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Methodology article

An optimised recovery method for thermophilic Campylobacter from liver

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Abstract

Background: The past three decades have witnessed the rise of *Campylobacter* enteritis in man from virtual obscurity to notoriety, with present isolation rates superseding those of other enteric pathogens such as *Salmonella spp.* and *Shigella spp.* in most developed countries. Although campylobacters are not completely new to applied bacteriology, they have evaded traditional isolation techniques used for the isolation of pure cultures, apart from single isolations that were free from competing organisms. Offals, in particular liver have been decribed as both a source of campylobacters, as well as a route of transmission of this organism to human. Therefore, the aim of this study was to develop an optimum method for the recovery of *Campylobacter* spp. from porcine liver.

Results: Four isolation techniques (methods A-D) were compared in a small pilot study for their ability to successfully recover campylobacters from freshly eviscerated porcine liver. The optimum isolation method involved direct swabbing of the liver tissues followed by plating onto Preston Selective medium, which was superior to methods involving mechanical disruption to liver tissues, including direct plating and enrichment methods, with and without blood. Consequently, any isolation method that involves disruption of liver tissue e.g. homogenisation or stomaching, is not suitable for the detection of campylobacters from liver and hence it is recommended that employment of a direct swabbing technique without mechanical disruption of tissues in combination with selective plating to optimally recover campylobacters from freshly eviscerated liver.

Conclusions: Employment of a direct swabbing technique in combination with selective plating allow *Campylobacter spp.* to be optimally recovered from freshly eviscerated liver and therefore this technique is recommended when examining liver for the presence of this organism.

Background

The past three decades have witnessed the rise of *Campylobacter* enteritis in man from virtual obscurity to notoriety, with present isolation rates superseding those of other enteric pathogens such as *Salmonella spp.* and *Shigella spp.* in most developed countries.

Unlike the salmonellae and other enteric pathogens, the majority (ca. 99%) of clinical reports concerning *Campylobacter* are sporadic and *Campylobacter* enteritis outbreaks are rare. The lack of well-developed typing schemes has hindered the epidemiological investigations seeking natural reservoirs of the organism and modes of transmission from these sources to man. Only about 15% of clinical isolates are identified to species level thus making epidemiological investigations extremely difficult to perform.

Campylobacters are not completely new to applied bacteriology. They have evaded traditional isolation techniques used for the isolation of pure cultures, apart from single isolations that were free from competing organisms. Until the development of a selective medium by Skirrow [1], these organisms were known mainly by veterinarians as animal pathogens which were responsible for a wide variety of disorders in cattle, sheep and pigs [2]. Since the development of more sophisticated isolation techniques, the true disease potential of these organisms has become apparent and today campylobacteriosis is regarded as a zoonosis, which is capable of being transmitted to man by a wide range of domestic animals, their meat and offals.

Offals, in particular liver have been described as both a source of campylobacters [3–6], as well as a route of transmission of this organism to human [5]. Therefore, the aim of this study was to develop an optimum method for the recovery of *Campylobacter* spp from porcine liver.

Results and discussion

The isolation rates of the four methodologies are shown (Table 1). The swabbing method (method D) had the highest efficacy for the isolation of campylobacters and was statistically different from the other three methods ($P < 0.001$).

Although there are numerous techniques for the isolation of *Campylobacter spp.* from meats and foodstuffs, there is presently no accepted standard reference method for the isolation of this organism. Several studies have addressed this in an attempt to define the most suitable isolation technique, so that all results are comparable [7–10], however one single protocol may not be ideal for all types of foodstuffs. Therefore, it is important to optimise the isolation technique for the particular foodstuff being examined.

In the present study, four isolation methods were compared to obtain an optimal isolation technique for porcine liver. As previous studies have shown that a low proportion (<10%) of liver may be infected with *Campylobacter spp.*[3,4], an isolation method was sought which would allow rapid screening of a large number of livers, which was both specific and sensitive.

