory (2, 6).

The purpose of this study was to determine the degree of correlation and reliability of such methods in the routine analyses of animal feeds, animal by-products, and feed ingredients.

MATERIALS AND METHODS

Samples. Over a 6-month period, 1,013 samples representing feather meal, fish meal, meat meal, soybean meal, and complete feeds were collected aseptically in 18-oz Whirl-Pak bags (Scientific Products, Inc., Detroit, Mich.) and submitted to our laboratory.

Culture method. Of each sample, 30 g was inoculated into 150 ml of Tetrathionate Broth (Difco) containing 1.5 ml of a 1:1,000 (v/v) solution of Brilliant Green dye and 9 ml of a 10% solution of Tergitol no. 7 (Union Carbide Corp., New York, N.Y.). The samples were incubated at 37 C for 20 to 23 hr. They were then swirled and allowed to stand for 30 min to 1 hr to allow the heavier particles to settle out. One loopful of this culture was streaked on Brilliant Green-agar containing 8 mg of sodium sulfadiazine per 100 ml of medium and S S Agar (Difco). After incubation for 24 hr, suspicious colonies were transferred to Triple Sugar Iron Agar (Difco). Serological identification and biochemical confirmation were carried out according to the recommended procedure (7).

Fluorescent-antibody method. Portions (10 ml) were withdrawn from the Tetrathionate Broth and centrifuged for 5 min at 310 × *g* to remove the larger suspended materials. The supernatant fluid was decanted into 15-ml conical tubes and centrifuged at 2,200 × *g* for 15 min. The supernatant fluid was discarded and the sediment was resuspended in 0.5 ml of FA phosphate buffer solution [7.67 g of NaCl, 626 g of $NaHPO_4$, and 21 g of K_2HPO_4 in 1 liter of distilled water (*p*H 7.2)]. A loopful of this suspension was placed within the inscribed circle of a Trident-Floro Slide (Aloe Scientific Co., St. Louis, Mo.) and air-dried.

After air-drying, the antigens were fixed on the slides by immersing for 20 min in a solution containing one part Formalin to nine parts of phosphate-buffered saline. After fixation, the slides were immersed in phosphate-buffered saline and agitated vigorously, followed by successive washing in a bath containing absolute alcohol, equal volumes of alcohol-xylene, and xylene. They were then allowed to air-dry (4).

Fluorescent staining. The antiserum used throughout this study was a globulin fraction, absorbed, *Salmonella* polyvalent O (somatic) antiserum including groups A through H which had been conjugated with fluorescein isothiocyanate (Sylvana Co., Millburn, N.J.).

The titer was determined by diluting 1:3, 1:4, 1:5, and 1:6. The highest dilution giving 4+ fluorescence (1:5) with several serotypes of known *Salmonella* was used to stain the fixed antigens prepared from the samples. Twenty isolates of *Proteus* sp., six of *Escherichia coli*, and three of *Pseudomonas* sp., which were found to agglutinate a commercially prepared poly-O antiserum, failed to fluoresce at this titration. A drop of this titered conjugate was placed on each smear and incubated in a moist chamber for 30 min at 35 C. The slides were removed, rinsed in a phosphate-buffered saline solution, and then washed successively in absolute alcohol, equal volumes of alcohol-xylene, and xylene.

Microscopic. The microscope used throughout this

TABLE 1. *Comparison between FA and culture methods in detecting Salmonella*

Product	Agreement[a]		False positive	False negative	Total samples	Significance of disagreement	Per cent positive
	Positive	Negative					
Fish meal	5	190	7	1	203	$P < 0.10$	3.0
Soybean meal	5	113	12	2	132	$P < 0.05$	5.3
Feather meal	42	93	19	0	154	$P < 0.01$	27.3
Meat meal	148	202	17	15	382	NS[b]	42.7
Chows	0	49	2	0	51	NS	0.0
Miscellaneous	5	81	1	4	91	NS	9.9
Total	205	728	58	22	1,013	$P < 0.01$	22.4

[a] Results were obtained by both methods.
[b] Not significant.

experiment was a Wild M20, attached to a Wild Dual Illuminator. The light source was an Osram HBO 200 mercury-vapor lamp. A BV12 exciter filter, BG38 heat-absorbing filter, and an OG1 barrier filter were used. A Wild Aplamatic Condenser (bright field) was employed along with a Fluotar 50× oil objective and two 15× eyepieces.

RESULTS AND DISCUSSION

Samples (1,013) were analyzed by both the FA and cultural methods. The overall agreement between the methods was 92.1%. Table 1 gives the number of samples agreeing, both positive and negative, as well as the number showing false positive and false negative, as determined by the cultural method. Also listed are the total number of samples tested, the per cent positive, and the significance of disagreement. Since we did not overlook the possibility that some of the so-called false positives were actually positive and we, therefore, had failed to isolate *Salmonella* by the cultural method, we subcultured 40 of the 58 false positive samples. Of these, 16 were proven positive.

The significance of disagreement was calculated by the Chi-square test and the difference appears to be real at the probability levels indicated. Disagreement between methods appeared to be greatest in feather meal; this was due entirely to false positives.

One of the problems encountered using the FA technique is that of nonspecific staining by other members of the *Enterobacteriaceae* family (1, 2). Some researchers have reported eliminating this cross-reaction by absorbing with different strains of *E. coli* (1, 4). Little or no problem was expected in this respect, since no cross-fluorescence had been observed when determining the titer of the conjugated antisera. These results may support the concept that the FA technique is more sensitive in detecting *Salmonella* than are culture methods.

TABLE 2. *Comparison between method and subculture on 73 samples of meat meal*[a]

No. of samples	Subsample 1[b]		Subsample 2[c]	
	FA	Culture	FA	Culture
51	−	−	−	−
11	+	+	+	+
6	+	+	−	−
2	−	−	+	+
2	+	+	+	−
1	+	−	−	−

[a] Results are expressed as positive (+) or negative (−).
[b] Agreement between methods 97.9%.
[c] Agreement between samples 86.2%.

The greatest number of false negatives (15 of the 22) were found in the meat meal; however, this material constituted only 37% of the total number of samples tested. There was no obvious reason for having missed these positives by FA, although a few times we seemed to have had some trouble separating the antigens from the meal during centrifugation; on occasion this material has had a tendency to obscure some of the weaker fluorescing organisms.

There is an apparent difference in the results obtained between subsamples when the same method is used. The extent of this difference could have a bearing on the acceptable difference between methods. Samples of meat meal (73) were subdivided into two 30-g samples and each sample was analyzed for *Salmonella* both by the FA technique and the cultural method in an attempt to answer this question (Table 2). Agreement between methods was achieved 97.9% of the time, whereas the samples agreed only 86.2% of the time.

The agreement of 97.9% between methods on these 73 samples of meat meal was somewhat better than that achieved on the 382 samples of meat meal (91.6%) included in the initial test (Table 1). No false negatives occurred in these 73 samples. Perhaps this was due to the discarding of any slides that showed excessive amounts of product material and the subsequent preparation of new ones from the same sample.

We are of the opinion that the FA technique is an acceptable method, but in some cases it may require cultural confirmation. It takes less time to obtain results, it is a less expensive test (after the initial investment for equipment), and it will allow a greater number of samples to be tested.

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