ON METHODS FOR THE ISOLATION OF SALMONELLA FROM CHICKENS.

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SUMMARY.

The efficiency of some selective media for isolating Salmonella from chickens was investigated.

From each of 268 birds the liver, lung and intestinal mucosa and contents were cultured. These were sown on to direct plates of MacConkey, bismuth sulphite and S.S. agar and into tetrathionate broth.

S. pullorum was recovered most frequently from the lung, while most isolations of other species of Salmonella were made from the intestine.

An efficient yet economical method for routine isolations of Salmonella from chickens was adopted.

INTRODUCTION.

One of the common causes of mortality in young chickens in Queensland is Salmonella infection. As no consistent lesions are found, post-mortem diagnosis of these infections rests upon the isolation of Salmonella from the chickens.

The incidence of these diseases is highest in chickens under three weeks of age, so all such birds submitted to the Animal Health Station for diagnosis are examined bacteriologically. Older chickens are cultured at the discretion of the pathologist. These routine examinations require a method of culturing which will produce the maximum recovery of Salmonella organisms consistent with economy of time and media. It was therefore decided to determine the efficiency of various selective media and combinations of media for the isolation of Salmonella, particularly *Salmonella pullorum*. Previous experience at the Station, together with reports by Mallmann, Ryff and Matthews (1942) and Bushnell and Porter (1945), indicated that, of the available media, some combinations of MacConkey, S.S., and bismuth sulphite agar in conjunction with tetrathionate broth enrichment would be suitable media for trial.

MATERIALS AND METHODS.

The bismuth sulphite, S.S. and Kligler iron agar used were Difco products.

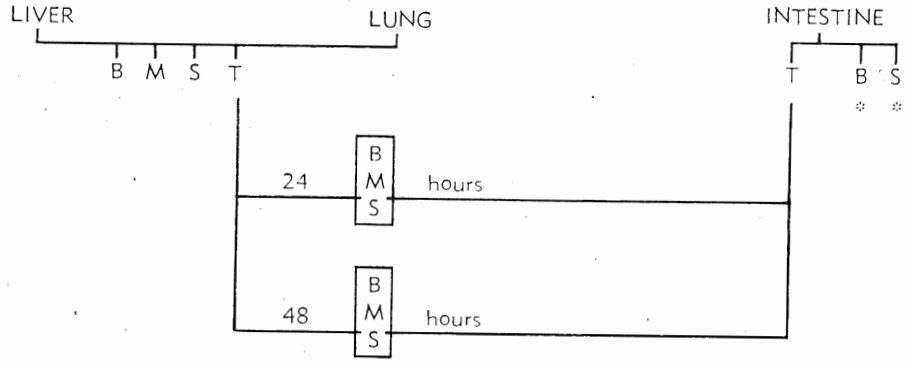
MacConkey agar was made up using the Difco (1948) formula but substituting 17 gm. agar per litre for the 13 gm. specified.

Tetrathionate broth was made to the formula of Mackie and McCartney (1948). Before use, 0·2 ml. of iodine solution was added to 10 ml. of base. This solution was prepared by grinding together in a mortar 6 gm. of iodine and 5 gm. of potassium iodide, then dissolving the mixture in 20 ml. distilled water.

Urea agar was made according to the method of Christensen (1946).

Sick chickens were submitted by officers of the Poultry Branch, Department of Agriculture and Stock, or by the owners. When four or more birds were presented, four only were cultured. When less than four birds were submitted, all of them were cultured. Sick birds were used where possible in preference to dead ones to reduce the number of contaminating organisms.

From each bird one lobe of the liver was detached with sterile forceps and the cut surface smeared on portion of half plates of MacConkey, S.S., and bismuth sulphite agar. The forceps were flamed thoroughly. One lung was then removed and smeared on portion of the other half of these plates. The smeared portion served as a primary inoculum and was stroked out with a sterile wire loop. The intestine was removed and laid on clean paper, then opened lengthwise with sterile scissors to expose the contents. A representative sample of the contents and of the mucosa was collected by scraping with a sterile nickel spatula. This was sown on S.S. and bismuth sulphite agar whole plates, and into tetrathionate broth. The plates were stroked out, incubated for 24 hours at 37°C. and examined. Tetrathionate broths were incubated at 37°C. and were plated on to half plates of MacConkey, S.S., and bismuth sulphite agar after 24 hours' and again after 48 hours' incubation. All plates containing no Salmonella-like colonies were incubated for a further 24 hours at 37°C. The method can be represented schematically thus:—



B = Bismuth sulphite agar; S = S.S. agar;
M = MacConkey agar; T = Tetrathionate broth.

* Here the inoculum was spread on the whole plate. Two inocula were spread separately on each of the other plates.

It will be seen that 11 plates and two tetrathionate broths were used for each bird.

Colonies fermenting lactose on MacConkey or S.S. agar were not identified.

Several suspicious colonies from each plate were sown onto Kligler iron agar and urea agar slopes, which were incubated at 37°C. overnight. Urea positive slopes indicative of Proteus were discarded.