Only naturally contaminated livers were used in this study to assess the most effective isolation method, as employment of spiked specimens would not have truly reflected the sites of adherence, the natural adherence mechanism and the density of cells normally found in the wild-type state. Consequently, as it was difficult to obtain a large number of positive specimens, this small pilot study did show that the direct swabbing technique was the most sensitive isolation method employed (Table 1). Preston agar was selected as the selective plating medium, as this medium has been shown previously to be the optimum medium for the isolation of wild-type campylobacters from porcine liver [3]. Campylobacters isolated from porcine liver were not considered to be sub-lethally stressed, as direct plating onto selective media did not cause a decrease in isolation rates. The swab method was shown to be sufficiently sensitive for the detection of *Campylobacter spp.,* as isolation was on a presence/absence basis. The swab technique allowed the intrinsic contamination of liver to be assessed by selective sampling of deep tissue as opposed to surface tissue. Swabbing was also the most economical in terms of time and materials. Therefore swabbing onto selective media was considered to be the optimal method of recovery of campylobacters from liver.

Table 1: Comparison of isolation methods (A-D) for campylobacters from freshly eviscerated porcine liver.

				ISOLATION METHOD (% livers positive)			
Plant Code	No. visits	No. Herds examined	No. livers examined	A (Direct Isolation)	B (Enrichment with blood)	C (Enrichment without blood)	D (Swabbing technique)
A	2	15	20	5	10	10	15
B	1	2	10	10	0	0	20

