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EXAMINATION OF QUEENSLAND STOCK FEEDS AND FERTILIZERS OF ANIMAL ORIGIN FOR SALMONELLA

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SUMMARY

Of 51 samples of stock feeds and fertilizers manufactured in Queensland examined for salmonellas, 23 were positive. Eighteen different species were found. *S. anatum,* **the most frequently isolated, was present in 11 samples (22%), of which 8 were meat-and-bone meal, 2 were meatworks fertilizer and 1 was blood-and-bone fertilizer.** *S. typhimurium* **was not isolated.**

No salmonellas were isolated by direct plating of samples. Enrichment in tetrathionate broth resulted in 7 and 8 isolations on SS agar plated after 24 and 48 hours' incubation, respectively, using 1-g samples; 16 isolations were made from both 24 and 48 hours' plating using 10-g samples. The comparable figures for BG agar were 6 and 10 for 1-g and 7 and 11 for 10-g samples.

The use of both 1-g and 10-g samples resulted in the isolation of more species than from either alone. The use of two types of solid media also increased the number of different species isolated.

I. INTRODUCTION

Salmonellosis has always been an important source of loss to the animal industries of Queensland, particularly in pigs and cattle (Simmons and Sutherland 1950; Simmons 1951; Simmons, Connole, and Elder 1963).

Many authors have recently drawn attention to the presence of salmonellas in the components of, and in complete, animal feeds and have stressed the possible importance of these materials in the dissemination of salmonellas. Boyer, Bruner, and Brown (1958), Moran (1960) and Stenberg (1961) examined complete feeds; Walker (1957), Clarenburg (1959), Kovacs (1959), Watkins, Flowers, and Grumbles (1959), Gray, Harley, and Noble (1960) and Thomas (1961) examined meat-and-bone meals as separate components; and Gray, Lewis, and Gorrie (1958), Report (1959, 1961) Newell *et al.* (1959), Dixon and Wilson (1960), Röhr (1960) and Morehouse and Wedman (1961) examined both component parts and complete feeds.

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With the co-operation of the Standards Branch of the Department of Primary Industries, samples of meat-and-bone meal and fertilizers containing meat products, manufactured in Queensland, were examined for salmonellas. This paper gives the results of examination of 51 samples.

II. MATERIALS AND METHODS

Samples.—Table 1 shows the types of samples examined and the areas in Queensland where they were manufactured. The first 18 samples were described either as fresh (1, 3, 4, 6–16), stored 4½–9 months (5) or old stock (17, 18). This information was not stated for the other samples.

TABLE 1

GEOGRAPHICAL DISTRIBUTION, TYPES AND NUMBERS OF SPECIMENS CONTAINING SALMONELLAS

Type of Sample	Where Made	No. of Samples	Positive	Negative
Blood and bone fertilizer	Brisbane ..	2	2 (3, 5)*	0
	Rockhampton	1	1 (29)	0
Meatworks fertilizer ..	Brisbane ..	2	2 (8, 11)	0
	Gayndah ..	1	1 (15)	0
	Bowen	1	0	1 (18)
Meat-and-bone meal ..	Brisbane ..	7	2 (6, 47)	5 (1, 2, 4, 12, 22)
	Warwick ..	6	1 (15)	5 (14, 23, 24, 42, 43)
	Beaudesert ..	4	0	4 (19, 31, 36, 46)
	Bundaberg ..	4	4 (20, 35, 44, 51)	0
	Toowoomba ..	3	2 (38, 39)	1 (26)
	Oakey	3	0	3 (37, 41, 45)
	Townsville ..	3	3 (32, 33, 48)	0
	Roma	2	1 (21)	1 (40)
	Mareeba ..	1	1 (16)	0
	Gladstone ..	1	1 (27)	0
	Rockhampton	1	1 (30)	0
Bone flour	Brisbane ..	1	0	1 (7)
Liver meal	Brisbane ..	1	1 (9)	0
	Rockhampton	1	0	1 (28)
	Townsville ..	1	0	1 (50)
Blood meal	Brisbane ..	1	0	1 (10)
	Bowen	1	0	1 (17)
	Townsville ..	1	0	1 (49)
Bone and salt lick ..	Rockhampton	1	0	1 (25)
Whale protein meal ..	Tangalooma ..	1	0	1 (34)
TOTAL	..	51	23	28

*Sample numbers are given in parentheses.

