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The Stability of Small Number of Campylobacteria in Four Different Transport Media

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Aho, M., M. Kauppi and J. Hirn: The stability of small number of campylobacteria in four different transport media. Acta vet. scand. 1988, 29, 437–442. – Four different transport media (SIFF, Cary-Blair, RAPW and brucella broth with charcoal and FBP) were evaluated for their ability to support small number of campylobacteria. The best medium was SIFF, although Cary-Blair medium was almost equally efficient. It was possible to store less than 1 000 CFU (5/7 strains) for 1 week at room temperature in SIFF medium and less than 100 000 CFU (5/7 strains) for 3 days at room temperature in Cary-Blair medium. On the basis of the results of this study SIFF medium is recommended for use with samples having low campylobachter concentrations.

microbiological methods; food hygiene.

Introduction

Campylobacteria are nowadays considered as a very important cause of human enteritis. In the developed countries campylobacteria are involved in 5–10 % of all human enteritis cases and in the developing countries even more (*Blaser & Reller* 1981). In many developed countries there has been an increasing number of campylobacter enteritis cases so that they now actually exceed cases of salmonella in some regions (*Walder* 1982). According to *Sechter & Rogol* (1986), it may be that developments in methodology are one of the main reasons for the increased detection of campylobacteria. In Finland about 700–800 cases are diagnosed annually (*Pönkä & Kosunen* 1987). During the years 1985–1987 there were 4 widespread campylobacter outbreaks in Finland, 3 waterborne (*Rautelin et al.* 1986 and 2 unpublished) and 1 foodborne (*Visakorpi et al.* 1986), with a total of 182 diagnosed cases.

Many difficulties are encountered in isolating and transporting campylobacteria because:

(i) they die slowly at temperatures between +10°– +30°C (*Blaser et al.* 1980, *Doyle & Roman* 1981, *Gill & Harris* 1983, *Abram & Potter* 1984);

(ii) they are sensitive to atmospheric oxygen (*Fletcher & Plastridge* 1964, *George et al.* 1978, *Smibert* 1978, *Hoffman et al.* 1979);

(iii) they are sensitive to drying, except under refrigerated conditions (*Luechtefeld et al.* 1981, *Doyle & Roman* 1982a);

(iv) concentrations of NaCl exceeding 2 % are inhibitory to them (*Hänninen* 1981b, *Doyle & Roman* 1982b);

(v) acidic environments lower than pH 5.0 are inhibitory to campylobacteria, organic acids being more inhibitory than inorganic ones (*Doyle & Roman* 1981, *Christopher et al.* 1982, *Gill & Harris* 1983), and

(vi) they are sensitive to disinfection, particularly to chlorine which is widely used in

the food industry (*Luechtefeld et al.* 1981, *Oosterom et al.* 1983, *Wang et al.* 1983b).
There is an urgent need for a simple and effective method of transporting of samples and pure cultures. *Wang et al.* (1980) recommended a semi-solid brucella agar with 10 % ovine blood and without antibiotics and *Rosef et al.* (1983) an SIFF medium for transport of pure campylobacter cultures. SIFF medium is a modification of Stuart medium, with increased osmotic pressure and added starch (*Sandven et al.* 1982). *Luechtefeld et al.* (1981) transported turkey caecal contents in a Cary-Blair medium with decreased agar concentration (*Cary & Blair* 1964). *Wang et al.* (1983a) evaluated many media and they concluded that the best transport medium for human faeces at 25°C is alkaline peptone water with thioglycolate and cystine (RAPW medium). *Sjögren et al.* (1985) recommended the use of a semi-solid motility test medium (*Chan & Mackenzie* 1982) for transport of human faecal samples. They took 2 samples, first one to be cultured directly in the selective enrichment broth and then another to be pre-enriched for 24 h. All these experiments were carried out with either pure cultures or samples with a high campylobacter concentration.
The purpose of this study was to evaluate the stability of small number of campylobacteria at room temperature in 4 different media in order to simulate transport of samples.

