# Rapid quantification of viable Campylobacter on chicken carcasses by real-time PCR and propidium monoazide as a tool for quantitative risk assessment

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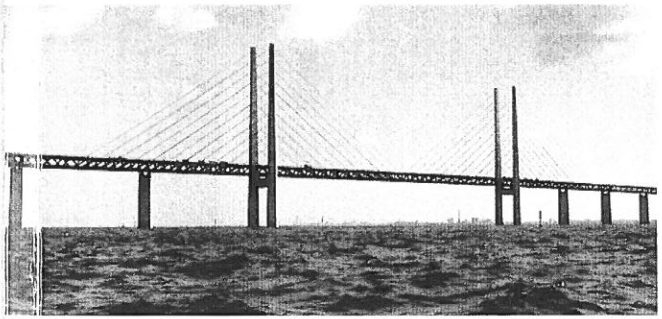

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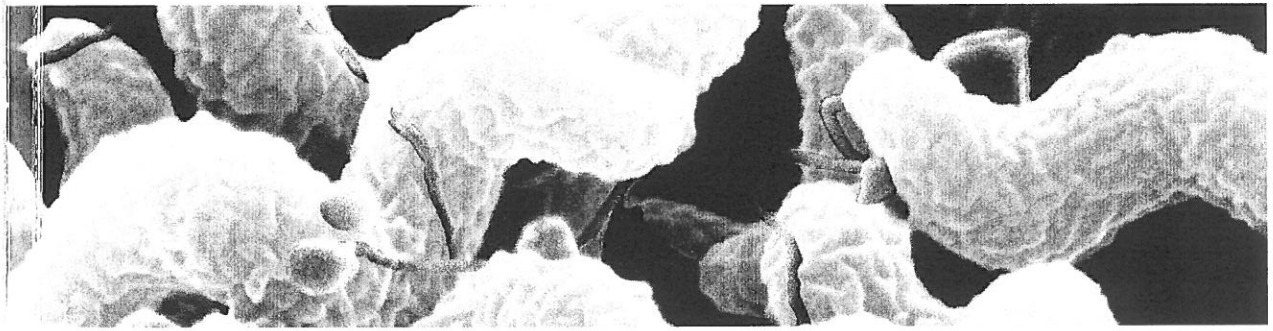

Final Programme & Abstract Book

**PSC1.05** **Lipooligosaccharide gene locus classes of *Campylobacter jejuni*: A predictor for genotype and virulence potential?**

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**Introduction:** Besides its role in human enteric illnesses, *C. jejuni* is a predominant infectious trigger of acute post-infectious neuropathies, such as Guillain-Barre´ syndrome. Significant interest in studying the structure and biosynthesis of the core lipooligosaccharide (LOS) of *C. jejuni* has resulted from its potential role in these paralytic disorders. LOS class types in *C. jejuni* strains isolated from chicken meat has hardly been studied. In addition, the role of LOS class variation in the invasion potential of *C. jejuni* strains from chicken meat still needs to be explored.

**Method:** In this study we present the results of PCR screening of five LOS locus classes (A, B, C, D, and E) for a collection of 116 *C. jejuni* isolates from chicken meat ($n$= 76) and sporadic human cases of diarrhea ($n$= 40). We correlated LOS classes with clonal complexes (CC) assigned by multilocus sequence typing (MLST). Finally, we evaluated the invasion potential of a panel of 52 of these *C. jejuni* isolates for Caco-2 cells.

**Results:** PCR screening showed that 87.1% (101/116) of isolates could be assigned to LOS class A, B, C, D, or E. Concordance between LOS classes and certain MLST CC was revealed. The majority (85.7% [24/28]) of *C. jejuni* isolates grouped in CC-21 were shown to express LOS locus class C. The invasion potential of *C. jejuni* isolates possessing sialylated LOS (n= 29; classes A, B, and C) for Caco-2 cells was significantly higher ($P < 0.0001$) than that of C. jejuni isolates with nonsialylated LOS (n= 23; classes D and E). There was no significant difference in invasiveness between chicken meat and human isolates. However, *C. jejuni* isolates assigned to CC-206 (correlated with LOS class B) or CC-21 (correlated with LOS class C) showed statistically significantly higher levels of invasion than isolates from other CC. Correlation between LOS classes and CC was further confirmed by pulsed-field gel electrophoresis.

**Conclusion:** We showed that simple PCR screening for *C. jejuni* LOS classes could predict certain MLST CC and add to the interpretation of molecular-typing results. Our study corroborates that sialylation of LOS is advantageous for *C. jejuni* fitness and virulence in different hosts. The modulation of cell surface carbohydrate structure could enhance the ability of *C. jejuni* to adapt to or survive in a host.

**PSC1.06** **Rapid quantification of viable *Campylobacter* on chicken carcasses by real-time pcr and propidium monoazide as a tool for quantitative risk assessment**

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A number of intervention strategies against *Campylobacter* contaminated poultry focus on post-slaughter reduction of the number of cells, emphasizing the need for rapid and reliable quantitative detection of only viable *Campylobacter*. We present a new and rapid quantitative approach for enumeration of foodborne *Campylobacter*, combining real-time PCR (Q-PCR) with a simple propidium monoazide (PMA) sample treatment. In less than 3 hours, this method generates a signal from only viable and viable but non-culturable (VBNC) *Campylobacter* with an intact membrane. The method performance was evaluated by assessing the contribution to variability from individual chicken carcass rinse matrices, species of *Campylobacter*, and the efficiency of DNA extraction with differing cell inputs. The method was compared with culture-based enumeration on 50 naturally infected chickens. The cell contents correlated with Ct-values ($R^2$ = 0.993), with a quantification range from $1\times10^2$–$1\times10^7$ CFU/ml. The correlation between the *Campylobacter* counts obtained by PMA-PCR and culture on naturally contaminated chickens was high ($R^2$ = 0.844). The amplification efficiency of the Q-PCR method was not affected by chicken rinse matrix or by species of *Campylobacter*. No Q-PCR signals were obtained from artificially inoculated chicken rinse when PMA sample treatment was applied. In conclusion, this study presents a rapid tool for producing reliable quantitative data on viable *Campylobacter* in chicken carcass rinse. The proposed method does not detect DNA from dead *Campylobacter*, but recognises the infectious potential of the VBNC state, and is thereby able to assess the effect of control strategies, and provide trustworthy data for risk assessment.