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A comparison of procedures for the isolation of campylobacters from seagull faeces

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SUMMARY

Two enrichment broths (Preston and Roman & Doyle's) and four solid media (Preston, Skirrow's, Butzler's and Blaser's) were compared to determine their relative efficiencies in recovering campylobacters from 389 freshly voided seagull faeces, 276 of which were found to contain campylobacters by one or more of the procedures used.

A combination of enrichment in Preston medium followed by plating on to Preston agar gave the highest number of isolates (263). Enrichment in fluid media was shown to be an important part of the technique, as only 85 (30·8 %) of the 276 isolations were made as a result of direct plating.

Very little difference was seen between the two forms of enrichment ($P > 0{\cdot}5$) but of the four selective media, Butzler's was significantly less efficient than any of the other three ($P < 0{\cdot}01$), because it failed to grow more than a few strains of *Campylobacter coli* and the NARTC group, which together made up nearly two-thirds of the total number of *Campylobacter* spp. isolated.

INTRODUCTION

A wide variety of animals has been shown to carry campylobacters. Among birds, carriage rates of 20–90 % have been found in the following: chickens (Simmons & Gibbs, 1979; Svedham, Kaijser & Sjogren, 1981; Fricker, Girdwood & Munro, in prep.), turkeys (Leuchtefeld & Wang, 1981), pigeons and rooks (Fenlon, 1981), migratory waterfowl (Leuchtefeld *et al.* 1980), wader species (Fricker & Metcalfe, in prep.) and seagulls (Skirrow & Benjamin, 1980; Fenlon, 1981). It would appear, therefore, that birds are an important natural reservoir of campylobacters. In poultry there is strong evidence for a direct link between the consumption of undercooked meat and human disease (Brouwer *et al.* 1979; Skirrow, Fidoe & Jones, 1981; Itoh *et al.* 1982; Mouton *et al.* 1982). Circumstantial evidence has also been presented for the indirect transfer of campylobacters from birds to man. Khan (1982) detected the build-up of a single serotype of *Campylobacter jejuni* in a flock of waterfowl and the mud of a riverside meadow, then in five dogs which frequented the area and finally in seven human contacts.

Seagulls have been implicated in the spread of salmonellae to domestic animals (Williams *et al.* 1977; Johnston, Maclachlan & Hopkins, 1979; Johnston *et al.* 1981)

and since these birds frequently carry campylobacters they may well play a role in the infection of farm animals with these organisms. Roosting gulls can cause serious deterioration of water quality in storage reservoirs (Fennel, James & Morris, 1974) and transfer of campylobacters from gulls to man via water is a possibility, particularly where failures in water treatment occur. Campylobacters have frequently been isolated from natural water, and workers in Southampton found them only in the presence of *Escherichia coli*, Type 1 (Pearson *et al.* 1977; Knill, Suckling & Pearson, 1978), their findings being confirmed by Bolton *et al.* (1982). It would appear therefore that these campylobacters are derived from animal or avian sources and do not occur as free-living saprophytes. The role of birds, in particular seagulls, in the dissemination of campylobacters, requires further investigation.

Clearly the value of any such investigation depends upon the reliability of the methods used. To date only a few comparisons of culture media for the isolation of campylobacters have been reported (Patton *et al.* 1981; Bolton *et al.* 1983; Wells, Bopp & Blaser, 1982). Not only is it important to assess the performance of media with different types of sample, but it is important to recognize any bias a particular medium may have for the isolation of different campylobacter species.
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With these points in mind we have evaluated the performance of four selective agars and two enrichment broths for the isolation of campylobacters from seagull faeces.

