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Studies on the isolation of *Salmonella dublin*

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SUMMARY

Abattoir drain swabs, bovine faeces and a few other veterinary samples were examined for the presence of *Salmonella dublin*. Three selective agar media and four enrichment broths were investigated. The two most efficient plating media were deoxycholate citrate agar and brilliant green MacConkey agar. Wilson and Blair's bismuth sulphite agar (de Loureiro's modification) was least successful. Selenite F broth, whether incubated at 37 or 43° C., was better than the other enrichment broths used which contained a triphenyl methane dye as one of the selective ingredients.

INTRODUCTION

The separate identity of *S. dublin* and *S. enteritidis* was established in 1930 by White. Since then, the geographical distribution of *S. dublin* infections has been accurately recorded. A high incidence of the serotype has been noted in several continents and the distribution has been recorded by Gibson (1965). A recent paper (Hugh-Jones 1970) found that *S. dublin* was most frequently isolated from adult cattle in Wales and in Devon. The data were taken from the investigation of specimens submitted to Veterinary Investigation Centres in England and Wales in 1969. The serotype is widespread and presents a major veterinary problem in certain countries. Reliable isolation techniques are thus of interest to public health and veterinary microbiologists. The present investigation seeks to examine the efficiency of some selective and enrichment media in the isolation of an important salmonella species.

MATERIALS

The samples used in this study were bovine faeces, abattoir drain swabs and seven mixed veterinary specimens (four bovine placentas, two bovine vaginal mucus, one bovine gut).

The selective media investigated were deoxycholate citrate agar, brilliant green MacConkey agar and de Loureiro's (1942) modification of Wilson and Blair's bismuth sulphite agar. The enrichment media examined were selenite F broth, Muller–Kauffmann tetrathionate broth, magnesium chloride malachite green broth and SGB enrichment broth (Difco Code No. 0661-01-9). The selective agars, tetrathionate and selenite broths were prepared according to Harvey & Price (1974). The magnesium chloride malachite green broth was made to the formula

of Rappaport, Konforti & Navon (1956) and the Difco enrichment medium was prepared to the manufacturers' instructions.

METHODS

Selective media and enrichment media were investigated separately. In the first study the enrichment broth (selenite F) was kept constant and the plating media were compared. In the second investigation the selective medium was constant (deoxycholate citrate agar) and the relative efficiency of the enrichment broths was examined. Abattoir drain swabs were cultured in selenite F using 100 ml. of single-strength medium added to the Moore's swabs in the 8 oz. (220 ml.) screw-capped jars in which they were submitted to the laboratory (Moore, 1948). The inoculum of veterinary material used in enrichment broths was two drops (0·04 ml.) of a suspension of 1 g. of the sample in 1 ml. of peptone water. Coarse particles were allowed to settle in the suspension before inoculating the enrichment media. Enrichment broths were incubated for 24–72 hr. Subcultures at 24, 48 and 72 hr. were made for the abattoir drain swabs and one subculture at 24 hr. was used with veterinary samples. Incubation temperatures were 37° C. and 43° C., depending on the enrichment broth. Temperatures are recorded in the tables. Selective agars were incubated at 37° C. for 24–48 hr. Deoxycholate citrate and brilliant green plates were examined at 24 hr. and Wilson and Blair agars after 24 and 48 hr. incubation. Suspicious colonies were picked for further examination and all strains were identified at the Salmonella Reference Laboratory, London.

RESULTS

The results obtained with abattoir drain swabs are recorded in Table 1. Thirty-five positive samples were examined, 32 were plated on three selective agars and three on two selective media only. The solid media used were deoxycholate citrate agar, brilliant-green MacConkey agar and bismuth sulphite agar. The enrichment broth in this investigation was selenite F and this was incubated at 43° C. Subcultures were made after 24, 48 and 72 hr incubation. Table 1 illustrates the advantages of subculture at different times, but it must be noted that the 24 hr. incubation period of enrichment was the most successful with deoxycholate citrate and brilliant-green MacConkey plating agars. Deoxycholate citrate agar was the most efficient selective medium and with the three subcultures all 35 samples were found positive. Brilliant green MacConkey agar produced 80% of the possible positives while Wilson and Blair's bismuth sulphite agar was a relative failure in the isolation of *S. dublin*. Only 3/32 samples were found positive on this medium.

