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In-line milk filter analysis: *Escherichia coli* O157 surveillance of milk production holdings

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Abstract

Escherichia coli O157 is a major etiological agent of food-borne illness. Bovine animals are recognized reservoirs for this organism and represent a significant source from where these pathogens can enter the food chain. Food products derived from these animals are convenient vehicles, and are often the focal point(s) of infection. As a useful strategy to provide herd-level surveillance and to investigate for the presence of this pathogen in a population of Irish dairy cattle, milk filters from 97 farms were analysed by conventional culture and other methods. Five hundred and thirty-six milk filters were evaluated over a 2-year period. Filters from 12 of the 97 farms (12%) were found to contain *E. coli* O157, based on culture methods. Sixteen verocytotoxigenic *E. coli* O157 organisms were recovered and characterized in detail. The farm families in each case were consuming raw milk from their respective herds. The potential risk to public health associated with the detection of *E. coli* O157 and the local consumption of raw milk are discussed.

Keywords: Bacteriology; *E. coli* O157; In-line milk filters; Multiplex-PCR

Introduction

Escherichia coli is a recognized commensal bacterium that colonizes the animal gastrointestinal tract. Within this species are a number of pathogenic groups, most notably the verocytotoxigenic *E. coli* O157 (VTEC), an important human pathogen (Riley et al., 1983). Humans infected with VTEC may develop disease ranging from mild diarrhoea, haemorrhagic colitis (HC), thrombotic thrombocytopenic purpura (TTP) and some may develop haemolytic uremic syndrome (HUS) (Griffin and Tauxe, 1991). In the United States, HUS is the major cause of acute renal failure in children (Doyle et al., 1997). Increasing numbers of human infections and outbreaks associated with VTEC-O157 have been reported in England and Wales and the United States (Willshaw et al., 2001; Tarr et al., 1997; Renter et al., 2003). These outbreaks are often linked with the consumption of contaminated food, including meat, milk and their derived products, along with direct or

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indirect contact with ruminant animals (Pritchard et al., 2000; Willshaw et al., 2001). Epidemiological data and studies of foods associated with human illness suggests that the infective dose of VTEC-O157 can be very low (typically 10–100 cells), together with an incubation period in the range between 1 and 10 days, with a median of 3–4 days (Griffin, 1998). VTEC has a significant impact on public health, in Ireland, and is currently non-notifiable when detected in animals, indicating the heightened requirement for real-time surveillance to identify clusters, control transmission and safeguard public health.

Bovine animals are a known reservoir for *E. coli* O157 (Paiba et al., 2003; Griffin and Tauxe, 1991; Wells et al., 1991) and the gastrointestinal tract appears to be transiently colonized with these organisms (Besser et al., 1997). A pattern of prolonged periods of low prevalence shedding interspersed by short periods of high prevalence shedding has recently been reported in an Irish study (Minihan et al., 2003). In addition, Minihan et al. (2003) also showed that VTEC-O157 could be recovered from environmental samples. The impact of these observations on food safety and the attendant risk to public health are obvious. Good hygienic farming practices will contribute to a reduction of this pathogen in bovine herds and their environments (Shere et al., 1998). Data relating to VTEC-O157 in raw milk is limited and further investigation has been recommended (Heuvelink et al., 1998). Raw milk is a source for the transmission of this organism to immuno-naïve individuals (Haack et al., 2003). To determine the prevalence of this pathogen in lactating animals and their environment in real-time requires careful cohort surveillance.

In-line milk filters are an integral part of modern milking systems, trapping debris, large particles of organic material and foreign objects preventing entry to the bulk milk tank and ensuring that the milk is therefore free of any visible dirt. Residues retained in the milk filter are regarded as 'real-time' indicators of the faecal shedding from a cohort of animals and their environment. Assuming satisfactory teat hygiene and preparation is performed prior to milking, along with good farming practices, each milk filter has the capacity to filter milk from approximately 150 cows (Mac Millan, 2002). Microbiological examination of these milk filters may provide a useful means of detecting VTEC-O157 in milk at farm level from large numbers of cohort animals. Milk filter residue examination is an indication of the status of the milk production holding.

In-line milk filters are a simple, cost-effective and convenient tool to screen a cohort of animals for VTEC and other zoonotic pathogens. The objective of this study was to determine base-line values for the prevalence of *E. coli* O157 and associated virulence markers in raw milk from a sub-set of milk producing commercial dairy herds in the southern region of Ireland. DNA fingerprint profiles were determined to facilitate the development of a database for epidemiological comparison.

Materials and methods

Farm selection

The southern region of Ireland is a dairy intensive area with 25% of the national dairy output and 29% of the gross agricultural output. Ninety-seven dairy farms in the greater County of Cork were randomly recruited to the study. These farms were selected from a liquid milk holdings database maintained at the Veterinary Directorate-Cork County Council. This database was developed for the purposes of implementing the statutory requirements of Statutory Instrument (SI) No. 9 of 1996 and contained all of the necessary data relevant to structural standards, animal health, milk quality and registration status in each holding. Participation in this study was on a voluntary basis. Each farmer was fully briefed on the objectives of the study by the researcher accompanied by a veterinary inspector. In addition, each farmer was asked to complete a detailed questionnaire. Hygiene protocols to be implemented for the collection and dispatching of the milk filter samples to the laboratory at the Veterinary Directorate were demonstrated. This study was conducted over a 2-year period beginning in September 2001.

The participating herds were surveyed at 4 monthly cycles over a 2-year period from 2001 to 2003. Year 1 comprised of 3 cycles: A–C, (A = September–December 2001; B = January–April 2002 and C = May–August 2002). Year 2 comprised of cycles D–F (D = September–December 2002; E = January–April 2003 and F = May–August 2003). In-line milk filters were collected by arrangement from all 97 farms and represented each lactating animal at that time. Prior to sampling farm personnel involved in this study were given appropriate instruction on good hygiene practices.

In-line milk filters

Milk filters used were spun from non-woven cotton. The sock-like filters had a pore size in the range of 60–300 μm (5.2 cm wide and of varying lengths typically 23.2, 36.4, or 56 cm).

E. coli O157 culture methodology

Detection of *E. coli* O157 in milk filter samples was performed according to the Food and Drug Administration Bacteriological Methods with minor modification