DEVELOPMENT OF METHODS FOR THE ISOLATION AND DETECTION OF *CAMPYLOBACTERACEAE* IN THE MEAT FOOD CHAIN

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Introduction

Campylobacter spp. are recognised as one of the leading causes of food-related illness and are the most common cause of gastric-infections in Ireland. In 2004 alone, there were 1711 reported cases of human *Campylobacteriosis* in the Republic (CIR 43.7/100,000), with males under the age of five carrying the highest burden of infection (HPSC, 2004).

The isolation and identification methods used to examine clinical and food samples for *Campylobacter* are designed to primarily detect *Campylobacter jejuni* and *Campylobacter coli* and are biased towards these species. However, an isolation procedure incorporating the use of Hydrogen enriched atmosphere together with filtration onto blood agar and incubation at a lower temperature (37 °C) reportedly facilitates recovery of a much wider range of *Campylobacteraceae* for clinical specimens (Lastovica and Le Roux, 2000). Using these new methodologies, evidence is accumulating from studies carried out in South Africa and Europe that there are other *Campylobacter* strains of importance associated with gastric illness, including *Campylobacter concisus* and *Campylobacter upsaliensis*. These emerging species are being isolated at high rates and now appear to be as clinically significant as *C. jejuni* and *C. coli* (Enberg *et al.*, 2000; Abenhus *et al.*, 2002; Maher *et al.*, 2002).

The distribution and reservoirs of emerging *Campylobacteraceae* in the food chain have not yet been established. It is therefore essential to modify current detection methods for the isolation of a wider range of *Campylobacteraceae* from food and environmental samples. This will contribute to effective monitoring and surveillance and allow for a better understanding of the epidemiology and transmission routes of this pathogen. The following summarises the methods and techniques investigated, to establish new isolation methods capable of recovering a wide range of *Campylobacter* from meat.

Materials and Methods

Campylobacter strains (n=59) of human, clinical and animal origin were used in this study. All strains were supplied by the Danish Food and Veterinary Research Institute in Copenhagen, Denmark, and the Red Cross children's Hospital in Cape Town, South Africa and stored in 80% Glycerol at – 80 °C.

Enrichment: *Campylobacteraceae* spp. were inoculated into a range of different enrichment media {Hunts Enrichment Broth (Hunt *et al.*, 1992), Bolton broth (Oxoid, Basingstoke, U.K) and *Campylobacter* Enrichment Broth (CEB) (Lab M, Surrey, UK). Each broth was incubated in an atmospheric cabinet (Don Whitley) supplied with 2.5% O_2, 7% H_2, 10% CO_2, and 80.5% N_2 (personal communication, Keevil, W. University of Southampton) for 0-72 h at 37 °C. During the enrichment period, *Campylobacter* were plated out at various time intervals onto two agar types; Tryptose Blood Agar with 5% lysed horse blood (TBA) and Anaerobe Basal Agar with 5% lysed horse blood (ABA) (Oxoid). Agar plates were incubated under the same conditions for 48 h to allow for enumeration. Morphology and motility of each sample was assessed at every time interval using NanoOrange Staining (Molecular Probes, the Netherlands) (Alonso *et al.*, 2002).

Isolation: Meat pieces (25 g) were inoculated with control *Campylobacter* strains at levels of 1.0 $\log_{10}$ cfu ml $^{-1}$ and 2.0 $\log_{10}$ cfu ml $^{-1}$. Samples were placed in a filter stomacher bag with 225 ml CEB and 5% lysed horse blood and incubated for 24 or 48 h in the gas atmosphere described above at 37 °C. A range of options were investigated for recovery of *Campylobacter* from the enrichment broth, alone and in combination, including stomaching versus pulsification (Microgen Bioproducts, U.K); centrifugation of enrichment broth to remove food debris, at various times and speeds; membrane filtration (various filter types and pore sizes: Cellulose nitrate 0.45μm, cCellulose acetate 0.45 μm, Cellulose nitrate 0.6 μm, Mixed ester 0.6 μm and Polycarbonate 0.6 μm) and the volume of enrichment broth to be transferred to the membrane surface (0.2, 0.3, 0.4 ml).

Validation: Using the optimised enrichment and isolation method described above, beef, pork and chicken (25g) were inoculated individually with 59 *Campylobacteraceae* strains to levels of $\log_{10}$ 1.0 cfu ml $^{-1}$ and $\log_{10}$ 2.0 cfu ml $^{-1}$ and recovery was tested.