Meat meal is defined as being made from meat. It may contain blood and bone but should not contain more than 5% P_2O_5. Meat-and-bone meal contains meat and bone and may contain blood; it is defined as containing more than 5% P_2O_5. Meatworks fertilizer is made from blood, bone and offal. Bone flour is 100% bone. Liver meal contains liver and lung. Blood meal is 100% blood. Bone and salt licks contain not more than 36% salt; the rest is bone.

Samples were taken in new tins, not sterilized, by means of a trier at either the factory or point of sale. The same trier was used for all samples taken on a particular day. A representative sample was taken from a certain number of bags, the number sampled depending on the total number of bags. The top of one bag, the middle of the next and the bottom of the next were sampled in rotation.

Media.—Tetrathionate broth was dispensed in 50-ml quantities and 1 ml of iodine solution was added just before use. Salmonella–Shigella agar plates (S.S.–Baltimore Biological Laboratory) and Brilliant Green agar (BG–Bacto-Brilliant Green Agar Difco) were poured into 100-mm diam. plates. Urease production was tested by inoculating slopes of urea agar (Christensen 1946).

Acid production from glucose, mannitol, maltose, sucrose, dulcitol and lactose was determined using 1% of the test solution in peptone water containing Andrade's indicator. A Durham tube was included in the glucose tube to detect any gas produced. Production of indole was determined using Ehrlich's reagent, after growing the organisms for 24 hr in tryptone water. Potassium cyanide growth test (KCN) was done as described by Edwards and Ewing (1955).

Method of examination.—The specimens were examined once, except for samples 11, 13, 15 and 17, which were each examined three times. The results given are a composite of the three examinations. One gram of each sample was weighed into one sterile 4-oz bottle and 10 g into each of two sterile 4-oz bottles, using a sterilized spatula for each sample.

Nutrient broth (100 ml) was poured into one of the bottles containing 10 g of the sample; the bottle was shaken and then incubated for 2 hr at 37°C. A loopful of the contents was then sown onto BG agar and SS agar, using half of a plate for each sample.

Tetrathionate broth (50 ml) was poured into the other two bottles containing 1 and 10 g respectively of the sample; the bottles were shaken and then incubated at 37°C. After 24 and 48 hours' incubation, a loopful of each was sown on to half of a plate each of BG agar and SS agar.

All plates were examined after 24 hours' incubation at 37°C for non-lactose fermenting organisms, and single colonies of this type with a maximum of 6 per plate were inoculated into nutrient broth and onto urea agar slopes. After 24

hours' incubation the broths of those colonies which did not hydrolyse urea were inoculated into sucrose medium and onto blood agar. Isolates not fermenting sucrose were then inoculated into the glucose, maltose, mannitol, dulcitol and lactose media and into tryptone water.

After 24 hours' incubation, isolates which produced acid and gas from glucose, acid in mannitol, maltose, and dulcitol but did not produce indole were tested against "O" antisera of Salmonella groups B, C, D and E on a slide, using the growth from the blood plate as antigen.

Isolates which did not agglutinate any of the antisera were inoculated into KCN medium and their growth or failure to grow recorded daily for 4 days' incubation. Any of which did not grow were regarded as possibly a salmonella. All isolates considered to be salmonellas were sent to the Salmonella Typing Centre, Adelaide, South Australia.

III. RESULTS

The results of examining 51 samples of meat-and-bone meal products for salmonellas are given in Tables 2-4.

