International Journal of Food Microbiology 130 (2009) 131–134

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Enrichment culture for the isolation of *Campylobacter* spp: Effects of incubation conditions and the inclusion of blood in selective broths

Lisa K. Williams [a,*], Frieda Jørgensen [b], Rose Grogono-Thomas [a], Tom J. Humphrey [a]

[a] Zoonotic Infections Group, Division of Veterinary Pathology Infection and Immunity, School of Clinical Veterinary Science, University of Bristol, Langford, Bristol, BS40 5DU, UK
[b] Foodborne Zoonosis Unit, Health Protection Agency South West, University of Bristol, Langford, Bristol, BS40 5DU, UK

ARTICLE INFO

Article history:
Received 13 October 2008
Received in revised form 9 January 2009
Accepted 20 January 2009

Keywords:
Campylobacter spp.
Enrichment culture
Blood free enrichment culture

ABSTRACT

Isolation of *Campylobacter* spp. using enrichment culture is time consuming and complex. Reducing the time taken to confirm the presence or absence of *Campylobacter* spp. would have many advantages for diagnostic, commercial and research applications. Rapid techniques such as real-time PCR can detect campylobacters from complex samples but blood in enrichment culture can inhibit the PCR reaction, if applied directly to enriched samples. The aim of this study was to investigate the effect of blood in enrichment culture on the isolation of campylobacters from chicken caeca, carcass rinses and bootsock (gauze sock walked through a broiler chicken house) samples using Bolton broth. The effect of incubation temperature (37 °C or 41.5 °C for 48 h, or 37 °C for 4 h then transfer to 41.5 °C for 44 h) and method of generating atmosphere (incubation of container in jar gassed with microaerobic atmosphere or incubation of container with small headspace and tightly screwed lid in an aerobic atmosphere) with and without blood on isolation from chicken carcass rinses and chicken faeces was also investigated. The presence of blood in enrichment culture did not improve the isolation of campylobacters from chicken faeces or bootsock samples but significantly improved recovery from chicken carcass rinse samples. There was no significant effect of the method used to generate incubation atmosphere. Isolation rates did also not depend significantly on whether broths were incubated at 37 or 41.5 °C for 24 or 48 h. Overall, the presence of blood in such media is not essential, although isolation can vary depending on sample type and enrichment method used.

1. Introduction

Campylobacter spp. is the most commonly reported bacterial cause of infectious intestinal disease in England and Wales (Health Protection Agency (HPA) 2008) with 46,603 laboratory cases reported to the HPA, Centre for Infection in 2006 (HPA 2008). Most infections in humans are caused by *Campylobacter jejuni* and *Campylobacter coli,* although *Campylobacter upsaliensis* is important in the developing world (Humphrey et al., 2007). The majority of cases of human campylobacteriosis in the industrialised world have been attributed to poultry (Studahl and Anderson, 2000; Rodrigues et al., 2001; Neimann et al., 2003; Wingstrand et al., 2006). Detection of campylobacters from samples, which include food, faeces and environmental samples, is done by either direct plating onto selective agars or by enrichment culture the latter usually providing better recovery when few target cells are present. Isolation from complex samples types such as food, water and environmental samples is considered to be difficult and time consuming, as numbers of campylobacters can be low and other factors such as pH, oxidative stress and temperature inhibit recovery (Verhoeff-Bakkenes et al., 2008; Garenaux et al., 2008; Yamasaki et al., 2004). It is important that detection is both sensitive and robust from such sample types, so that for example, the effect of measures put in place on farms or at the processing stage can be determined. The standard enrichment protocol ISO 10272-1 (Anon, 2005) recommends the use of Bolton broth for enrichment but other enrichment methods are widely used (Final report: FSA project B15005 Sampling regimes and microbiological methods for detecting thermophilic *Campylobacter* spp. in poultry on the farm before slaughter, 2006). Several studies (Josefsen et al., 2003; Humphrey et al., 1995; Jørgensen et al., 2002; Mason et al., 1999; Humphrey et al., 1996; Food Standards Agency 2003; Paulsen et al., 2005) have compared enrichment methods using food, water and environmental samples and found that both Bolton and Exeter broths were superior to other broths such as Preston. The limitations of reliably isolating and identifying campylobacters by conventional culture may be addressed by the application of molecular techniques directly to enrichment culture, however; blood in enrichment culture can inhibit the PCR process (Josefsen et al., 2004). Molecular methods have developed rapidly over the past few years for *Campylobacter* spp. (Linton et al., 1997;

* Corresponding author. Tel.: +44 117 928 9245; fax: +44 117 928 9505.
E-mail address: lisa.williams@bristol.ac.uk (L.K. Williams).

0168-1605/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2009.01.018

Inglis and Kalischuk, 2003; Wang et al., 2002; Klena et al., 2004) and are now commercially available and used although not usually in routine testing.

The blood contained in broths offers a challenge to the application of molecular methods directly to the enrichment after incubation. To date, there has been little work on blood-free enrichment culture, with only a few studies examining the effect of removing blood (Thunberg et al., 2000; Josefsen et al., 2004; Sails et al., 2003). The incubation temperature of enrichment broths may also affect isolation of campylobacters. Traditionally incubation of these media was carried out at 42 °C for isolation of thermophilic *Campylobacter* spp. including *C. jejuni* and *C. coli* (Scates et al., 2003) but now the ISO method recommends incubation of Bolton broth at 37 °C followed by 42 °C while manufacturers recommend 37 °C for Preston and Exeter broths.