Materials and methods

Organisms.
Campylobacter jejuni biotype 1 (NCTC 11385), *C. jejuni* biotype 2 (NCTC 11392), *C. coli* (NCTC 11353) and *C. laridis* (NCTC 11352) were obtained from the Public Health Laboratory Service, London, U.K. *C. jejuni* B33 (broiler origin) and *C. jejuni* 9000 (human origin) were obtained from Dr. M.-L. Hänninen, Department of Food and Environmental Hygiene, College of Veterinary Medicine, Helsinki, Finland. *C. jejuni* 100 (bovine origin) was isolated by the authors at the National Veterinary Institute, Helsinki, Finland.

Preparation of cultures
Organisms were grown for 20 h at 43°C in brucella broth (Difco Laboratories, Detroit, Mich., U.S.A.) supplemented with FBP (ferrous sulfate, sodium metabisulfite and sodium pyruvate, all at 0.5 g/l). All strains were preserved in the same broth supplemented with 10 % glycerol at –70°C. Frozen and freeze-dried strains were cultured twice before use. The microaerophilic atmosphere above the liquid (90 ml in a 200 ml flask) and agars was achieved by evacuating gas boxes to –800 mbar and then filling them with a gas mixture containing 5 % O_2, 10 % CO_2 and 85 % N_2. Evacuation and filling was carried out twice. After the incubation period when campylobacteria were in the early stationary phase, broth was diluted with 0.1 % buffered peptone water (BBL Microbiology Systems, Cockeysville, MD., U.S.A.).

Transport and culture media
Transport media. The following 4 media were used: SIFF medium (*Sandven et al.* 1982), Cary-Blair medium (*Cary & Blair* 1964), RAPW medium (*Wang et al.* 1983a) and Brucella broth with charcoal and FBP (see composition below*).
All transport media were portioned 10 ml in test tubes and autoclaved for 20 min at 120°C.
Enrichment broth and selective agar. Modified Skirrow enrichment broth and selective agar (*Skirrow* 1977, *Blaser et al.* 1979, *Hänninen* 1981a). The modification was addition 2 mg/l of amphotericin B (E.

R. Squibb & Sons Ltd., Liverpool, U.K.) to the media.

Broth was portioned 90 ml in 200 ml flasks and agar 25 ml on ∅ 9 cm Petri dishes with nodules.

Test procedure

Brucella broth containing campylobacteria in early stationary phase was diluted to give dilutions of approximately 10, 100, 1 000, 10 000 and 100 000 CFU/ml of campylobacteria. 1 ml of each dilution was added to a transport medium. Quantitative examination of the brucella broth dilutions was carried out on Skirrow spread plates. Transport media were sealed and stored for one, three and seven days at room temperature in the dark. Whole medium (10 + 1 ml) was enriched in a Skirrow broth for 20 h at 43°C under a microaerophilic atmosphere. Two loopfulls of incubated Skirrow broth were plated on a Skirrow plate. Skirrow plates were incubated for 44 h at 43°C under a microaerophilic atmosphere. Campylobacteria were recognized by examining wet mounts by microscopy for curved rods exhibiting darting motility and by catalase and oxidase tests.

* brucella broth (Difco)	28.0
bacteriological charcoal (Oxoid Ltd., Basingstoke, Hants., U.K.)	4.0
$FeSO_4*7H_2O$ (E. Merck, Darmstadt, F.R.G.)	0.5
$Na_2S_2O_3*5H_2O$ (Merck)	0.5
sodium pyruvate (Boehringer Mannheim GmbH, F.R.G.)	0.5
agar no. 1 (Oxoid)	5.0
aqua dest.	1 000 ml
pH was adjusted to 7.2	

Results and discussion

The results obtained are shown in Table 1.