Slide agglutinations with the growth produced on the Kligler or urea agar slopes were done with polyvalent "O" Salmonella serum. Positives were

grouped with Group A, B, C, D, E sera. Suspicious colonies were also sown in nutrient broth to inoculate 1·0 per cent. carbohydrate media (glucose, sucrose, maltose, mannité, lactose) and tryptone water (for the indole test). Generally one set of "sugars" was used for each organ cultured. When more than one serological group was found on a plate, a colony of each group was sown into nutrient broth. The broths were incubated for 5-6 hours and each was used to inoculate a set of " sugars."

One culture per bird of every serological group found was sent to Miss N. Atkinson at the Institute of Medical and Veterinary Science, Adelaide, for specific serological identification.

RESULTS.

Eighty batches containing a total of 268 chickens were examined.

Table 1 shows the isolations of Salmonella on each medium from each source for the 44 birds which were infected with one or more types of Salmonella. From 14 chickens, *S. pullorum* was isolated. Other Salmonella were isolated from 32 birds.

Table 1.

THE DISTRIBUTION OF RECOVERIES OF SALMONELLA.

Number of Bird.	Direct Plates.		Tetrathionate. Liver and Lung.		Direct Plates. Intestine.	Tetrathionate. Intestine.	
	Liver.	Lung.	24 Hours.	48 Hours.		24 Hours.	48 Hours.
	M S B	M S B	M S B	M S B	B S	M S B	M S B
1						S	S S
2	P P P	P P P	P P	P P P		P P P	P P P
3		P P P	P P	P P P	P P	P P	P P P
4						S S S	S S S
5		S				S S	S S S
6	S S S	S S S	S S	S S			
7	S S S	S S	S S	S S S	S	S S	S S S
8						S S S	S S S
9			S S S	S S S		S S S	S S S
10	S		S S S	S S S			S S
11							S
12	P P P	P P P	P P		P P	S S	S S S
13	P P	P P P	P P		P	S S S	S
14	P P P	P P P	P P P	P P P	P P	P P P	P P P
15		P P		P P P	P		
16		P P P	P P	P P P	P		
17					P		
18		S S	S S S	S S S		S	S S S
19		S					

Table 1—continued.

THE DISTRIBUTION OF RECOVERIES OF SALMONELLA.

Number of Bird.	Direct Plates.		Tetrathionate. Liver and Lung.		Direct Plates. Intestine.	Tetrathionate. Intestine.	
	Liver.	Lung.	24 Hours.	48 Hours.		24 Hours.	48 Hours.
	M S B	M S B	M S B	M S B	B S	M S B	M S B
20	S		S S	S S S	S S	S S S	S S S
21	S S	S S	S S S	S S S	S S	S S S	S S S
22	S		S S S	S S S	S	S S S	S S
23	S		S S	S S S	S S	S S S	S S S
24		P P P	P P P	P P P			
25	P P P	P P P	P P P	P P P	P		
26	P P P	P P P	P P P	P P P			
27	P P P	P P P	P P P	P P P	P P	P P P	
28			S S S	S S S		S S S	S S S
29							S S S
30			S S	S		S S	S S S
31			S S S	S S S		S	S
32						S S	
33							S S S
34			S S	S S	S S	S S S	S S S
35						S S S	S S S
36						S S S	S S S
37						S S S	S S S
38					S	S S S	S S S
39	S S		S S S	S S S		S S S	S S S
40				S S S			S
41	P P P	P P P	P P P	P P P	P		
42	P P P	P P P	P P P	P P P	P P	P P P	P P P
43	S		S S	S S S		S S	S S S
44	S	S	S S	S S S		S S	S S S
S. pullorum ..	8 9 9	13 13 12	9 11 11	11 11 11	9 7	4 5 5	4 4 4
Other Salmonella	6 6 4	4 5 3	16 15 9	16 16 15	5 6	20 23 19	23 27 26
Total ..	14 15 13	17 18 15	26 26 20	27 27 26	14 13	24 28 24	27 31 30

The horizontal lines separate birds from different batches.

P = S. pullorum. M = MacConkey agar.

S = Salmonella other than S. pullorum. S = S.S. agar.

Blank = No Salmonella isolated. B = Bismuth Sulphite agar.

S. pullorum.

One of the 14 isolations of *S. pullorum* was made on only one of the 20 possible platings* used on the bird. This was on the direct plating of intestine on S.S. agar (Table 1, Chicken No. 17).

The other 13 isolations were made from varying combinations of the three sources† (liver, lung, and intestine). The lung was one of the sources in each case.

From the direct plating of the lung, the organism was recovered on both MacConkey and S.S. agar 13 times, and on the bismuth sulphite agar 12 times.

The liver sown on direct plates yielded fewer isolations.

Tetrathionate broth sown with pooled lung and liver was instrumental in recovering a large proportion of the *S. pullorum* isolated. From platings of this broth after 24 hours' incubation, the organism appeared somewhat erratically on the three solid media. However, after 48 hours' incubation and subsequent plating of the tetrathionate broth, *S. pullorum* grew in each of 11 cases on all three solid media.

Isolations from the intestine, either on direct plates or after tetrathionate enrichment, were few in number.

Other Salmonella.

