GENETIC DIVERSITY OF *ESCHERICHIA COLI* IN BEEF CATTLE FAECES FROM PASTURE TO FEEDLOT

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Background

There is speculation that intestinal carriage of pathogens and the degree of shedding by cattle has some correlation with *Escherichia coli* O157:H7 contamination of dressed carcasses (Elder *et al.* 2000). Thus, genotypic typing methods have been adopted to evaluate the effects of diet on *E. coli* O157:H7 shedding (Buchko *et al.* 2000) and to investigate the transmission of this organism from cattle to humans (Louie *et al.* 1999). Conventional typing based on phenotypic characteristics is of limited use for identifying genetic diversity in *E. coli* populations, as all *E. coli* isolates cannot be distinguished by such methods (Caugant *et al.* 1985). However, isolates may be distinguished by molecular techniques which include pulse-field gel electrophoresis (PFGE), randomly amplified polymorphic DNA analysis (RAPD), ribotyping and restriction fragment length polymorphism (Jarvis *et al.* 2000; Pacheco *et al.* 1996; Picard *et al.* 1991; Vogel *et al.* 2000). Pulsed-field gel electrophoresis has been used to establish the extensive diversity of *E. coli* strains recovered from the colons of dairy cattle (Jarvis *et al.* 2000).

The current study was designed to develop a molecular method to characterize *E. coli* strains associated with beef cattle faeces during pasture grazing and after transfer to a feedlot.

Objective

The objectives were to develop a RAPD typing method to determine the diversity of *E. coli* strains in beef cattle and to examine the impact of pasture grazing and feedlot finishing on diversity.

Methods

Hereford x Angus cross cattle (15 steers, 15 heifers) were put on pasture when weaned at ten months of age. After five months the cattle were transferred to a feedlot and fed a barley silage/grain ration. At monthly intervals, faecal samples were extracted from the rectum of each animal, homogenized in 0.1% peptone water and incubated on SD 39 agar after hydrophobic grid membrane filtration (Entis and Lesner, 1997). Twenty-four presumptive *E. coli* colonies were randomly selected from each sample at each sampling time for molecular typing.

RAPD analysis of the *E. coli* isolates was performed as described by Pacheco *et al.* (1996). The primer used in RAPD analysis has the sequence "CCGCAGCCAA". Template DNA was prepared from *E. coli* isolates by suspending one colony in 50 µl sterile water and boiling for 15 min, then centrifuging at 4000 rpm for 2 min to pellet cellular debris. A 25 µl PCR mixture consisted of 5 µl DNA, 2.5 mM $MgCl_2$, 8 pmole primer, 300 µM of each dNTP's and 1U *Taq* DNA polymerase (Sigma-Aldrich Canada Ltd. Oakville, ON). Amplification was performed as follows: 1 cycle of 5 min each at 94°C, 36°C and 72°C, followed by 30 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min. Amplified DNA fragments were separated on 1.8% agarose gels and bands of DNA were recorded digitally using a Kodak EDAS290 system (Eastman-Kodak, Rochester, NY).

The RAPD patterns of *E. coli* isolates from five animals at the three periods of cattle placed on pasture (Time 1); cattle after 6 months on pasture (Time 2), and cattle after 1 month in a feedlot (Time 3) were compared using Dice similarity coefficient. Subsequently a dendrogram was constructed using a UPGMA method on Molecular Analyst Fingerprinting Software, version 1.61 (Bio-Rad Laboratories, Hercules, CA).

Results and Discussion

The similarity of RAPD patterns from *E. coli* isolates taken from each individual animal over the three time periods was compared on a single dendrogram (data not shown). Within an individual animal, and within each time period, the majority of *E. coli* isolates shared close genetic relatedness (Dice similarity coefficient >80%), whereas across the time periods and between animals the *E. coli* populations were clearly distinct. Similar findings were reported in an earlier study which suggested that individual cattle harbor genetically diverse *E. coli* populations that are unique to the individual animal (Jarvis *et al.* 2000).

Figures 1 and 2 illustrate the trend of changes in *E. coli* populations over time by presenting the RAPD analysis from one of the animals sampled. Figure 1 provides the RAPD patterns of 24 *E. coli* isolates from an individual animal at three different sampling periods. Figure 2 is a dendrogram constructed from the four predominant *E. coli* RAPD patterns taken from times 1, 2, and 3 noted in Figure 1. These four RAPD patterns represent the predominant, distinct (Dice similarity coefficient <80%) patterns observed at the three time periods. The dendrogram suggests that there is a shift in the *E. coli* population over time, and that a predominant strain is present at each time. Similar trends were observed in RAPD analysis from the *E. coli* populations in the other four beef cattle surveyed (data not shown).