Salmonellas were isolated from 23 (Nos. 3, 5, 6, 8, 9, 11, 13, 15, 16, 20, 21, 27, 29, 30, 32, 33, 35, 38, 39, 44, 47, 48 and 51) of the 51 samples.

TABLE 2

NUMBER OF NON-LACTOSE FERMENTING COLONIES EXAMINED AND NUMBER SUBSEQUENTLY IDENTIFIED AS SALMONELLAS

	Tetrathionate								Direct Plating	
	1g				10g					
	24 Hours		48 Hours		24 Hours		48 Hours			
	SS	BG	SS	BG	SS	BG	SS	BG	SS	BG
NLF Colonies	120	75	205	55	189	51	227	73	23	32
Salmonellas	24	24	30	22	61	30	45	23	0	0

NLF = Non-lactose-fermenting organism.
SS = Salmonella–Shigella agar.
BG = Brilliant Green agar.

TABLE 3

NUMBERS OF SAMPLES FROM WHICH SALMONELLA SPECIES WHERE ISOLATED

Specimen	No. of Samples
S. anatum	11
S. oranienburg ..	6
S. senftenberg ..	5
S. bredeney ..	4
S. cambridge ..	3
S. newington ..	3
S. derby	2
S. chester	2
S. give	2
S. meleagridis ..	2
S. taksony	1
S. orion	1
S. singapore ..	1
S. enteritidis ..	1
S. adelaide	1
S. newport	1
S. manila	1
S. bareilly	1
Total	48

Table 2 shows the number of non-lactose fermenters and the number of salmonellas isolated by each method of culture. A total of 1150 non-lactose fermenting colonies was examined; 277 (24%) were salmonellas.

No salmonellas were isolated from plating samples directly onto selective media (Table 2).

Twelve samples yielded salmonellas when 1-g aliquots were enriched in tetrathionate broth before plating on selective media and 21 when 10-g aliquots were used. Only 9 samples yielded salmonellas in both 1-g and 10-g quantities. Salmonellas were isolated from 46 SS and 34 BG agar plates, but only 28 pairs of SS and BG agar plates were both positive. Twenty-eight of the plates inoculated from tetrathionate broth incubated for 24 hr yielded salmonellas, compared with 52 plates sown from tetrathionate broth which had been incubated for 48 hr.

Table 3 shows the number of samples from which particular species of salmonellas were isolated. *S. anatum* was the most frequently isolated, followed by *S. oranienburg, S. senftenberg* and *S. bredeney.*

Table 4 shows the species isolated from each sample by each culture technique. Each species is represented once per plate, although more than one strain of each species may have been isolated from the plate. Seven strains died before they were identified serologically and are listed as unidentified.

TABLE 4

SALMONELLA SPECIES ISOLATED BY DIFFERENT CULTURE METHODS

Sample No.	1 g Tetrathionate				10 g Tetrathionate			
	24 hours		48 hours		24 hours		48 hours	
	SS	BG	SS	BG	SS	BG	SS	BG
3				*S. cambridge*				
5				*S. chester* *S. anatum*				
6							1 unidentified *S. anatum* *S. derby*	
8					*S. bredeney* *S. cambridge*		*S. bredeney*	
9			*S. taksony*		*S. taksony*		*S. taksony*	*S. taksony*
11	*S. anatum**	*S. anatum*		*S. bredeney*	*S. bredeney** *S. anatum* *S. newington**			
13	*S. oranienburg*	*S. oranienburg*	*S. cambridge*		*S. bredeney* *S. anatum*	*S. bredeney**	*S. bredeney* *S. anatum*	*S. bredeney**
15					*S. anatum**	*S. anatum** *S. senftenberg*	*S. senftenberg*	*S. senftenberg*
16	*S. enteritidis*	*S. enteritidis*	*S. enteritidis*	*S. enteritidis*	*S. enteritidis*	*S. enteritidis*	*S. enteritidis*	
20					*S. give*		*S. give*	
21								*S. senftenberg*

