OPTIMISATION OF THE ISOLATION OF *CAMPYLOBACTER SPP.* FROM RETAIL PACKS OF RAW POULTRY

Madden, R. H.[1, 2], Moran, L.[1] and Scates, P.[1]

[1]Food Microbiology Branch, DARD, Newforge Lane, Belfast BT9 5PX, Northern Ireland. [2]Department of Food Science, Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX, Northern Ireland.

Background

In Northern Ireland (NI) *Campylobacter* spp. are the principal cause of gastro-enteritis (Anon. 1995) with *Campylobacter jejuni* responsible for 90-95% of cases and *Campylobacter coli* causing the majority of the remainder, as is the case in most developed countries. Local surveys of campylobacters in retail packs of poultry have shown levels of 65% (Flynn *et al.* 1994) and 57% (Wilson, 2002). In order to conduct a survey to determine the current level the most effective current isolation procedures were required. Accordingly one of the most commonly used campylobacter enrichment broths, Preston broth, was selected for comparison in a trial with a much newer medium, Bolton broth. Locally Preston broth had shown up to 100% efficiency in recovering campylobacters from pigs (Madden *et al.* 2000).

Objectives

This investigation sought to compare Bolton (Oxoid, Basingstoke, UK) and Preston (Bolton and Robertson, 1982) enrichment broths for their ability to recover campylobacters from retail packs of raw poultry using the manufacturers protocols. Both overall recovery rates and species diversity were to be evaluated. With the more efficient medium defined a study could then be undertaken to determine the effect of enrichment broth volume (90ml or 225ml) on recoveries of campylobacters. The standard 1:9 ratio of samples to broth (w/v) was to be used in both cases. Use of the lower volume would significantly reduce costs where large numbers of samples were to be analysed. Finally the consequences of the choice of incubation temperature, 37^{O}C or 42^{O}C, and duration, 24h or 48h, on the subsequent isolation rates of campylobacter species were to be investigated.

Materials and methods

All media used were obtained from Oxoid, Basingstoke, UK, and all chemicals from Sigma-Aldrich, Fancy Road, Poole, UK.

Sampling of retail poultry packs.

Retail packs of chilled poultry were purchased from local supermarkets and butchers. In supermarkets the EC processor codes and pack 'sell by ' dates were used to ensure a diverse range of samples. Analyses commenced within 2h of purchase.

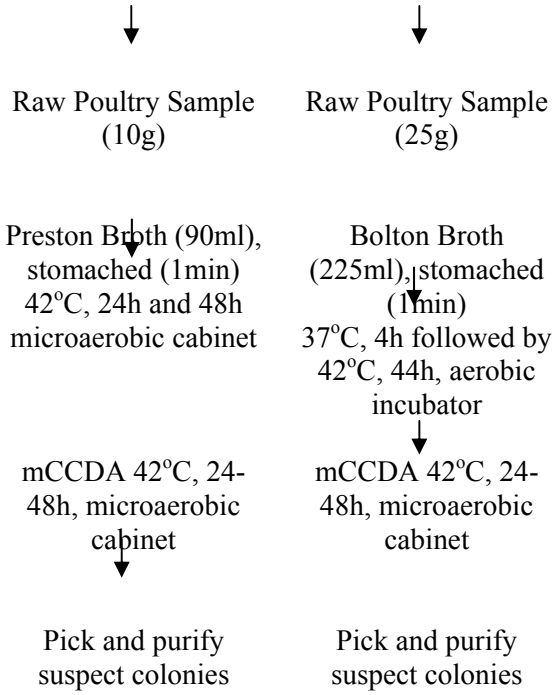
Comparison of methods for the isolation of *Campylobacter* spp.

