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The use of Preston enrichment broth for the isolation of 'thermophilic' campylobacters from water

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SUMMARY

The application of Preston enrichment broth to isolation of 'thermophilic' campylobacters from water has been investigated. Enrichment substantially increased the yield of such organisms. The optimum timing for subculturing the broth was after 48 h incubation. Despite the addition of aerotolerant supplement to the broth the number and variety of isolates was greater when the broth was incubated microaerically than when it was incubated in air.

INTRODUCTION

Individual cases and outbreaks of campylobacter enteritis have been reported where water was the suspected source of infection (Pearson *et al.* 1977; Tiehan & Vogt, 1978; Blaser, Penner & Wells, 1982; Mentzing, 1982). If the epidemiology of such infections is to be studied it is important to have sensitive methods for the isolation of 'thermophilic' campylobacters from water. We have previously recorded that the Preston enrichment broth (Bolton & Robertson, 1982) is useful in this respect but pointed out that its optimum conditions of use were still unknown (Ribeiro, Gray & Price, 1982). The present studies were set up to extend our earlier findings and examine some of the variables in order to determine the most appropriate way of using this medium.

Additionally, Blaser and his colleagues (1982) found multiple serotype involvement in water-associated outbreaks. Preliminary findings showed that multiple biotypes could be isolated from small volumes of water (Ribeiro & Price, 1983) so during the latter part of the studies isolates were biotyped to confirm this.

METHODS

Samples. The water tested was collected in 500 ml volumes from the River Taff at Pontypridd and several 50 ml aliquots processed on the day of collection. The processing has been described previously (Ribeiro *et al.* 1982). Half-filter membranes (equivalent to 25 ml of filtered water) were used to inoculate the media.

Media. The selective medium was Skirrow's (1977) medium. Enrichment was performed using the Preston broth in 15 ml quantities with aerotolerant supplement – ferrous sulphate, sodium metabisulphite and sodium pyruvate – at a final concentration of 0·025% (George *et al.* 1978).

Incubation temperatures. All plates and bottles were incubated at 43 °C.

Study 1: *Direct versus enrichment culture.* This was an extension of previous work (Ribeiro *et al.* 1982), complementary halves of filter membrane being placed on selective plates and into enrichment broths.

Study 2: *Multiple subculture.* Two 50 ml aliquots (producing four half-membranes for inoculation) were filtered from each sampling. Each half-filter was placed in enrichment broth which was incubated in a microaeric* atmosphere containing 10 % carbon dioxide with daily subculture to Skirrow's medium for 4 days. The selective medium was incubated for 48 h in the same atmosphere.

Study 3: *Enrichment in a microaeric atmosphere versus enrichment in an aerobic atmosphere.* Three or four 50 ml aliquots of water were filtered on each occasion (producing six or eight half-membranes for inoculation). Duplicate Preston broths were inoculated with complementary halves of the membranes. One was incubated as in Study 2 and the other in an aerobic atmosphere. Subcultures were made and incubated as described above.

Biotyping. The abbreviated scheme described by Skirrow & Benjamin (1980), with minor modifications in methodology for testing hydrogen sulphide production, was used. Specifically the iron medium was pre-reduced, the organism was grown up in an atmosphere of 90 % hydrogen and 10 % carbon dioxide, the test was kept at 37 °C rather than room temperature and incubation was extended overnight if the test was negative at 4 h.

RESULTS

Direct versus enrichment culture

In addition to the 64 specimens included in the earlier report (Ribeiro *et al.* 1982) a further 64 were examined. None of the 128 were positive direct but 34 (26·6 %) were positive on subculture of the enrichment broth after 24 h. A further three (2·3 %) became positive after 48 h enrichment.

Multiple subculture

The isolation rate following subculture at different times is shown in Table 1. Overall the highest rate was obtained after 48 h incubation. A comparison of the results of 24 and 48 h subcultures and of 48 h and 72 h subcultures is shown in Tables 2 and 3. The paired McNemar Test (McNemar, 1947) showed that the difference between 24 and 48 h subculture isolation rates did not achieve statistical significance, but that between 48 h and 72 h rates did.