Table 2 records results obtained with bovine faeces. Selenite F broth incubated at 43° C. was used for enrichment. Only one subculture was made after 24 hr. incubation. The plating media in this study were deoxycholate citrate agar, brilliant-green MacConkey agar and bismuth sulphite agar. Table 2 is arranged in two parts. Section A shows the combinations of the three plating media found positive or negative and section B records the results in terms of three fourfold

Table 1. *Isolation of* Salmonella dublin *from abattoir drain swabs after enrichment in selenite F broth at 42° C. for 24–72 hr. and subculture on three different selective media*

Culture positive after enrichment for (hr.):	Number of cultures positive on: Deoxycholate citrate agar	Brilliant-green MacConkey	Bismuth sulphite agar*
24, 48, 72	12	9	0
24, 48	7	7	0
48, 72	0	1	0
24	13	9	0
48	2	2	0
72	1	0	3
Total positive on each medium	35	28	3
Positive after enrichment for			
24 hr.	32	25	0
48 hr.	21	19	0
72 hr.	13	10	3

* Three samples were not subcultured on bismuth sulphite agar.

Table 2. *Isolation of* Salmonella dublin *from bovine faeces after enrichment in selenite F broth at 43° C. for 24 hr. and subculture on three different selective media*

A

Combinations of results found: Deoxycholate citrate agar (DCA)	Brilliant-green MacConkey (BGM)	Bismuth sulphite (BSA)	No. in each combination
+	+	+	16
+	+	−	17
+	−	−	3
+	−	+	2
−	+	−	1

Total samples positive 39.

B

DCA \ BGM	+	−	Total
+	33	5	38
−	1	0	1
Total	34	5	39

$\chi^2 = 1{\cdot}5$
$P < 0{\cdot}2$

DCA \ BSA	+	−	Total
+	18	20	38
−	0	1	1
Total	18	21	39

$\chi^2 = 17{\cdot}2$
$P < 0{\cdot}001$

BGM \ BSA	+	−	Total
+	16	18	34
−	2	3	5
Total	18	21	39

$\chi^2 = 11{\cdot}3$
$P < 0{\cdot}001$

Table 3. *Isolation of* Salmonella dublin *from bovine faeces and mixed veterinary specimens after enrichment in several media for 24 hr. at 37 or 43° C. and subculture on deoxycholate citrate agar*

A

	Enrichment media and temperatures					
	Selenite F		SBG	MG $MgCl_2$	M-K tet.	
	37°	43°	37°	37°	43°	No. in each combination
	+	+	+	+	+	34
	+	+	−	+	+	12
	+	+	+	+	−	6
	+	+	+	−	+	2
	+	−	+	+	+	1
	+	+	−	+	−	5
	+	+	−	−	+	11
	+	+	+	−	−	2
	+	+	−	−	−	14
	−	+	−	−	+	1
	+	−	−	−	−	1
	−	−	−	+	−	1
Total positive	88	87	45	59	61	90

B

		M-K tet.			SBG			MG $MgCl_2$		
		+	−	Total	+	−	Total	+	−	Total
Selenite F 43° C.	+	60	27	87	44	43	87	57	30	87
	−	1	2	3	1	2	3	2	1	3
	Total	61	29	90	45	45	90	59	31	90
		$\chi^2 = 22{\cdot}3$			$\chi^2 = 38{\cdot}2$			$\chi^2 = 22{\cdot}78$		
		$P < 0{\cdot}001$			$P < 0{\cdot}001$			$P < 0{\cdot}001$		

The materials used in this study were 83 bovine faeces, 4 bovine placentas, 2 samples of bovine vaginal mucus and 1 bovine gut. SBG = Selenite brilliant green; M-K tet. = Muller–Kauffmann tetrathionate: MG$MgCl_2$ = Malachite green magnesium chloride medium.

tables so that the efficiency of the three media can be indicated. The values of χ^2 and P are given for each pair. It will be noted that there is no significant difference in performance between deoxycholate citrate agar and brilliant-green MacConkey agar, but both these media performed significantly better than de Loureiro's modification of Wilson and Blair's bismuth sulphite agar.

