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Evidence of Genomic Instability in *Campylobacter jejuni* Isolated from Poultry

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Poultry isolates of *Campylobacter jejuni* derived from a survey of meat processing batches were genotyped by pulsed-field gel electrophoresis (PFGE) of chromosomal DNA to establish the clonal relationships between single-colony isolates. In the majority of batches studied, one or two genotype patterns predominated. However, in one batch (batch A), 21 single-colony isolates gave 14 different PFGE genotypes. The banding patterns obtained with *Sma*I were sufficiently different to distinguish between genotypes, although the patterns also produced many common bands. The question of whether these isolates represented different clones or had a common clonal ancestry was addressed by additional genotypic and phenotypic methods. Restriction length polymorphism of PCR products obtained from the flagellin genes showed an identical flagellin genotype for all of these isolates. In contrast, unrelated control isolates resulted in different flagellin genotypes. Moreover, all 14 different PFGE genotypes of batch A had identical Penner serotypes and identical or similar biotypes and phage types. It was concluded that the isolates were of clonal origin and that the diversity in the PFGE banding patterns had most likely originated from genomic rearrangements. However, the PFGE genotypes were shown to be stable upon subculturing in vitro and after in vivo passage in chickens, and natural transformation between isogenic mutants carrying antibiotic markers did not occur in vivo in a chick colonization model. The possible mechanisms for the hypothesized genomic recombinations and the conditions that allow, induce, or select for such events are discussed.

Campylobacter jejuni is a common cause of human acute bacterial enteritis. This bacterium can be isolated from the gastrointestinal tracts of most domestic animals but appears to be highly adapted to the avian gut. Epidemiological studies indicate that the handling and consumption of raw or undercooked chicken pose a significant risk for human infection. Several factors contribute to the high incidence of contamination of poultry. Firstly, chickens can be colonized in the gut and, more specifically, in the cecum at very high levels (maximally about 10^{10} organisms per g of cecal content) without symptoms. Secondly, once some birds in a flock have acquired *Campylobacter*, the whole flock usually becomes colonized. In this way, many flocks of chickens are infected on the day of slaughter; e.g., in the United Kingdom up to 90% of the flocks can be infected (7). Thirdly, during slaughter and processing, cross-contamination of previously *Campylobacter*-negative carcasses may occur.

Flock colonization is generally restricted to one strain or a limited number of strains (3, 11, 16), and typing techniques indicate that certain subtypes may predominate in poultry. This predominance may be a reflection of enhanced survival of such strains in different environments, hosts, and hostile conditions, and their relative absence in human infections suggests a lower level of virulence (12). In order to investigate potential relationships between survival mechanisms and infection, more epidemiological data are required, especially at the subspecies level. Therefore, a survey was initiated to determine the rate of contamination of poultry produced in different European countries with *Campylobacter* and *Salmonella* species and to investigate a possible link to locally occurring human infections (9). The original survey was then extended by typing the *Campylobacter* isolates at the subspecies level in order to identify epidemiological trends.

In the past, subtyping of *Campylobacter* has been confined to serotyping. However, phenotyping methods such as serotyping are increasingly being replaced by methods based on molecular genetic techniques. The method selected for genotyping in our survey was pulsed-field gel electrophoresis (PFGE) of chromosomal DNA digested with rare cutting enzymes (19, 28). This method proved suitable for the identification of individual strains and therefore for the recognition of epidemiological trends within chickens and poultry samples. The general finding was that PFGE genotypes of isolates within a batch were either identical or considerably different (9a).

In the course of this epidemiological survey, our attention was drawn to one particular batch in which the genotypes of the isolates were shown to be similar but not identical. The clonal relationship of these isolates was determined. Aside from PFGE genotyping, single-locus PCR-restriction fragment length polymorphism (RFLP) and several phenotypic typing methods, including serotyping, were applied to establish the genotypic and phenotypic similarities of the isolates. The evidence presented here suggests that these isolates were of clonal origin but had undergone genomic rearrangements. Attempts to reproduce such events experimentally were unsuccessful. The mechanisms by which such events could have occurred in vivo or in vitro are discussed.

MATERIALS AND METHODS

Bacterial strains. *Campylobacter* species were isolated from processed poultry from meat processing plants in Germany, The Netherlands, and France. Meat batch A (The Netherlands) comprised 30 packets of poultry collected in numerical order directly after packaging. Swabs were taken from the outside of the meat, a fresh cut inside the meat, the packet wrapping, and the dripping fluid and

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