APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2008, p. 2321–2331 Vol. 74, No. 8
0099-2240/08/$08.00+0 doi:10.1128/AEM.02269-07

Development of a Strain-Specific Molecular Method for Quantitating Individual *Campylobacter* Strains in Mixed Populations▽

Karen T. Elvers,[1]* Christopher R. Helps,[1] Trudy M. Wassenaar,[3] Vivien M. Allen,[1] and Diane G. Newell[2]

University of Bristol, Langford, Bristol BS40 5DU, United Kingdom[1]; Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, United Kingdom[2]; and Molecular Microbiology and Genomics Consultants, Tannenstrasse 7, 55576 Zotzenheim, Germany[3]

Received 5 October 2007/Accepted 8 February 2008

The identification of sites resulting in cross-contamination of poultry flocks in the abattoir and determination of the survival and persistence of campylobacters at these sites are essential for the development of intervention strategies aimed at reducing the microbial burden on poultry at retail. A novel molecule-based method, using strain- and genus-specific oligonucleotide probes, was developed to detect and enumerate specific campylobacter strains in mixed populations. Strain-specific oligonucleotide probes were designed for the short variable regions (SVR) of the *flaA* gene in individual *Campylobacter jejuni* strains. A 16S rRNA *Campylobacter* genus-specific probe was also used. Both types of probes were used to investigate populations of campylobacters by colony lift hybridization. The specificity and proof of principle of the method were tested using strains with closely related SVR sequences and mixtures of these strains. Colony lifts of campylobacters were hybridized sequentially with up to two labeled strain-specific probes, followed by the generic 16S rRNA probe. SVR probes were highly specific, differentiating down to 1 nucleotide in the target sequence, and were sufficiently sensitive to detect colonies of a single strain in a mixed population. The 16S rRNA probe detected all *Campylobacter* spp. tested but not closely related species, such as *Arcobacter skirrowi* and *Helicobacter pullorum*. Preliminary field studies demonstrated the application of this technique to target strains isolated from poultry transport crate wash tank water. This method is quantitative, sensitive, and highly specific and allows the identification and enumeration of selected strains among all of the campylobacters in environmental samples.

Campylobacter jejuni is a major reported cause of acute bacterial diarrheal disease in humans in the industrialized world. In England and Wales, the prevalence of human campylobacteriosis peaked in 2000, when 57,674 cases were reported to the Health Protection Agency; since then there has been a decline, and early provisional data for 2006 indicate that there were 46,603 notified cases of this disease (http://www.hpa.org.uk/infections). In the United States, an estimated 2.4 million people are affected each year (27). Although campylobacteriosis is generally self-limiting, the socioeconomic cost of this disease is high in terms of the burden on health resources and in terms of the cost to industry associated with time away from work (39). Thus, control and prevention of campylobacteriosis are essential to reduce this significant public health problem.

C. jejuni can asymptomatically colonize most wild and domestic birds. As highlighted by epidemiological studies, the handling and consumption of raw or undercooked poultry meat are major sources of campylobacteriosis. Once the first birds in a flock become infected, campylobacters spread rapidly. Thus, in some countries, including the United Kingdom, up to 90% of broiler flocks can be campylobacter positive at slaughter (5, 17, 22). The level of cecal colonization in broilers can be up to 10^9 organisms per g of cecal contents. Carcass contamination is related to the within-flock prevalence of campylobacter colonization (1). During processing, defeathering (1) and evisceration (7, 40) may increase the levels of contamination of poultry carcasses and the abattoir environment. Subsequently, carcasses from *Campylobacter*-negative flocks can also become contaminated either when they are placed in soiled transport crates (47) or via cross-contamination when they follow carcasses from a *Campylobacter*-positive flock through the abattoir (20). Determining the survival and persistence of *Campylobacter* at different sites is essential for the development of farm-to-fork strategies for the control and prevention of food-borne campylobacteriosis and, in particular, to inform quantitative risk assessment models. This determination needs to be performed at the strain level, preferably using a rapid and cost-effective method.

Molecular approaches have been used extensively in attempts to understand the epidemiology of campylobacteriosis. *C. jejuni* is both phenotypically and genotypically diverse, and a range of genotypic methods have been developed for this organism, including *fla* restriction fragment length polymorphism typing (2, 10), pulsed-field gel electrophoresis (PFGE) (24, 52), *flaA* short variable region (SVR) sequence typing (29), and multilocus sequence typing (MLST) (15). These methods and others have all revealed the presence of diverse *Campylobacter* genotypes in many environments, such as abattoirs (31) and poultry farms (13, 23, 38). It may be anticipated that the persistence and survival of *C. jejuni* in such environ-

* Corresponding author. Mailing address: Division of Farm Animal Science, School of Clinical Veterinary Science, University of Bristol, Langford, Bristol BS40 5DU, United Kingdom. Phone: 44 (0)1173319131. Fax: 44 (0)1179289324. E-mail: Karen.Elvers@bristol.ac.uk.
▽ Published ahead of print on 15 February 2008.