Modified charcoal cefoperazone deoxycholate agar (mCCDA) was used as the plating medium with both Preston and Bolton broth. Figure 1 shows the enrichment protocols as used to compare Preston and Bolton broths (n=40), The former was based on a previous local study (Scates *et al.* 2003) and the latter was as described by Bayliss *et al.* (2000). In addition Preston medium was evaluated with 25g of sample and 225 ml of broth whilst Bolton broth (90ml) was inoculated with 10g of sample. The samples in Bolton broth were enriched for both 24h and 48h in a microaerobic atmosphere (85% N_2, 10% CO_2 and 5% O_2 in a Don Whitley Scientific Mk III anaerobic cabinet (Don Whitley Scientific, Shipley, UK)) to allow the effect of the incubation time on recoveries to be observed. Levels of contamination on mCCDA were assessed by using a standard plating technique and scored according to the apparent level of non-campylobacter growth in each streak (Bayliss *et al*. 2000). Presumptive *Campylobacter* isolates were characterised using a PCR method in order to determine genus (Linton *et al.* 1996) and a multiplex PCR method (Vandamme *et al.* 1997) to speciate *C. jejuni* and *C. coli.*

Local practice has been to incubate the enrichment broth under microaerobic conditions, as well as the selective solid medium. A comparison of microaerobic and aerobic incubation of 10g samples (n=21) in Bolton broth (90ml) was conducted to determine if this practise had any effect on recoveries .

With the selection of the optimal medium and sample size a trial sampling of 100 retail packs was undertaken using two incubation regimes, 37°C, 48h and 37°C, 4h followed by 42°C, 44h.

Figure 1. Comparison of the two methods compared for the isolation of *Campylobacter* spp. from retail packs of raw poultry. All incubations were microaerobic.



Results and discussion

Forty retail packs of poultry were studied to compare recoveries of campylobacters using the manufacturers recommended protocols, Figure 1. Included in the trial was a comparison of the effect of using 90ml and 225ml of both enrichment broths with a 1:9 sample:broth (w/v) ratio. Analysis of variance showed significantly more samples were positive for campylobacters using Bolton broth (n=27) than with Preston (n=24) using the protocols in Figure 1 (p=0.004). This is in agreement with findings of Bayliss *et al.* (2000) who used 25g samples inoculated into 225ml volumes of both enrichment broths. Bolton broth was more efficient at suppressing non-campylobacter flora than Preston broth which, when incubated for 24 and 48 h, yielded 2.4 and 3.6 times more contaminants respectively.

There was no statistically significant difference (p=0.439) between recoveries of campylobacters from 10g samples incubated in 90ml, as opposed to 25g in 225ml, with either medium. Hence the more economical lower volume could be adopted with no significant loss of sensitivity with either Preston or Bolton broth. This result may reflect the relatively high level of contamination of retail poultry with *Campylobacter* spp. (Jorgensen, 2002)

One *Campylobacter* isolate was obtained from each positive sample obtained using the two enrichment media, and two sample weights, and identified, Table 1. Again Bolton broth was seen to be superior as it allowed the detection of a significant number of *C. coli* isolates. Note that mixed cultures of *C. jejuni* and *C. coli* were detected by this procedure indicating that the preliminary picking and re-streaking procedure did not yield a pure culture. This is a frequent observation with this genus in our laboratory, indicating that repeated streaking is essential to ensure that a pure culture is obtained for subsequent studies.

Normally in our laboratory all *Campylobacter* incubations are conducted in a microaerobic atmosphere therefore the effect of microaerobic versus aerobic incubation was investigated. Samples of raw poultry (n=21) were inoculated into Bolton broth, using 10g inoculum, and incubated at 37°C for 4h followed by 44h at 42°C both aerobically and microaerobically. Microaerobic incubation of the enrichment broth was

seen to give the higher recovery of campylobacters with 62% of samples positive, whilst in air 52% of samples were positive. Thus microaerobic incubation of Bolton broth was seen to be the more efficient method .

Table 1. Identification of *Campylobacter* isolates from Preston (n=48) and Bolton broth (n=58) using PCR-based procedures. Bolton broth was superior, as it allowed the isolation of a wider range of species and detected more positive samples

.

	Multiplex PCR speciation			
Enrichment medium	*C. jejuni*	*C. coli*	Both *coli* and *jejuni*	Genus only positive
Preston broth (24h)	88%	0%	8%	4%
Bolton broth (48h)	63%	38%	6%	12%

However the incubation temperature of an enrichment broth had been shown to markedly influence the types of campylobacters subsequently isolated (Scates *et al*. 2003), hence incubation at 37^{O}C as well as 42^{O}C could prove beneficial and a trial to compare the two temperatures was designed. This necessitated determining the optimal incubation time for Bolton broth at the lower temperature, and the study was also used to compare the effect of incubation time on recoveries from the broth incubated at 42^{O}C, using the protocol of Figure 1. Samples of raw poultry (n=21) were prepared and streaked out onto mCCDA after 24 and 48h, 37^{O}C, and 42^{O}C. Plates were incubated at the same temperature as the enrichment broths. The 24h incubation time gave higher recoveries, at both temperatures, whilst 48h incubations allowed significantly more non-campylobacters to grow on the mCCDA plates. This is similar to the results seen in previous work with Preston broth where campylobacter recoveries from porcine ileal contents were higher with shorter incubation times (Madden *et al.* 2000).

