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Campylobacter genotypes from food animals, environmental sources and clinical disease in Scotland 2005/6

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ABSTRACT

A nationwide multi-locus sequence typing (MLST) survey was implemented to analyze patterns of host association among *Campylobacter jejuni* and *Campylobacter coli* isolates from clinical disease in Scotland (July 2005–September 2006), food animals (chickens, cattle, sheep, pigs and turkey), non-food animals (wild birds) and the environment. Sequence types (STs) were determined for 5247 clinical isolates and 999 from potential disease sources (augmented with 2420 published STs). Certain STs were over represented among particular sample sets/host groups. These host-associated STs were identified for all sample groups in both *Campylobacter* species and host associated clonal complexes (groups of related STs) were characterized for *C. jejuni*. Some genealogical lineages were present in both human disease and food animal samples. This provided evidence for the relative importance of different infection routes/food animal sources in human disease. These results show robust associations of particular genotypes with potential infection sources supporting the contention that contaminated poultry is a major source of human disease.

1. Introduction

Campylobacter is the most common bacterial cause of gastroenteritis in the industrialized world, with economic costs estimated at US $4.3 billion in 1997 (Buzby et al., 1997). Human campylobacteriosis is caused principally by *C. jejuni* and *C. coli* (Friedman et al., 2000). Consumption of contaminated chicken, red meat, water, milk, and contact with pets and farm animals have been implicated as potential sources of human *Campylobacter* infection (Friedman et al., 2000; Kapperud et al., 2003). Recent work focuses upon characterizing the relative contributions of different sources to the human disease burden (Sheppard et al., 2009). Microbial typing techniques can allow isolates to be grouped on the basis of genotype, potentially enabling the identification of host-associated lineages from possible food chain sources (Sheppard et al., 2009).

It is known that *Campylobacter* populations differ among host species and environmental niches (McCarthy et al., 2007). In the past this was poorly understood in large part because of the lack of uniform and common typing approaches allowing comparison of different studies. Population studies combined with epidemiological analyses of bacteria require the characterization of large numbers of isolates and to achieve this classification should be (i) unambiguous, (ii) reproducible among laboratories, (iii) rapid/scalable (high-throughput), and (iv) cost effective. Nucleotide sequences are the ideal data for detailed population and evolutionary analyses, being both highly discriminatory and reproducible (Maiden, 2006).

Multi-locus sequence typing (MLST) approaches have become increasingly common and have been effectively implemented for research on the population structure and molecular epidemiology of numerous bacterial species (Maiden et al., 1998) including *Campylobacter* (Dingle et al., 2001a,b; Miller et al., 2005). This information can be shared *via* publicly accessible online databases (Jolley et al., 2004a,b) and there is an increasing body of literature in which MLST is applied to *Campylobacter* isolates of human, animal and environmental origin (Clark et al., 2005; Colles et al., 2003; Dingle et al., 2001a,b; Dingle and Maiden, 2005; D'Lima et al., 2007; Fearnhead et al., 2005; French et al., 2005; Karenlampi et al., 2007; Kinana et al., 2006, 2007; Mickan et al., 2007; Miller et al., 2006; Ogden et al., 2007; Salis et al., 2003a,b; Sheppard et al., 2008; Sopwith et al., 2006). It is possible to differentiate between *C. jejuni* and *C. coli* populations from some food animal sources using MLST data (Colles et al., 2008; McCarthy et al., 2007; Miller et al., 2006) and there is, therefore, the potential to improve the understanding of *Campylobacter* ecology and to identify the sources of human infection.

The aim of this study was to implement a high-throughput MLST genotyping protocol to enable the national-scale characterization of *C. jejuni* and *C. coli* genotypes from clinical cases of campylobacteriosis, food animals and environmental sources. The data can then be used to test the extent to which host/source associated genetic lineages exists

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and describe genotypes that are principally found among certain isolate groups. Comparison of this data to clinical isolates provides insight into the potential reservoirs for human infection which will in turn allow the introduction of effective mitigation strategies.

2. Materials and methods

2.1. Microbiology

Clinical stool (5674), animal faeces (2644) and food samples (394) were collected as part of a Food Standard Agency Scotland funded *Campylobacter* MLST project in Scotland (CaMPS). Feed animal and environmental isolates represented a large dataset from a wide range of hosts, geographical areas and time periods. A structured sampling protocol involved the rotation of sampling in a 15-month period (July 05–September 06) between rural (North East and South West) and urban (Aberdeen, Edinburgh and Glasgow) locations. Rural farms were selected randomly and urban sampling centred on areas where animal faeces coincided with human activity (parks) and food sampling of various meat types (retail chicken and livers from ox, sheep and pig) from retail outlets including supermarkets and butchers.