The aim of this study was to investigate the effect of blood in enrichment media on the detection of campylobacters from caeca, chicken carcass rinses and bootsock (gauze sock walked through a broiler chicken house) samples using Bolton broth, as recommended in the ISO 10272-1 method. In the second part of the study effect of incubation temperature (37 or 41.5 °C for 48 h or 37 °C for 4 h then transfer to 41.5 °C for 44 h), and the method of achieving atmosphere (containers were either incubated with <1 cm headspace and tightly capped lids incubated aerobically, hereafter referred to as initially aerobic atmosphere, or with loose lids incubated microaerobically, hereafter referred to as microaerobic atmosphere) was investigated using modified Bolton (mBolton) or modified Exeter (mExeter) broth with or without blood.

2. Materials and methods

2.1. Chicken carcass rinses

Samples were purchased from two independent retailers located in the South West of England. Samples were processed on the day of collection. Neck-skin (25 g) was aseptically removed from each individual chicken and placed into a small stomacher bag. The whole carcass was then put in a large stomacher bag. A 300 ml volume of 0.85% (*w/v*) saline (Oxoid Ltd, Basingstoke, UK) was poured through the vent into the abdominal cavity of the carcass. Most of the air was removed from the bag and the entire carcass rinsed in saline for 1 min. The rinse solution was then poured into the smaller bag containing the neck skin and this was stomached for one further minute using a lab blender 400 (Colworth, London, UK).

2.2. Bootsock samples

A white gauze bootsock, soaked in 20 ml saline immediately before use, was put onto a rubber boot. A plastic layer was placed between the rubber boot and the bootsock to minimise contamination from the former to the latter. The bootsock was then walked through the broiler house, ensuring that all areas of the house floor were covered. The bootsock was removed, placed into a polythene bag and transported to the laboratory at ambient temperature on the day of sampling. The non-contaminated top part of each bootsock was removed with sterile scissors; the remaining soiled area was cut in two and placed into a filter-stomacher bag. Saline (50 ml) was added to the bag and the sample homogenised for 30 s using a lab blender 400 (Colworth).

2.3. Caeca

Caeca from broiler chickens were removed at the processing plant following slaughter (20–30 individual caeca/batch) and placed into a polythene bag. These were transported to the laboratory under chilled conditions and a pooled sample (0.3 g from each caecum) for each batch was prepared.

2.4. Faecal samples

Freshly voided faecal samples were collected from the broiler house floor. Samples from each house were pooled and mixed thoroughly.

2.5. Enrichment culture

In this study, samples were enriched in Bolton, mBolton and mExeter broth with and without the addition of lysed defibrinated horse blood; an overview of each broth is outlined in Table 1. Chicken carcass rinses (25 ml of the rinse solution) and bootsock samples (25 ml of the saline solution) were transferred to 250 ml sterile plastic containers and enrichment broth (225 ml) was added to each container. A 1 g sub-sample from each pooled caecal and faecal sample was placed in a 100 ml sterile container and ~90 ml enrichment broth with or without the addition of lysed defibrinated horse blood (Oxoid), was added to each. Regardless of container size, <1 cm of headspace remained in the containers and lids were tightly capped. In the first experiment, using Bolton broth, all enrichments were incubated at 37 °C for 4 h and then transferred to 41.5 °C for 42 to 44 h in aerobic conditions. In the second experiment, using mBolton and mExeter broths, enrichments were prepared with and without the addition of lysed defibrinated horse blood and were incubated at the following temperatures 37, 41.5 and 37 °C for 4 h and then transferred to 41.5 °C for 42 to 44 h in either an initially aerobic (O_2) atmosphere with tightly capped lids and <1 cm of headspace or a microaerobic (MA) atmosphere (5% O_2, 10% CO_2 2% H_2 in a balance of N_2) with loose lids in an anoxomat jar (Launch Diagnostics LTD, Longfield, UK).

Following incubation for 48 h for Bolton broth and 24 and 48 h for mBolton and mExeter broths, 10 µl of each sample was streaked onto mCCDA and the plates incubated at 37 °C in a microaerobic atmosphere for 48 h. Presumptive campylobacter colonies were confirmed by growth on duplicate plates of Columbia Blood Agar with 5% (v/v) defibrinated horse blood (COLBA; Oxoid Ltd). The two plates were incubated under different conditions at 37 °C for 48 h; one aerobically and one under microaerobic conditions. Any isolates that were aerotolerant were presumed not to be campylobacter and did not undergo further confirmatory tests. Presumptive positives were confirmed by microscopy and by positive oxidase reaction.

Table 1
Enrichment methods for the isolation of *Campylobacter* spp. All media and supplements supplied by Oxoid limited, Basingstoke, UK unless stated otherwise and volumes described are for 1 l

	Base	Lysed horse blood (SR050C)	Supplements	Recommended Incubation time and temperature
Bolton broth	Bolton broth (CM985)	50 ml	2 vials Bolton broth supplement (SR0183)[a]	37 °C for 4 h, 41.5 °C for 44 h
mBolton broth	Bolton broth (CM985)	50 ml	2 vials modified Bolton broth supplement (SR0208E)[b]	37 °C for 4 h, 41.5 °C for 44 h
mExeter broth	Bolton broth (CM985)	10 ml	2 vials Campylobacter growth supplement (FBP: SR084E)[c] 1 vial Campylobacter enrichment (Exeter) (SV59 Mast Diagnostics, Merseyside UK)[d]	37 °C for 48 h

Concentrations in 1 l of medium:
[a] 20 mg Cefoperazone, Vancomycin, Trimethoprim, 50 mg Cycloheximide.
[b] 20 mg Cefoperazone, Vancomycin, Trimethoprim, 10 mg Amphotericin B.
[c] 0.25 g Sodium pyruvate, Sodium metabisulphite, Ferrous sulphate.
[d] 10 mg Trimethoprim, 5 mg Rifampicin, 2500 iu Polymyxin B, 15 mg Cefoperazone, 2 mg Amphotericin.