A survey of 100 samples of retail packs of poultry was then undertaken using the optimised procedures of 10g of sample and 90ml of Bolton broth incubated at 37^{O}C and 42^{O}C for 24h and streaked on mCCDA with the plates being incubated at the same temperature as the enrichment broth. Plates were examined after 24 and 48h. Overall 82% of samples were positive, with incubation at 37^{O}C giving 73% and 42 ^{O}C 75% .

Conclusions

Bolton broth was seen to be better than Preston broth for detecting Campylobacter spp. in retail packs of raw poultry. The former medium also detected significantly more *C. coli* than did Preston broth. The use of a smaller sample size, 10g as opposed to 25g, had no significant effect on recoveries of campylobacters when used at a 1:10 ratio (w/v) with the enrichment broth, but markedly reduced costs. Incubation of Bolton broth for 24h gave better recoveries of *Campylobacter* spp. with fewer contaminants than did 48h.
Incubation of Bolton broth at 37^{O}C and 42^{O}C (with an initial 4h incubation at 37^{O}C), yielded similar number of positive samples but combining the results of both incubations significantly increased the total number of positive samples found.
Overall, therefore, the optimal method in terms of efficiency and cost was the use of 10g sample added to 90ml of Bolton broth and incubated for 24h at either 37^{O}C or 42^{O}C (with an initial 4h incubation at 37^{O}C). The use of both incubation temperatures would be necessary to maximise recoveries.

References

Anonymous 1995. The health of the public in Northern Ireland. DHSS, HMSO, Belfast, Northern Ireland.

Bayliss, C.L., MacPhee, S., Martin, K.W., Humphrey, T.J. and Betts, R.P. 2000. Comparison of three enrichment media for the isolation of *Campylobacter* spp. from foods. *J. Appl. Micro.* 89:884-891.

Bolton, F.J. and L. Robertson. 1982. A selective medium for isolation of *Campylobacter jejuni/coli*. Journal of Clin. Path. 35:462-467.

Flynn, O.M.J., Blair, I.S. and McDowell, D.A. 1994. Prevalence of *Campylobacter* spp. on fresh retail chicken wings in Northern Ireland. J. Food Prot. 57:334-336.

Linton, D., Owen, R.J. and Stanley, J. 1996. Rapid Identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. Res. Micro. 147, 707-718.

Madden, R.H., Moran, L. and Scates, P. 1998. Frequency of occurrence of *Campylobacter* spp. in red meats and poultry in Northern Ireland and their subsequent sub-typing using polymerase chain reaction fragment length polymorphism and the random amplified polymorphic DNA method. J. Appl. Micro. 84:703-708.

Madden, R.H., Moran, L. and Scates, P. 2000. Optimising recovery of *Campylobacter* spp. from the lower porcine gastrointestinal tract. J. Micro. Methods. 42:115-119.

Scates, P., Moran, L. and Madden, R.H. (2003). Effect of incubation temperature on isolation of *Campylobacter jejuni* genotypes from foodstuffs enriched in Preston broth. Appl. Environ. Micro. 69:4658-4661.

Vandamme, P., Van Doorn, L.-J., Al Rashid, S.T., Quint, W.G.V., Van der Plas, J., Chan, V.L. and On, S.L.W. 1997. *Campylobacter hyoilei* Alderton *et al* 1995 and *Campylobacter coli* Veron and Chatelain 1973 are Subjective Synonyms. Int J. Syst. Bact. 47:1055-1060.

Jorgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D.R.A., Bolton, F.J., Frost, J.A., Ward, L., Humphrey, T.J. 2002. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods, Int. J. Food Micro. 76:151-164.

Wilson, I.G. (2002). *Salmonella* and *Campylobacter* contamination of raw retail chickens from different producers: a six year study. Epidem. Infect. 129:635-645.