Identification: Cultures were confirmed as *Campylobacteraceae* by use of the new O.B.I.S L-ala biochemical identification kits (Smith *et al.*, 2006) (Oxoid, Basingstoke, U.K) in conjunction with the KOH test to determine Gram reaction (Halebian *et al.*, 1981). *Campylobacter* latex agglutination kits (Microgen Bioproducts, UK) were used to establish the thermophillic/non-thermophillic status of each strain. Catalase, oxidase and hippurate testing were also carried out.

Polymerase Chain Reaction: PCR protocol is currently under optimization which allow for speciation of a wide range of known and emerging *Campylobacters*. The method allows for differentiation of species based on nucleotide sequence

differences in a common house keeping gene (Klena *et al.*, 2004). Once complete the procedure will allow for broad speciation using a minimum of multiplex reactions.

Results and Discussion

The method developed involved placing a meat samples (25g) in a filter stomacher bag with 225 ml CEB + 5% lysed horse blood and pulsifying it for 15 s. The sample was then placed in an atmospheric cabinet supplied with 2.5% O_2, 7% H_2, 10% CO_2, and 80.5% N_2 for 24 h at 37 °C. After incubation, 10 ml of broth was centrifuged at 2,500 rpm for 10 s followed by a dilution series. 200μl of supernatant was transferred to a mixed ester membrane (pore size 0.6 μm) on the surface of an ABA plate (with 5% lysed horse blood). The filters were removed after 15 s and plates were incubated for up to 6 days in the above described atmosphere and examined at intervals throughout the incubation period. Suspect colonies were confirmed using a biochemical matrix developed by Lastovica *et al.*, (2000) with the addition of the OBIS L-ala biochemical test and Latex agglutination. Speciation of *Campylobacter* positive isolates was achieved using an optimised PCR protocol (Klena *et al* ., 2004).

Conclusion

The above described protocol has been applied to examine the *Campylobacter* populations in fresh meat in Ireland over a 12 month period and the initial results indicate that in addition to *C. jejuni* and *C. coli*, a wider range of *Campylobacter* species are recoverable from these meats.

References

Alonso J.L., Mascellaro S., Moreno Y., Ferrus M. and Hernandez J. (2002). Double-staining method for differentiation of morphological changes and membrane integrity of *Campylobacter coli* cells. *Applied and Environmental Microbiology,* 68(10): 5151-4.

Aabenhus R., Permin H., On SL. and Andersen L.P. (2002). Prevalence of *Campylobacter concisus* in diarrhoea of immunocompromised patients. *Scandanavian Journal of Infectious Disease,* 34(4): 248-52.

Engberg J., On S.L.W., Harrington C.S. and Gerner-Smidt P. (2000). Prevalence of *Campylobacter, Arcobacter, Helicobacter* and *Sutterella* spp. in human faecal samples as estimated by a re-evaluation of isolation methods for *Campylobacter*. *Journal Clinical Microbiology,* 38: 286-291.

Halebian, S, Harris, B, Finegold, S.M, Rolfe, R.D (1981). Rapid methods that aid in distinguishing Gram-negative anaerobic bacteria. *Journal of Clinical Microbiology,* 13(3): 444-448

Health Protection Surveillance Centre (2004). Annual Report 2004.

Hunt, J.M (1992). "*Campylobacter*". FDA Bacteriological Analytical Manual, 7th Edition. P 77-94

Lastovica A.J. and Le Roux E. (2000). Efficient isolation of *Campylobacter* from Stools. *Journal of Clinical Microbiology*, 2798-2799.

Klena, J.D, Parker, C.T, Knibb, K, Ibbitt, C, Devane, P.M.L, Horn, S.T, Miller, W.G, Knonkel, M.E. (2004). Differentiation *of Campylobacter coli, jejuni, lari* and *upsaliensis* by a multiplex PCR developed from nucleotide sequences of the lipid A gene lpxA. *Journal of Clinical Microbiology* 42(12): 5549-5557.

Maher M., Finnegan C., Collins E., Ward B., Carroll C. and Cormican M. (2002). Evaluation of culture methods and a DNA probe-based PCR assay for detection of *Campylobacter* species in clinical specimens of faeces. *Journal of Clinical Microbiology,* 41(7): 2980-2986.

Smith, C.M, Colbourne, N.R, Stephens, P.J, Druggan, P (2006). A simple and rapid biochemical screening test for the differentiation of *Campylobacter* species from other contaminating organisms. Presented at Conference on Emerging *Campylobacter* in the Food Chain. Croke Park, Dublin, Feb 8th 2006

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