

Evaluating methods for the molecular epidemiology of *E. coli* O157:H7

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The epidemiology of *Escherichia coli* O157:H7 (O157) among cattle herds suggests a point source for periodic introduction of new strains into herds. An assessment of the relationship between geographic and genetic distance between isolates will provide insight into the means of transmission of O157 into cattle herds.

Pulsed-field gel electrophoresis (PFGE) following digestion with *Xba*I is widely used to subtype O157. However, PFGE with a single enzyme has not been critically evaluated for establishing relatedness. We reasoned that, if PFGE with one enzyme is a good measure of relatedness, genetic similarity measures generated from two enzymes should be highly correlated.

Sixty-two epidemiologically unrelated bovine isolates of O157 were selected. After digestion with either *Xba*I or *Bln*I, PFGE was performed according to the PulseNet protocol. Cluster analysis of the Dice similarity indices based on the unweighted pair group method using arithmetic averages (UPGMA) was done in Bionumerics (Applied Maths). Dendrograms from both enzymes were compared, and the correlation between similarity matrices from both enzymes was calculated in Microsoft Excel.

Pearson's product moment correlation (r) between the two similarity matrices was 0.513. This moderate positive correlation suggests that PFGE based on a single enzyme provides a crude measure of true genetic similarity. A likely reason for the relatively poor correlation is that, for each enzyme, some matching bands do not contain homologous genetic material. Results of comparison of matching bands using Southern

blotting of PFGE gels indicate that the occurrence of “matching” bands that contain non-homologous DNA is common between isolates of *E. coli* O157:H7.

To further assess the correlation between similarity indices generated by two or three restriction enzymes, a subset of 14 isolates was used for PFGE following separate digestions with *NheI*, *PacI*, *SfiI* and *SpeI*. Average correlations between unique triplets were greater than those between unique pairs of enzymes. An extrapolation of the data indicated that average similarities generated by six separate digests would provide a correlation of .8.

Further work will evaluate alternative methods by comparison with PFGE, and the method or methods determined to be the most accurate will be used for geographic/genetic analysis.