The efficiency of four different enrichment broths is shown in Table 3. Each fluid medium received the same inoculum of naturally infected material. Selenite F broths were incubated at both 37° and 43° C. Other broths were incubated at the temperatures recommended for their use. The combinations of media positive or negative for *S. dublin* are recorded in the upper part of the table (A) and the lower part, (B), examines the more important paired comparisons of media arranged as fourfold tables so that χ^2 values may be calculated. Selenite F, the only medium not containing brilliant green or malachite green, was significantly more efficient than each of the other three media, which contained dye. SBG

enrichment was least successful in the isolation of *S. dublin*. No difference was found in the results obtained with selenite F incubated at 37 and 43° C.

DISCUSSION

Veterinary samples, or environmental samples from abattoirs, are regularly examined in this laboratory. It is necessary therefore to be familiar with reliable techniques for the isolation of *S. dublin*. Infections caused by this serotype are common in Wales (Hugh-Jones, 1970).

The technique we prefer is enrichment in selenite F broth incubated at 37 or 43° C. Subcultures are made to deoxycholate citrate agar at 24 hr., or at 24, 48 and 72 hr if time permits. It is unusual, however, for us to use more than one subculture and that at 24 hr. is optimum (Table 1). Brilliant-green MacConkey agar (Harvey, 1956) is also useful, but the colony size of *S. dublin* on this medium is often smaller than that of other salmonella serotypes (Harvey & Price, 1967). This is illustrated in Plate 1, in which colonies of *S. dublin* and *S. typhimurium* are photographed on the same plate of brilliant-green MacConkey agar. Unless this colonial peculiarity is recognized, false negative results may be reported. It is best to use deoxycholate citrate and brilliant-green MacConkey agars in parallel. On this point we agree with Magee & Hinton (1974). Wilson and Blair's bismuth sulphite agar (de Loureiro, 1942) was a failure in the isolation of *S. dublin* (Tables 1, 2).

A comparison of enrichment media established that selenite F broth, which does not contain brilliant green, was superior in efficiency to those enrichment broths in which brilliant green or malachite green were incorporated. The relative order of efficiency of the fluid media was very similar to that found with *S. typhi* – a serotype known to be sensitive to brilliant green (Harvey 1956).

The bias of these liquid media against the successful isolation of *S. dublin* and *S. typhi* is merely an extreme example of a wider problem of selective bias by an enrichment medium for or against the isolation of a serotype. We have recorded this on previous occasions (Harvey, 1973; Harvey & Price, 1974). If samples are examined which may contain a wide variety of salmonella serotypes, optimum results are obtained by the use of two enrichment media and in Cardiff we favour selenite F broth and Muller–Kauffmann tetrathionate broth. Both media are incubated at 43° C.

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REFERENCES

GIBSON, E. A. (1965). Reviews of the progress of dairy science. Section E. Diseases of dairy cattle. Salmonella infection in cattle. *Journal of Dairy Research* **32**, 97.

HARVEY, R. W. S. (1956). Choice of a selective medium for the routine isolation of members of the salmonella group. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **15**, 118.

HARVEY, R. W. S. (1973). Salmonella contaminated animal feed in relation to infection in animals and man. In *The Microbiological Safety of Food* (ed. Betty C. Hobbs and J. H. B. Christian), p. 9. London, New York: Academic Press.

HARVEY, R. W. S. & PRICE, T. H. (1967). The examination of samples infected with multiple salmonella serotypes. *Journal of Hygiene* **65**, 423.

HARVEY, R. W. S. & PRICE, T. H. (1974). Isolation of Salmonellas. *Public Health Laboratory Service Monograph Series*, no. 8. London: H.M.S.O. (In the Press).

HUGH-JONES, M. E. (1970). Salmonellosis and V.I.D.A., 1969, *Veterinary Record* **87**, 275.