Thirty-two isolations of Salmonella other than *S. pullorum* were made.

Isolations from direct plating of the three sources were few, the maximum for any one plating being six.

Tetrathionate enrichment yielded many more isolations. Again the platings of the tetrathionate incubated for 48 hours showed a slight increase in the number of recoveries over the platings made at 24 hours. The solid media did not differ significantly in the number of recoveries from these tetrathionate platings.

The apparently anomalous total of 46 isolations (Table 2) is due to the presence of *S. pullorum* together with other Salmonella in two birds (*see* Table 1).

These birds were from a flock infected with three species—*S. pullorum*, *S. worthington* and *S. meleagridis*. Two chickens (13, 18) from this flock yielded two of these species, while another chicken (13) yielded all three species. On several plates from these birds two different serological groups were represented, indicating the need for slide agglutinations to be carried out with several colonies on each plating.

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* The word "plating" is used to denote the sowing of material on a solid medium whether only half the plate or the whole plate was used.

† The word "source" is used to refer to the materials examined—namely, liver, lung, and intestinal contents.

Direct and Indirect Plating.

In Table 2, the number of isolations which would have been made if the direct plates only had been used is compared with the number of isolations resulting from the use of only the tetrathionate platings.

Table 2.

COMPARISON OF TOTAL ISOLATIONS MADE BY DIRECT AND INDIRECT METHODS.

Organism.	Number of Isolations.		
	Direct Plates only.	Tetrathionate Platings only.	Total (Direct + Tetrathionate).
S. pullorum	14	13	14
Other Salmonella ..	15	31	32
All Salmonella	29	44	46

DISCUSSION.

The lung appeared to be a favourable site for the isolation of *S. pullorum,* while the intestine was a poor source. For species of Salmonella other than *S. pullorum,* the intestine appeared to be the best source.

Comparison of direct and indirect plating emphasises the importance of preliminary enrichment in tetrathionate (Table 2). With Salmonella other than *S. pullorum,* tetrathionate enrichment was responsible for over twice the number of isolations that direct plates produced. With *S. pullorum,* direct plating was very effective, particularly in the case of the lung. Tetrathionate enrichment was almost equal in value. The results were examined with a view to devising an efficient method for isolating Salmonella which would be economical in the use of time and media.

Tetrathionate broth enrichment was responsible for the isolation of 44 strains of Salmonella, whereas direct plates were responsible for only 29 strains being isolated. The combination of media used in this experiment resulted in a total of 46 strains. The two strains which the tetrathionates did not detect occurred on direct plates, but in both cases on one plate only (Nos. 17, 19). Apparently the organisms were present in very small numbers. In addition, each strain was isolated from the least expected source. The *S. pullorum* was found on direct S.S. plates of the intestine and the other Salmonella came from the direct S.S. plate of lung. Although it is tempting from the viewpoint of economy to reduce the amount of media drastically and use only tetrathionate platings. the odd Salmonella may be missed. To reduce this apparently slight possibility, it was decided to include a direct plate. Supporting this move is the fact that should Salmonella grow on the direct plate, one day is saved in establishing a diagnosis. This is of importance in routine laboratory work. The direct plate which seemed to be most effective was the S.S. plate of liver and lung.

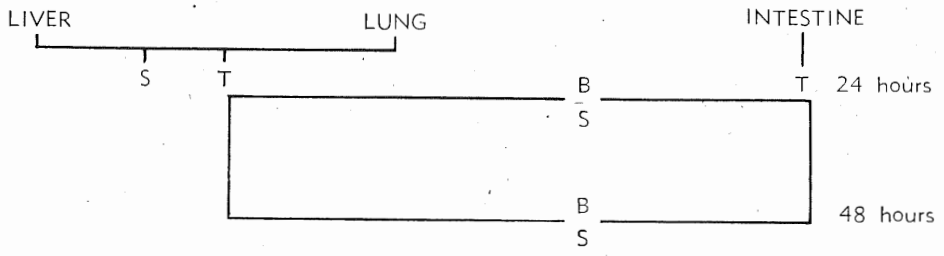
Examination of Table 1 revealed that plating of tetrathionates on MacConkey was unnecessary. The same results would have been obtained had this medium not been used. The method of culturing decided upon was as follows:—

From each bird the liver and lung were sown directly each on to half of an S.S. agar plate. These organs were pooled in a tetrathionate broth. The intestinal contents were placed in a second tetrathionate broth. After 24 hours' incubation, each of these broths was plated on to half an S.S. and half a bismuth sulphite agar plate. This was repeated after the broths had incubated for 48 hours.

The media used for each bird were therefore two tetrathionate broths, two bismuth sulphite agar plates and three S.S. agar plates.

This method would have achieved 45 of the 46 isolations made during the experiment.

The method may be represented schematically thus:—



B = Bismuth sulphite agar.
S = S.S. agar.
T = Tetrathionate broth.

Both bismuth sulphite and S.S. agar would still have to be used for plating tetrathionate broths, as on several occasions one of them failed to grow the organism. On each of these media Salmonella colonies were readily distinguished from all organisms except Proteus. The urea agar slope provided an efficient method of demonstrating the presence of this organism.

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