MATERIALS AND METHODS

In this study, two enrichment broths and four solid selective agars were used. The enrichment broths, which were distributed in 5 ml volumes in screw-capped bijoux, were those described by Bolton & Robertson (1982) and Doyle & Roman (1982). The four solid media used were Skirrow's (1977), Butzler's (Lauwers, De Boeck & Butzler, 1978), Blaser's (Blaser *et al.* 1978) and Preston medium (Bolton & Robertson, 1982). Table 1 shows the constituents of the media used. The solid media used were prepared according to the modifications recommended by Oxoid Ltd. Butzler's medium was prepared using blood agar base No. 2. (Oxoid CM271) containing 7% whole horse blood instead of the thioglycollate USP medium with 15% sheep blood described in the original formulation. Similarly, the Blaser's medium used was prepared using blood agar base No. 2 (Oxoid CM271) and 5% lysed horse blood. The aerotolerant supplement (FBP) described by Hoffman (Hoffman, Krieg & Smibert, 1979) was added to all media to give a final concentration of 0·05% of ferrous sulphate, sodium metabisulphite and sodium pyruvate.

A total of 389 freshly voided gull faeces were collected from a refuse tip on the outskirts of Glasgow between September 1982 and January 1983. Flocks of birds consisting almost entirely of herring gulls (*Larus argentatus*) were disturbed. Faeces which appeared fresh were collected on sterile cotton wool swabs and transferred into 5 ml of Nutrient Broth No. 2 (Oxoid CM67) containing FBP supplement. The specimens were cultured in the laboratory within about 30 min of collection.

The faecal samples were homogenized by vortex mixing for 30 s. Then 10 μl of

Table 1. *Composition of enrichment and selective plating media used in the isolation of campylobacter from seagull faeces*

	Enrichment media		Plating media			
	Preston Nutrient Broth No. 2 Oxoid	Doyle & Roman Brucella broth Gibco	Skirrow Blood Agar Base No. 2	Butzler Blood Agar Base No. 2	Blaser Blood Agar Base No. 2	Preston Nutrient Broth No. 2 +1·2% New Zealand Agar
Basal medium	CM67	1410250	Oxoid CM271			
Blood	5% lysed horse	7% lysed horse	5% lysed horse	7% whole horse	5% lysed horse	5% lysed horse
Bacitracin i.u./ml	—	—	—	25	—	—
Cephalothin μg/ml	—	—	—	—	15	—
Cephazolin μg/ml	—	—	—	15	—	—
Colistin μg/ml	—	—	—	10	—	—
Novobiocin μg/ml	—	—	—	5	—	—
Polymyxin B i.u./ml	5	20	2·5	—	2·5	5
Rifampicin μg/ml	10	—	—	—	—	10
Trimethoprim μg/ml	10	5	5	—	5	10
Vancomycin μg/ml	—	15	10	—	10	—
Amphotericin B μg/ml	—	—	—	—	2	—
Cyclohexamide μg/ml	100	50	—	50	—	100
Sodium succinate	—	0·3%	—	—	—	—
Cysteine hydrochloride	—	0·01%	—	—	—	—

the resulting suspension was plated on to each of the four solid media and the plates incubated in an oxygen reduced atmosphere, produced using a single Gaspak (Becton-Dickenson U.K.), in an anaerobic jar without a catalyst, at 43 °C for 48 h. Portions (1·0 ml) of the faecal suspension were also inoculated into each of the enrichment broths, which were incubated aerobically at 43 °C for 24 h. The enrichment broths were then subcultured on to each of the four solid media, which were incubated as described above.

Isolates were identified as *Campylobacter* spp. on the basis of colonial morphology, curved or spirillar appearance on a Gram-stained film and positive oxidase and catalase reactions. Each isolate identified in this way was plated out on blood agar in order to obtain single colonies. After overnight incubation at 37 °C, a single colony was selected and spread over the surface of a further blood agar plate using a sterile cotton wool swab. This plate was incubated at 37 °C for 24 h and the resultant growth used to carry out the biotyping tests described by Skirrow & Benjamin (1980, 1982), i.e. nalidixic acid sensitivity, hippurate hydrolysis, hydrogen sulphide production in FBP broth and sensitivity to triphenyltetrazolium chloride. Only one colony from each plate was identified in this way.

Statistical analysis of the results was carried out using MacNemar's test for paired samples and chi-squared.