Food and fresh faecal mammalian (25 g) and avian (<5 g) grab samples were collected. Smaller avian samples were collected using a sterile swab moistened with saline. Samples were transported chilled (4 °C) to the laboratory for immediate analysis. Faecal aliquots and swabs were homogenised (1:9) in *Campylobacter* enrichment broth (see below) and 0.1 ml decimal dilutions plated directly onto charcoal cefoperazone deoxycholate (CCD, CM0739, Oxoid, UK) agar for target enumeration. The presence or absence of *Campylobacter* was ascertained by incubating the remaining enrichment broth microaerobically (2% H_2, 5% O_2, 5% CO_2, balance N_2) (100-ml volumes of nutrient broth base (Mast, Bootle, UK) with 5% horse blood, growth supplement (Mast Selectavial SV61), amphotericin (2 μg/ml), cefoperazone (15 μg/ml) and trimethoprim (10 μg/ml)) at 37 °C. After 6–8 h enrichment, two additional antimicrobials (polymixin B (2500 IU/l) and rifampicin (5 μg/ml) were added, and the broths incubated for a further 5 days. All antimicrobials were purchased from Sigma-Aldrich UK. Enrichment broths (0.1 ml) were plated, after 2 and 5 days, on CCD agar (Oxoid, UK) and incubated microaerobically at 37 °C as in previous studies (Humphrey et al., 2005). Colonies were presumptively identified as *Campylobacter* microscopically (Gram stain) and by agglutination with Microscreen latex (Microgen, Camberley, UK) followed by PCR amplification using the pgm primers specific for *C. jejuni* and *C. coli* (Dingle et al., 2001a,b). Individual colonies from direct stool culture (clinical isolates) and enrichments (animal faeces and food isolates) were stored (−80 °C, nutrient broth with glycerol added to 15% (v/v)) for MLST. *Campylobacter* presence was subsequently confirmed by MLST of the remaining 6 loci and ST was used to assign species designation (*C. jejuni* or *C. coli*).

Clinical *Campylobacter* isolates were collected in the 15 health board regions in Scotland (Fig. 1). Isolates were received on charcoal transport swabs from the 28 diagnostic laboratories. As for animal faeces and food samples, clinical isolates from faeces were incubated microaerophilicly and cultured on mCCD. Cultured samples were divided providing cells for DNA extraction and archiving at −80 °C. in 15% glycerol. Sample information, for example source species or health board, were recorded and isolates were given a unique identifier code.

2.2. Multilocus sequence typing (MLST)

Isolates were recultured on CCD agar under microaerobic conditions (2% H_2, 5% O_2, 5% CO_2, balance N_2) for 48 h at 37 °C and DNA was prepared initially as a boilate in phosphate buffered saline or, later in the project, using a CHELEX resin method (BIO-RAD, USA). Template DNA arrays were prepared in 96-well polypropylene plates (Abgene, UK) and seven-locus MLST was carried with published primers (Miller et al., 2005) using a high-throughput protocol. The first phase was carried out using a Roboseq 4204 SE robot (MWG Biotech Inc.) and incorporated a disposable-tip liquid handling step in the transfer of

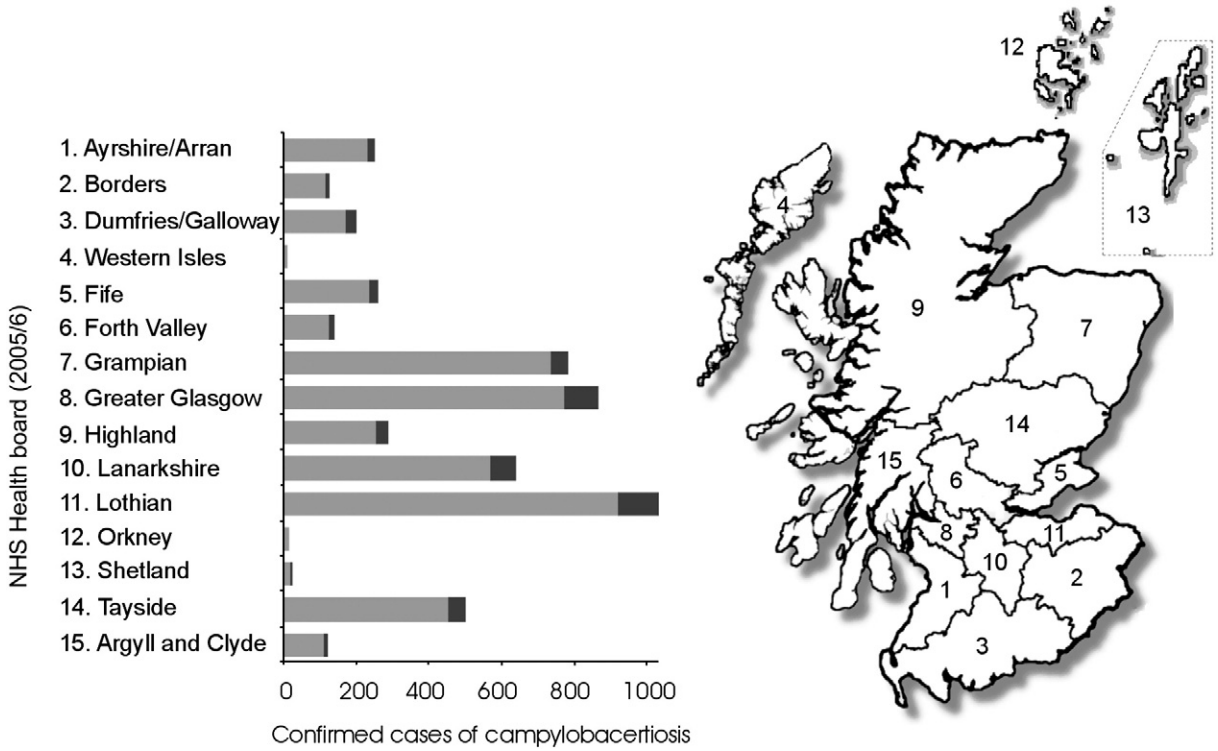

Fig. 1. Confirmed clinical cases of human campylobacteriosis in each NHS health board region in Scotland (2005/6). The Argyll and Clyde region has since been subsumed into the Greater Glasgow health board. Infection caused by *Campylobacter jejuni* and *Campylobacter coli* are indicated by the light and dark grey regions on the graph.