Enrichment in a microaeric atmosphere versus enrichment in an aerobic atmosphere

Of the 53 comparisons made between incubation of the Preston enrichment broth in aerobic and microaeric conditions, one or both of the methods was positive on 20 occasions. Microaeric incubation gave a positive result on 18 occasions (90 %), aerobic incubation on only eight (40 %). A comparison of these methods is shown

* The microaeric atmosphere was produced by evacuating an anaerobic jar without a catalyst to a pressure of −450 mm of mercury and refilling it with a gas mixture containing 90 % nitrogen and 10 % carbon dioxide.

Table 1. *Campylobacter isolation rates: on subculture of Preston broth after different times of incubation in a microaeric atmosphere*

	No. of specimens	Positive on any subculture (% of no. tested)	Positive on 24 h subculture (% of no. positive at any time)	Positive on 48 h subculture (% of no. positive at any time)	Positive on 72 h subculture (% of no. positive at any time)	Positive on 96 h subculture (% of no. positive at any time)
During Study 1	128	37 (28·9%)	34 (91·9%)	33 (89·2%)	N.T.	N.T.
During Study 2	80	37 (46·2%)	31 (83·8%)	36 (97·3%)	28 (75.7%)	21 (56·8%)
During Study 3	53	18 (34.0%)	17 (94·4%)	18 (100%)	18 (100%)	17 (94·4%)
Overall	261	92 (35·2%)	82 (89·1%)	87 (94·6%)	46/55 (83·6%)	38/55 (69·1%)

Table 2. *Comparison of campylobacter isolations after 24 h and 48 h enrichment in Preston broth incubated in a microaeric atmosphere.*

		48 h subculture +	48 h subculture −
24 h subculture	+	77	5
	−	10	169
$U = 1{\cdot}29$ (paired McNemar)...		N.S.	

Table 3. *Comparison of campylobacter isolations after 48 h and 72 h enrichment in Preston broth incubated in a microaeric atmosphere*

		72 h subculture +	72 h subculture −
48 h subculture	+	45	9
	−	1	78
$U = 2{\cdot}53$ (paired McNemar) ...		$P < 0{\cdot}01$	

Table 4. *Comparison of campylobacter isolations after microaeric and aerobic incubation of Preston broth*

		Incubated in air +	Incubated in air −
Incubated in microaeric atmosphere	+	6	12
	−	2	33
$U = 2{\cdot}67$ (paired McNemar) ...		$P < 0{\cdot}01$	

in Table 4. The figures are identical whether positive results on any subcultures are considered or only positive results on the 48 h subculture. The microaeric method is substantially better than the aerobic one.

Biotypes

Tables 5 and 6 show the biotype identification of the strains isolated. All four biotypes were isolated at some time during the study. Of the 35 strains isolated following microaeric incubation 13 (37.1 %) were N.A.R.T.C.s, 11 (31.4 %) *Campylobacter jejuni* biotype I, 3 (8·6 %) *C. jejuni* biotype II, 6 (17·2 %) *C. coli* and 2 (5·7 %) were not fully identified. Different biotypes could be isolated from separate 25 ml aliquots of water (see Table 5, 1b, 1c, 1d and 2b, 2d) and even from the same aliquot (see Table 6, 10c). Multiple biotypes were isolated more frequently when the broth was incubated in a microaeric atmosphere than when it was incubated aerobically.

Table 5. *Identity of campylobacter strains isolated during study* 2

Specimen*	Identity	Specimen*	Identity
1a	—	3a	*C. jejuni* I
1b	N.A.R.T.C.	3b	—
1c	*C. coli*	3c	—
1d	*C. jejuni* (not subtyped)	3d	—
2a	—	4a	*C. coli*
2b	N.A.R.T.C.1†	4b	*C coli*
	N.A.R.T.C.2†	4c	*C. coli*
2c	*Campylobacter* sp. (N.T.)	4d	*C. coli*
2d	*C. jejuni* II		

N.A.R.T.C., Nalidixic acid-resistant 'thermophilic' *Campylobacter*.
N.T., Not typed
* Each entry represents yield from half a filter (i.e. is equivalent to 25 ml of water).
† Where there were colonial variants of the same biotype these are entered separately.

