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OCCURRENCE OF *CAMPYLOBACTER* SPP IN NORTHERN IRELAND MEATS AND THEIR TYPING USING RFLP-PCR.

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SUMMARY. A survey for the presence of Campylobacter spp. on carcasses post slaughter, and in retail packaged meats, was undertaken. Carcasses of lamb (n=100) were sampled by swabbing while beef carcasses (n=100) had samples of neck meat removed. Sampling was less than four hours post-slaughter, and no campylobacters were found. Similarly retail packs of beef (n=50) and pork (n=50) were free of campylobacters. However 38% of raw poultry in retail packs (n=120) had campylobacters present. All packs were of the same brand and the rates of isolation of *Campylobacter jejuni* and *Campylobacter coli* were almost equal. Subsequent typing of the isolates was undertaken but thirteen of the original forty-five isolates lost viability in storage. About half of the survivors were untypable using Penner serotyping, illustrating the limitations of this conventional methodology. Using PCR-RFLP (polymerase chain reaction, restriction fragment length polymorphism) typing nine distinct types were detected in the thirty two viable cultures. It was seen that certain types recurred over the twelve months of sampling hence, given that broilers have a lifespan of about six weeks, either vertical transmission of these organisms is occurring or residual contamination of the rearing houses is re-infecting new flocks. The typing system evaluated should provide a useful tool in determining the exact source of these infections.
KEYWORDS. Campylobacter, meats, typing, PCR, RFLP, poultry, epidemiology.

BACKGROUND AND OBJECTIVES. Reports of salmonellosis in Northern Ireland exceeded those of campylobacter infections until 1990 but subsequently there has been a marked increase in campylobacter infections and *Campylobacter* spp. are now the major cause of food-borne infections. Since meats are often implicated in cases of campylobacter food-poisoning a survey of raw meats, both at the abattoir and in retail outlets, for the presence of campylobacters was undertaken. In conjunction with the meat survey genetic methods of typing campylobacters were evaluated to see if they could p rovide appropriate tools for investigating the sources of any isolates obtained during the survey. Conventional phenotyping and serotyping were also be conducted for comparative purposes.

MATERIALS AND METHODS. Carcasses were swabbed (100cm^2) and the swab was treated as the meat samples discussed below. Meat samples (10g) were added to 90ml Preston selective broth supplemented with FBP (iron, bisulphite, pyruvate) (George et al. 1978) and incubated microaerophilically (42^{0}C, 48h). The enrichment was then streaked onto Preston selective agar and re-incubated (42^{0}C, 24-48h) prior to selecting typical colonies and confirming the presence of campylobacters by the normal biotyping procedures (Bolton and Robertson, 1982). Penner serotyping was conducted by Dr Andrew Fox at Manchester Public Health Laboratory, England. Genetic typing was based on the method of Nachamkin et al 1994, with a primer amplifying part of the *Fla A* gene used in the PCR protocol, then the products being digested with Dde1 restriction endonuclease. Agarose gels (2%) were run for six hours (70V) to separate the digestion products and visualisation used ethidium bromide stain.

RESULTS AND DISCUSSION. No Campylobacter spp were found on lamb carcasses (n=100) and to improve recoveries samples of neck meat were taken from beef carcasses (n=100) but again all samples were negative. Retail packs of beef (n=50) and pork (n=50) also yielded no *Campylobacter* spp. however retail packs of poultry (n=120) yielded campylobacters in 38% of samples. *Campylobacter coli* and *Campylobacter jejuni* were found in almost equal numbers. As the survey was conducted over a period of one year cultures isolated were stored at -20^{0}C pending typing. However 13 out of a total of 45 cultures lost viability. Typing of the remainder yielded the results shown (Table 1). The large number of untypable strains (29%) illustrates the limitations of the serotyping employed, and

overall five serotypes were noted. However using PCR/RFLP nine distinct types were identified, showing greater discrimination, and some of the types recurred over the period of the survey. The first two numbers of isolate codes relate to the sample month with all samples coded 06X, for

Table 1. Characterisation of poultry *Campylobacter* spp.

SPECIES	ISOLATE CODE	PENNER SEROTYPE	PCR/RFLP TYPE	SPECIES	ISOLATE	PENNER SEROTYPE	PCR/RFLP TYPE
C. jejuni	CS081	55	1	*C. coli*	CS035	UT	4
	CS082	55	1		CS036	UT	4
	CS083	55	1		CS037	UT	4
	CS084	55	1		CS046	UT	5
	CS085	55	1		CS049	UT	5
	CS087	55	1		CS073	UT	6
	CS105	55	1		CS078	UT	7
	CS107	55	2		CS097	4	8
	CS004	23	2		CS101	4	9
	CS091	23	2		CS102	4	9
	CS099	23	2		CS104	4	9
	CS100	23	2		CS106	4	7
	CS092	UT[1]	2		CS109	4	7
	CS093	UT	2		CS110	4	7
	CS064	7	3				
	CS067	7	3				
	CS063	9	3				
	CS062	UT	3				

[1]untypable

example, being taken on the sixth month of the survey. Thus type 1 cultures were obtained on the eighth and tenth month of the survey and given a six week lifespan for broiler chickens it is apparent that this *C. jejuni* type could not be from cross-infection but would require either vertical transmission, e.g. due to shell contamination, or re-infection from the broiler house environment. Campylobacters have been detected in chicks on the day of assignment to broiler houses (Chuma *et al* 1994) but the source of these organisms was not defined. However Kapperud et al (1993) suggested that infected water was the single greatest factor affecting whether broilers became infected, and that strict hygienic routines should be adopted when workers enter the rearing houses. Evans (1992) suggested transmission through the egg appeared unlikely but that well-designed cross-sectional studies were called for to define the mode of infection. Given the results reported above the typing method evaluated would appear to be a potent tool for tracing *Campylobacter* species through the production cycle to retail packs, then on to consumers.

CONCLUSIONS. Surveys of lamb and beef at abattoirs produced no evidence of campylobacter contamination while at the retail level beef and pork were campylobacter free. However 38% of retail packs of chicken were contaminated. Using a PCR/RFLP typing method the poultry isolates were successfully typed, in contrast with conventional serology where several isolates were untypable. The typing method showed recurrence of specific types over a period of one year in the output of a single producer. This indicates recurrent infection whose source can now be investigated using the methodology developed. Prophylactic measures will then be proposed.

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