It was possible to maintain 6 out of 7 campylobacter strains in SIFF medium for 3 days at room temperature with an inoculum of less than 1 000 CFU. An inoculum of more than 100 000 CFU was needed for the strain 9000. 5 out of the 7 strains were still viable after 1 week in SIFF medium in the same conditions with an inoculum of less than 1 000 CFU. In SIFF medium campylobacteria survived in smaller number than in the other media tested (Student t-tests: SIFF/Cary-Blair $p < 0.05$, SIFF/RAPW $p < 0.001$ and SIFF/Brucella broth with charcoal and FBP $p < 0.01$).

For *C. jejuni* biotype 1 and *C. jejuni* biotype 2 the inoculum for survival was higher for 1 day transport than for 3 days transport. This difference may indicate that some kind of cold enrichment can enhance the isolation of campylobacteria as suggested by *Rubin & Woodard* (1983). Because methylene blue added to the SIFF medium changes color on oxidation it is easy to follow the diffusion of oxygen. This is important in monitoring the age and storage conditions of the media.

Almost as good results were obtained by Cary-Blair medium as when using SIFF medium. 4 out of 7 campylobacter strains were viable after 3 days at room temperature with an inoculum of less than 1 000 CFU, but only 2 out of 7 after 1 week.

Using RAPW medium and Brucella broth with charcoal and FBP, it was not possible to maintain small number of campylobacteria. In RAPW medium cysteine (0.25 g/l) may be the restrictive factor, although *Doyle & Roman* (1982c) and *Palumbo* (1986) recommended its use (0.1 g/l) particularly for isolating freeze-thaw stressed *C. jejuni*. Some problems were encountered in mixing charcoal in the brucella broth, which may have had some effect on the results.

Table 1. Smallest inocula of campylobacteria survising for one, three and seven days in SIFF, Cary-Blair, RAPW and brucella broth with charcoal and FBP media at room temperature in the dark. Enrichment was performed in Skirrow broth and plating on Skirrow agars.

	Campylobacter strain						
Time[a]	*C. jejuni* biotype 1	*C. jejuni* biotype 2	*C. coli*	*C. laridis*	B33	100	9000
SIFF medium[b]							
1	10^2	10^2	10	10^3	10	10	10^3
3	10	10	10^2	10^3	10^3	10^2	10^5
7	10	10	10^2	–	10^3	10^2	–
Cary-Blair medium[b]							
1	10^2	10^2	10	10^3	10	10	10^3
3	10^3	10^2	10^3	–	10^3	–	10^5
7	10^3	10^3	–	–	–	–	–
RAPW[b]							
1	10^2	10	–	–	10^2	10^3	–
3	10^2	10^2	–	–	10^3	–	–
7	10^4	10^2	–	–	–	–	–
Brucella broth with charcoal and FBP[b]							
1	10^3	10	10^5	10^3	10	10^2	10^3
3	10^3	–	–	10^4	10^2	10^4	10^5
7	–	–	–	–	10^4	–	–

[a] = storage time (days)
[b] = see text for formulae
– = inoculum > 10^5 CFU

On the basis of the results of this study, SIFF medium is recommended for use with samples with low campylobacter concentrations. *C. jejuni* biotype 1 and *C. jejuni* biotype 2 withstood the transport stress well, whereas *C. laridis* and the strain 9000 (human origin) were the most sensitive (Student t-test: comparison *C. jejuni* biotypes 1 and 2, *C. coli* and B33 against *C. laridis* and 9000, all $p < 0.01$).

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Sammanfattning

Stabiliteten av ett litet antal campylobacterier i fyra olika transport medier.

Fyra olika transport medier (SIFF, Cary-Blair, RAPW och brucella buljong med kol och FBP) evaluerades med avseende på deras engenskaper att hålla vid liv ett litet antal campylobakterier. Det var möjligt att förvara mindre än 1 000 CFU (5/7 stammar) under 1 vecka i rumstemperatur i SIFF medium och mindre än 100 000 CFU (5/7 stammar) under 3 dagar i rumstemperatur i Cary-Blair medium. På grund af den här undersökningen SIFF medium rekommenderas i bruk med prov med låg koncentration av campylobakterier.

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