RESULTS

Of the 389 samples examined, 276 were found to contain campylobacters by at least one of the procedures used. Of these, 85 were positive by direct plating on

Table 2. *Number of campylobacter isolations obtained from 389 seagull faeces by each procedure*

	Total	NARTC	*C. coli*	*C. jejuni 1*	*C. jejuni 2*
			Direct plating		
Skirrow	62	29	17	11	5
Butzler	20	1	0	13	6
Blaser	67	32	16	13	6
Preston	83	40	16	17	10
			Preston enriched		
Skirrow	249	116	42	59	32
Butzler	116	9	6	64	37
Blaser	256	123	39	58	36
Preston	263	127	40	62	34
			Roman & Doyle enriched		
Skirrow	239	112	42	56	29
Butzler	112	7	6	62	37
Blaser	250	119	36	60	35
Preston	250	121	37	60	32

to one or more of the selective agars used. Table 2 shows the number of campylobacter isolations given by each procedure.

No single method detected all positive samples. The greatest number of isolates was obtained with a combination of enrichment in Preston broth, followed by plating on to Preston agar. This combination yielded 263 compylobacter isolates, being 95·3 % of the total isolates obtained. The lowest number of isolations made after enrichment occurred using Butzler's medium after enrichment in Roman and Doyle's broth, a procedure which recovered campylobacter from only 112 samples (40·6 % of the total number of isolations).

Of the 85 isolations made by direct plating, 83 were detected using the Preston medium, compared with 67, 62 and 20 by Blaser's, Skirrow's and Butzler's respectively. Of the two isolations made by direct plating, which were not recovered on Preston medium, one was detected using Blaser's medium and the other using both Blaser's and Skirrow's.

Heavy growths of competing bacteria were not common after enrichment, although yeasts were frequently encountered on the Skirrow, Butzler and Preston agars after enrichment in either of the two broths used. Competing organisms were frequently predominant on Skirrow's and Blaser's media which had been inoculated directly with the faecal suspension; in particular *Proteus* spp. were a problem.

DISCUSSION

The results show quite clearly that an enrichment stage is essential for the reliable isolation of campylobacters from seagull faeces, although the differences obtained with the two enrichment broths compared here are small ($P > 0{\cdot}05$) – 273 isolations being made after enrichment in Preston medium and 266 with Roman and Doyle's broth.

Although the differences in recovery of campylobacters were not great when using Skirrow's, Blaser's and the Preston media ($P > 0{\cdot}5$), a significant reduction

in isolations was seen with Butzler's medium ($P < 0{\cdot}01$). This was due to the inability of Butzler's medium to recover the majority of *C. coli* and NARTC strains. This confirms the findings of Bolton and co-workers at Preston, who were unable to recover light inocula of these organisms on Butzler's medium (Bolton *et al.* 1983). This medium is therefore unsuitable for the isolation of campylobacters from seagull faeces and its use should be restricted to situations where *C coli* and NARTC strains are not important. It is interesting to note that after enrichment, more *C. jejuni* isolations (of both biotypes) were obtained on Butzler's medium than on any other. This can probably be explained by the failure of Butzler's medium to support the growth of most strains of *C. coli* and the NARTC group. *C. jejuni* would be more likely to be recovered from Butzler's medium from samples containing a mixture of NARTC or *C. coli* and *C. jejuni*, whereas the ability of the other three solid media to grow *C. coli* and members of the NARTC group would reduce the chances of a *C. jejuni* colony being selected for biotyping.

Since the Preston medium, when used both as an enrichment medium and as a plating medium, gave the highest number of isolations, it is the medium of choice for the examination of seagull faeces. Further work (to be reported elsewhere) suggests that the numbers of campylobacters in gull faeces is low, and this finding is supported in this study since direct plating yielded so few isolates. Direct plating tended to detect the presence of NARTC strains more frequently than *C. jejuni* or *C. coli*, and this suggests that they may be present in higher numbers. It is likely that the findings presented in this study can be applied to other situations where the number of campylobacters is small.

The growth of yeasts on Skirrow's, Butzler's and Preston media was far more pronounced than on Blaser's medium, which suggests that the use of amphotericin B may be more useful than cyclohexamide. Limited trials in this laboratory have shown that this is indeed the case when examining seagull faecal material.

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