Table 6. *Identity of campylobacter strains isolated during Study* 3

Specimen*	Microaeric isolations	Aerobic isolations	Specimen*	Microaeric isolations	Aerobic isolations
4a	—	N.A.R.T.C.	9a	*C. jejuni* I	—
4b	*C. coli*	*C. coli*1†	9b	*C. jejuni* I	—
	—	*C. coli*2†			
4c	—	*C. coli*	10*a*	N.A.R.T.C.	N.A.R.T.C
				C. jejuni I	—
5a	*C. jejuni* I	—	10b	N.A.R.T.C.1†	—
				N.A.R.T.C.2†	—
6a	*C. jejuni* I	—	10c	N.A.R.T.C.	N.A.R.T.C.
6b	N.A.R.T.C.	—		*C. jejuni* I	—
6c	*C. jejuni* II	—		*C. jejuni* II	—
6d	*C. jejuni* I	—	10d	N.A.R.T.C.	N.A.R.T.C.
				C. jejuni I	—
7a	*C. jejuni* I	—			
			11a	N.A.R.T.C.	—
8b	*C. jejuni* I	—	11b	N.A.R.T.C.	N.A.R.T.C.
			11c	N.A.R.T.C.	—
			11d	N.A.R.T.C.	N.A.R.T.C.

See footnote to Table 5.

DISCUSSION

Knill, Suckling & Pearson (1982) isolated 'thermophilic' campylobacters from 251 (53·3%) of 471 water samples collected in Hampshire using a membrane filtration technique with direct inoculation onto Skirrow's medium. In Study 1 using a similar technique none of 128 samples yielded these organisms. This striking difference may be accounted for by the fact that the Southampton workers cultured a whole filter through which 100 ml of water had been passed whereas in this study half-filters (equivalent to 25 ml of water) were cultured. An alternative explanation is that the samples from Southampton were more heavily contaminated than those from Cardiff. Campylobacters were present in the Cardiff samples as is confirmed by the substantial isolation rates when an enrichment technique was employed.

With any enrichment technique there is an optimum time for subculture. The second study was set up because no information was available on this point. It was found that the maximum yield was obtained with enrichment for 48 h (see Table 1). The yield at 48 h was significantly better than at 72 h (see Table 3). Although the yield at 48 h was better than at 24 h this did not reach statistical significance (see Table 2). Nevertheless it is felt that the optimum time for a single subculture is 48 h.

George *et al.* (1978) showed that their aerotolerant supplement permitted growth of *C. jejuni* on brucella agar in the presence of high concentrations of oxygen. It was felt that adding this supplement to the Preston broth might enable it to be incubated aerobically.

However, when a comparison was made (see Study 3) the yield after microaeric incubation was significantly better than that after aerobic incubation (see Table 4). This was true whether positive results from any subculture were considered or only positive results from the 48 h subculture, previously found to be optimal, were considered. These findings parallel those of George *et al.* with Brucella agar. Clearly, even with an enrichment broth, which might be expected to have an oxygen gradient in it, it is necessary to incubate in a microaeric atmosphere.

All the four biotypes of 'thermophilic campylobacter were isolated during these studies. Their relative frequency differed from that described by Khan (1982). It is likely this reflects different sources of faecal contamination of the water being tested, as it is known that different animal species may harbour the various types in different proportions (Skirrow & Benjamin, 1982). The relative predominance of N.A.R.T.C.s in this study suggests much of the contamination is of ovine origin. We believe that the contamination needs to be recent as retesting from the original sample held at ambient temperature in the laboratory overnight failed to produce campylobacters from four aliquots when all four aliquots tested initially were positive.

A previous communication from this laboratory (Ribeiro & Price, 1982) stated that individual aliquots of a single sample could yield different biotypes. It has now been found that multiple types can be isolated from a single 25 ml aliquot. The maximum yield so far is three biotypes in 25 ml. Clearly the Preston broth will permit growth of all the biotypes. The yield was much less when the broth was incubated aerobically. This was particularly true where *C. jejuni* was concerned, none of the 12 strains isolated by microaeric enrichment in Study 3 were grown by aerobic enrichment. By contrast, five of 10 N.A.R.T.Cs were isolated after aerobic incubation, with an additional isolation being made on aerobic incubation alone. This presumably indicates that the N.A.R.T.Cs are more oxygen-tolerant than strains of *C. jejuni*.

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