Diets containing barley may influence faecal shedding of *E. coli* by cattle (Buchko *et al.* 2000). In the present study, cattle were grazed on pasture then changed to a barley silage/grain ration in the feedlot. From our RAPD analysis we observed that the *E. coli* population within an animal is in flux during grazing on pasture and in feedlot. Therefore, preliminary results could not correlate changes in *E. coli* populations with a change in the feeding pattern.

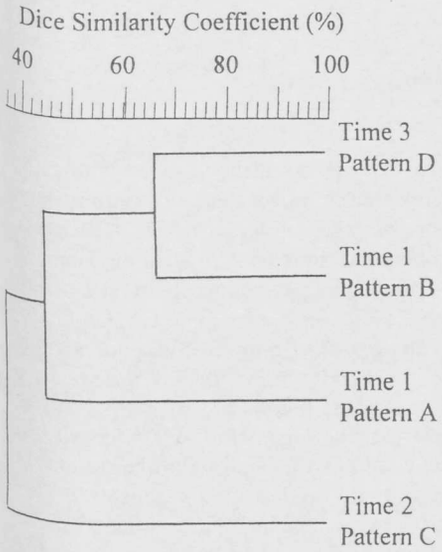

Figure 2. Dendrogram depicting genetic diversity of *E. coli* isolates from faecal samples of beef cattle. Pattern A-D are the predominant RAPD profiles seen in figure 1. Time 1, cattle placed on pasture. Time 2 cattle 6 months on pasture. Time 3, cattle 1 month on feedlot.

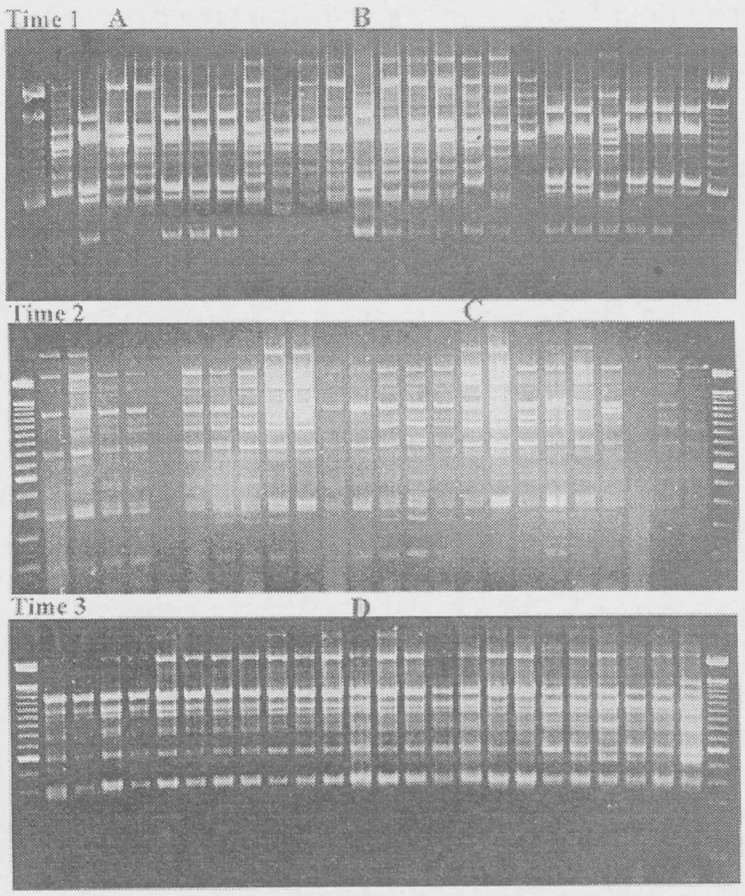

Figure 1. Agarose gels showing RAPD patterns of 24 *E. coli* isolates from faecal samples of a single animal. Time 1, cattle placed on pasture. Time 2, cattle 6 months on pasture. Time 3, cattle 1 month in feedlot. Pattern A-D are RAPD profiles used to construct dendrogram in figure 2.

Conclusions

RAPD analysis provides sufficient discriminatory power for characterizing diversity in an *E. coli* population. RAPD analysis indicated that the *E. coli* population changes over time in beef cattle, and generally only a few, predominant RAPD patterns were present in the isolates analyzed at each time period. Once the cattle go to slaughter, the methodology developed in this study will be used to determine origins of *E. coli* contamination of carcasses in the abattoir.

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