27					*S. bredeney*	*S. bredeney*		*S. bredeney*
29	*S. orion*	*S. orion*	*S. orion*		*S. orion*	*S. orion* *S. singapore*		
30					*S. meleagridis* *S. oranienburg*		*S. meleagridis*	
32					*S. oranienburg*	*S. oranienburg*	*S. oranienburg*	*S. oranienburg*
33	*S. anatum*	*S. anatum*	*S. anatum*	*S. anatum*	*S. adelaide*	*S. senftenberg*		*S. anatum*
35							*S. newington*	*S. newington* *S. anatum*
38			*S. oranienburg* *S. newport*		*S. meleagridis*	*S. meleagridis*	*S. meleagridis*	*S. meleagridis*
39					*S. manila* *S. anatum* *S. oranienburg* *S. newington*	*S. senftenberg*		
44							*S. derby*	
47							*S. anatum*	
48	*S. anatum*		*S. anatum*	*S. anatum*	*S. oranienburg*		*S. oranienburg* *S. senftenberg*	*S. oranienburg* *S. senftenberg* *S. bareilly*
51	*S. give* *S. anatum*	*S. give* *S. anatum*	*S. give* *S. anatum* 1 unidentified	*S. give* 2 unidentified	*S. chester** *S. anatum*	*S. chester**	*S. give* *S. anatum* 1 unidentified	2 unidentified

SS = Salmonella–Shigella agar. BG = Brilliant Green agar. * = Species not cultured on first examination.

IV. DISCUSSION

From Tables 2 and 3 it can be seen that the greatest number of non-lactose-fermenting organisms was isolated when the larger amount of meat meal sample was sown into tetrathionate broth. This is in agreement with the results of Thomas (1961). However, it is clear from Table 3 that there were species of salmonellas isolated from the smaller amount (1 g) which were not isolated when 10 g of material was used.

SS agar plates produced a greater number of isolations of salmonellas than BG agar plates, but occasionally, as in sample 21, the salmonellas were isolated only from BG agar plates.

About the same number of salmonellas was isolated from both 24-hr plates and 48-hr plates, but often salmonella species were isolated on one which were not isolated on the other. It is clear, therefore, that one culture method is not adequate for examining meat meal products for salmonellas. It is also clear that more than one non-lactose-fermenting isolate from each plate should be examined, as different types of salmonella can appear on a single plate.

Table 1 shows that salmonellas were distributed through most types of samples examined, except bone flour, blood meal, bone and salt lick and whale protein meal, but only a relatively small number of these were examined. Contaminated samples were not restricted to any part of the State of Queensland (Figure 1).