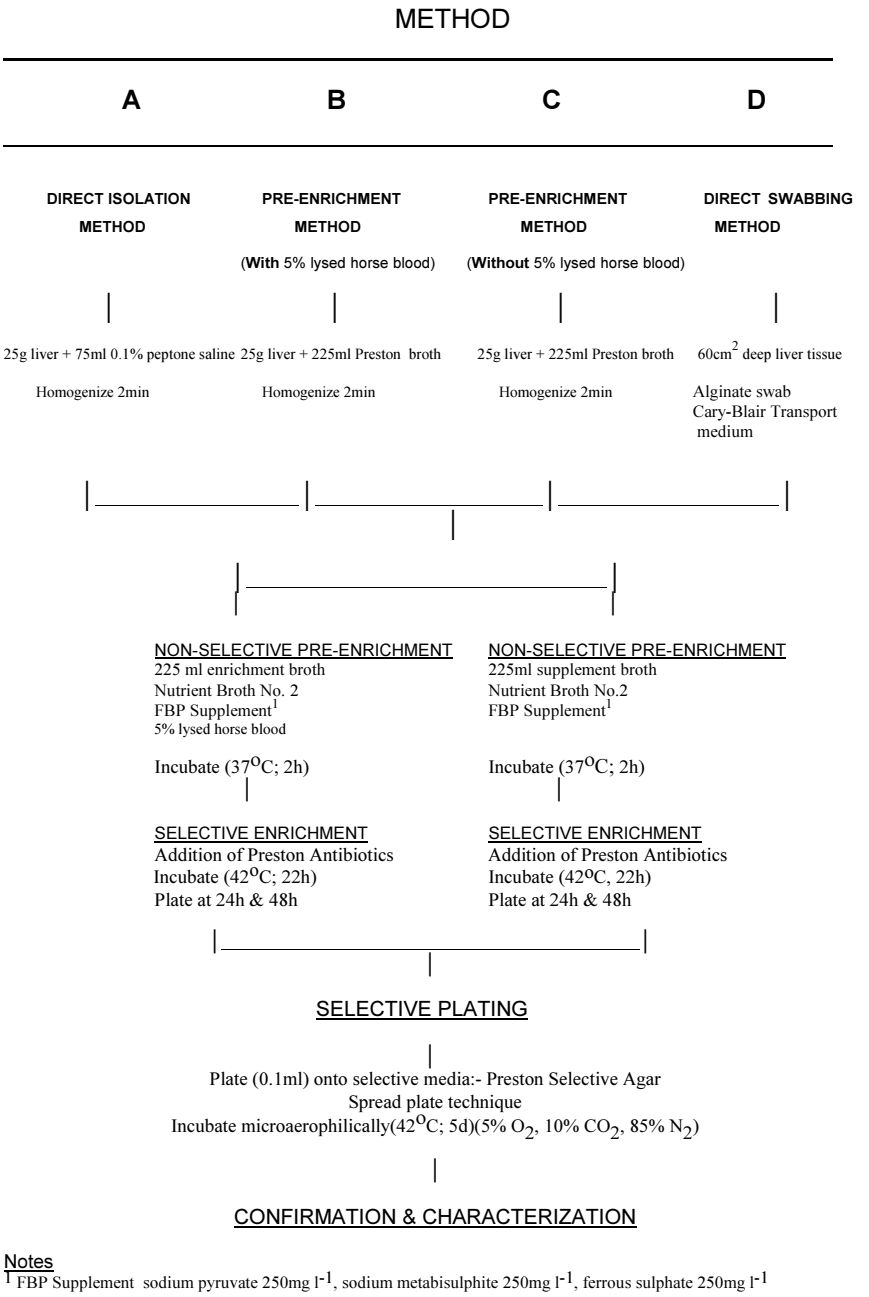

Figure 1
Schematic diagram of four isolation methods employed (A-D) for the detection of campylobacters from porcine liver.

Bolton and Robertson [11] and Bracewell *et al.*[12] concluded that the incorporation of an enrichment stage was superior to direct plating. Although enrichment procedures may enhance isolation rates, Turnbull and Rose [13] showed that eight samples were positive on direct plates yet were negative by enrichment procedures. This might indicate that some of the enrichment methods employed were not fully reliable or may included a cell disruption process such as homogenization or stomaching. Previously, it has been shown [14] that liver homogenates contain a heat-labile antagonistic factor, which is lethal to campylobcters even after six hours. As

the liver is the organ of detoxification, any intracellular inhibitory peptides or proteins, which are released during homogenization, may act antimicrobially and hence inhibit the growth and proliferation of campylobacters. Therefore, it is proposed that any isolation method should therefore not include a disruption stage, such as homogenization or stomaching, which are normal stages in isolation protocols and thus an examination of liver homogenates in the study design was not included.

Stern [7,8] demonstrated that the swab technique was capable of recovering 32 *Campylobacter* cells per cm^2 from lamb carcasses and this technique has been employed as an isolation technique by other workers [15]. In the present study, direct plating of cells onto selective media by swabbing was considered to be a rapid and sensitive isolation technique for *Campylobacter spp.* in porcine liver.

In conclusion, employment of a direct swabbing technique in combination with selective plating allow *Campylobacter spp.* to be optimally recovered from freshly eviscerated liver and therefore this technique is recommended when examining liver for the presence of this organism.

Materials & Methods

Four methods (methods A-D) were compared for isolating *Campylobacter spp.* (Figure 1). The detection of *Campylobacter spp.* was carried out both by taking liver samples (500 g) (methods A-C) and by directly swabbing the liver (method D). Livers were sampled as part of the "pluck" immediately post evisceration at the slaughter plant and prior to veterinary inspection. Liver samples (500 g) were taken aseptically from freshly eviscerated liver lobes and were transported to the laboratory under chilled conditions (4°C) and analyzed within 3 h of collection. Swabs were taken from deep liver areas immediately post evisceration and prior to veterinary inspection. For the swabbing method (Method D), samples were obtained by pre-moistening a sterile alginate swab in Cary-Blair transport medium [16] (Difco 9397-27-1, England), before swabbing an area (approx. 60 cm^2) of the deep tissue. Deep tissue swabs were obtained by making a large incision with a sterile boning knife, taking care not to rupture the gall bladder. Swabs were placed in Cary-Blair transport medium and transferred to the laboratory under chilled conditions (4°C). All swabs were examined within 3 h of collection.

30 samples were examined by each method after three visits over a two-month period to two EU-licensed pork processing plants in Northern Ireland (Plants A & B). Samples were taken from 17 herds of bacon pigs. Ten samples were taken at random on each plant visit constituting one batch. *Campylobacter spp.* were isolated from each sample by carrying out methods A-D in duplicate. Two replicate samples from all treatments (Methods A-D) were streaked onto Preston selective agar (Oxoid Ltd., England). Cultures were incubated as described (Figure 1). Presumptive positive colonies were streaked onto BA2 and incubated prior to characterization, employing several phenotypic tests as previously described [17]. All livers from which *Campylobacter spp.* were isolated were recorded as positive. Statistical analyses were performed to compare recovery by methods A-D employing Microsoft Excel employing a paired student's t-test with a one-tailed distribution. Statistical significance was noted whenever the probability (P) was less than 0.05 (5%).

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