MAGEE, M. F. & HINTON, M. (1974). The growth of *Salmonella dublin* and *Salmonella typhimurium* on MacConkey media containing brilliant green. *Medical Laboratory Technology* **31**, 175.

MOORE, B. (1948). The detection of paratyphoid carriers in towns by means of sewage examination. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **7**, 241.

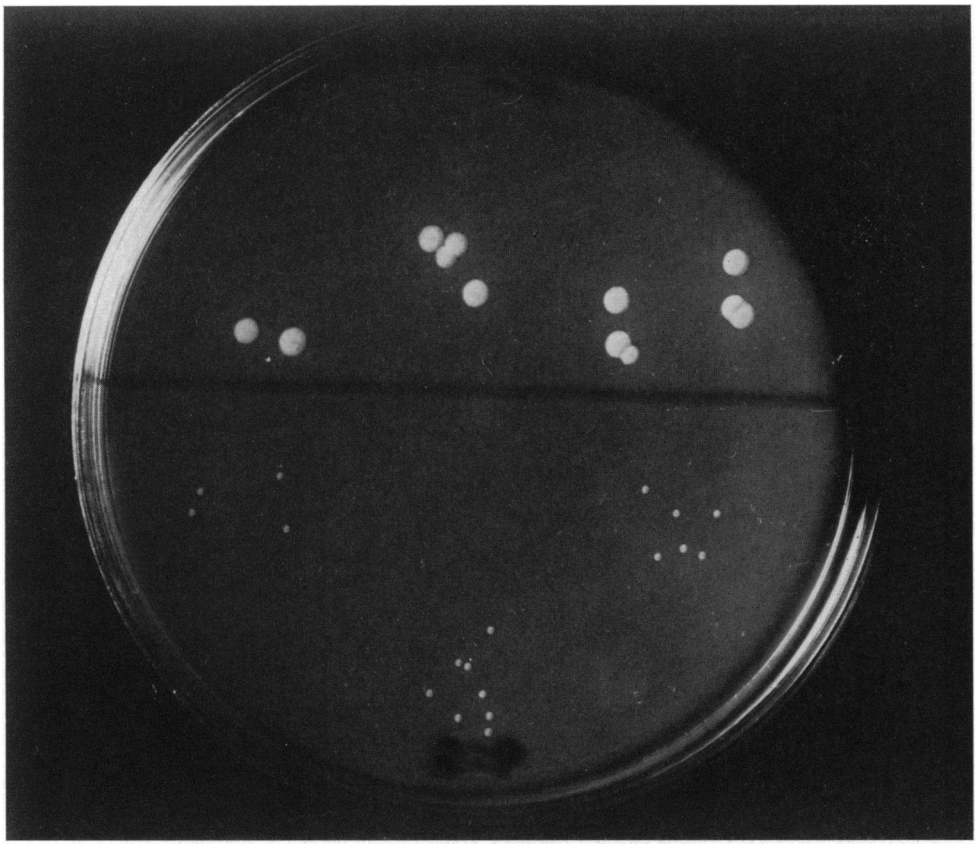
LOUREIRO, J. A. DE (1942). A modification of Wilson and Blair's bismuth sulphite agar (stabilized stock solutions). *Journal of Hygiene* **42**, 224.

RAPPAPORT, F., KONFORTI, N. & NAVON, B. (1956). A new enrichment medium for certain salmonellae. *Journal of Clinical Pathology* **9**, 261.

WHITE, P. B. (1930). Notes on organisms serologically related to *S. enteritidis* Gärtner. I. The Dublin and Tokyo types of salmonella. *Journal of Hygiene* **29**, 443.

EXPLANATION OF PLATE

Colonial size of *Salmonella dublin* and *Salmonella typhimurium* on brilliant-green MacConkey agar. Colonies above the line are *S. typhimurium*, those below the line are *S. dublin*. The inoculum used with both serotypes was $3 \times 0{\cdot}02$ ml. of a 10^{-6} dilution of a broth culture after 18 hr. incubation at 37° C. The selective agar plate was incubated at 37° C. for 24 hr.



R. W. S. HARVEY AND T. H. PRICE

(*Facing p.* 374)