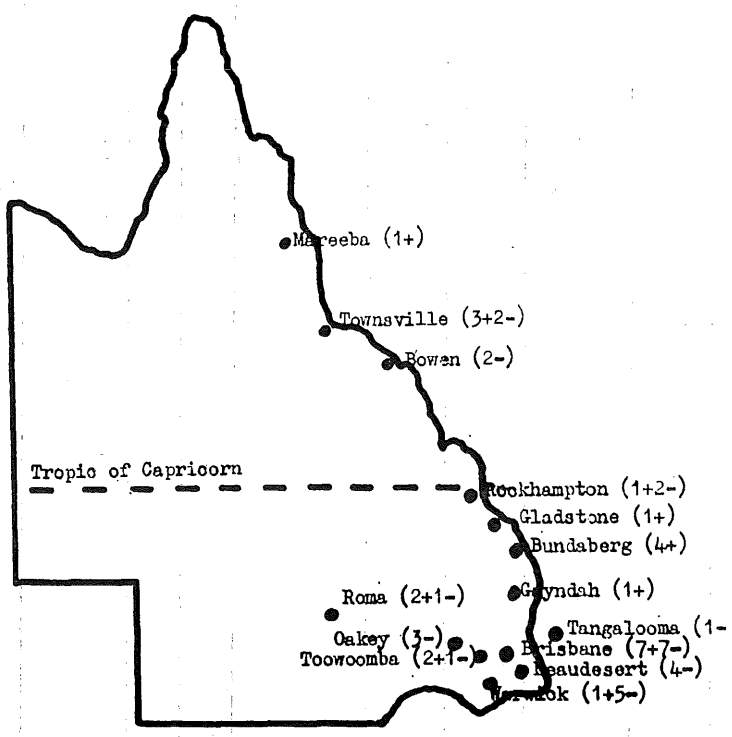

Fig. 1.—Geographical distribution of samples.
+ Samples from which salmonellas were isolated.
— Samples from which salmonellas were not isolated.

From our results, 45% of the samples were contaminated with salmonellas. This may be compared with the results of Gray, Harley, and Noble (1960), who gave a figure of over 90% for Australian bone meal, and 48% contamination of bone products, which included crushed bone, bone meal and bone flour (Report 1959).

S. typhimurium is far more frequently isolated than any other species from pathological specimens submitted to this Institute (Sutherland and Simmons 1950; Simmons 1951; Simmons, Connole, and Elder 1963). It is surprising, therefore, that *S. typhimurium* was not isolated from meat meal products. We have seen only three reports of *S. typhimurium* in this type of product. Walker (1959) found it twice in 40 samples of bone meal, but not in bone and meat meal, blood meal or meat meals. English workers (Report 1959) found it in 3 out of 78 samples of hoof and horn meal and in 19 out of 298 samples of bone products. A later Report (1961) states it was found in 1 out of 255 samples of meat-and-bone meal and in 1 of 219 samples of protein concentrate meal. Morehouse and Wedman (1961) reported that *S. typhimurium* was found most commonly in poultry products. From personal communication with Dr. Morehouse it was learnt that he has never isolated it from bone meal, and has isolated it once each from a combination of meat scrap and bone meal and meat scrap alone. Galbraith (1961), in a discussion on the public health aspects of salmonellosis, said it was difficult to say why *S. typhimurium* was not found in feeds manufactured from animals, and suggested perhaps the process of manufacture was more detrimental to some salmonella types than others.

Of interest in this regard is the report of Smith (1960) that *S. typhimurium* was isolated from bone meal by a filtration technique whereas the same meal cultured on numerous occasions by placing directly into liquid enrichment media did not result in the isolation of *S. typhimurium.*

The absence of *S. cholerae suis,* a common pathogen of pigs in Queensland (Simmons 1951), may be explained by the lack of pig meat constituents in the meat products and/or the use of tetrathionate broth, which may inhibit this species (Smith 1952).

Other species besides *S. typhimurium* showed an abnormal distribution when compared with those isolated from animal specimens at this Institute. For example, the four species most frequently isolated in this survey were *S. oranienburg* (6), *S. senftenberg* (5), *S. bredeney* (4) and *S. anatum* (11), whereas from animal specimens in 1951-1960, the four species most frequently isolated were *S. typhimurium, S. cholerae suis, S. bovis morbificans* and *S. derby* (Simmons, Connole, and Elder 1963). A similar discrepancy in results obtained in England has been commented on by English workers (Report 1959). Relevant to this point also may be the data given by Jones, Bennett, and Ellis (1961), indicating that the phage types of *S. typhimurium* isolated from abattoir effluents were often dissimilar from those isolated from human cases of *S. typhimurium* infection.

Gray, Lewis, and Gorrie (1958) reported data that gave additional evidence to the discrepancy between the species of salmonellas isolated from cases of bovine salmonellosis compared with those from bone meal fed to the animals.

It is apparent from the literature cited above that there is a great need for further investigation into the epidemiology of salmonellosis, especially that caused by *S. typhimurium* in domestic animals, before the role of infected feeds can be evaluated.

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