# RESEARCH PROJECT SUMMARY OUTLINE - FINAL REPORT
## (23rd August 2006)

**I.       Principal Investigator(s):** Rowland Cobbold PhD.

**II.      Institution:** College of Veterinary Medicine, Washington State University.

**III.     Project Title:** The role of super-shedders in determining feedlot pen prevalence of *E. coli* O157:H7.

## IV.    Stated Objectives:

1.  Demonstrate that removal of a super-shedder from a feedlot pen results in a reduction in the prevalence, mean count and duration of excretion of *E. coli* O157:H7 by other cattle in that pen.
2.  Demonstrate that addition of a super-shedder to a feedlot pen results in an increase in the prevalence, mean count and duration of excretion of *E. coli* O157:H7 by other cattle in that pen.
3.  Demonstrate transmission of *E. coli* O157:H7 from super-shedder cattle to in-contact cattle (i.e. pen-mates) using genetic similarity profiling.
4.  Demonstrate an association between presence of a super-shedder in a pen and the prevalence and identity of *E. coli* O157:H7 in the pen environment.

## V.     Background information about the need for this research.

Due to its significance to public health, it potential impact to beef trade and production security, as well as negative public perception issues, *E. coli* O157:H7 is a critical problem for the beef industry. Pathogen reduction through a series of interventions at various stages of the beef production process is currently considered the most effective means to reduce the risk of final product contamination. Control of *E. coli* O157:H7 at the pre-slaughter/harvest level is an important component in such a risk reduction strategy. Interventions targeted specifically towards integral aspects of *E. coli* O157:H7 epidemiology in cattle are more likely to be effective than empirically derived interventions whose mechanisms are unclear or which may have other ramifications to production, animal health or other food safety concerns. Although many aspects of the epidemiology of *E. coli* O157:H7 in livestock populations have been elucidated, critical gaps in our knowledge remain. Key among these (as outlined within the executive summary of the Beef Industry *E. coli* Summit Meeting, Jan 7-8) were reasons for the large degree of individual and group-level variation in *E. coli* O157:H7 excretion, and why existing pre-slaughter interventions such as pen cleaning, water tank washing, cattle segregation and diet changes have lead to inconsistent reductions in carriage.

NCBA-funded research completed immediately prior to the current research project examined the phenomenon of recto-anal junction (RAJ) colonization of cattle by *E. coli* O157:H7. The initial study concluded that the RAJ does indeed appear to be a significant

site for *E. coli* O157:H7 colonization, and that this colonization is associated with fecal excretion patterns. Tied to the phenomenon of RAJ colonization is the concept of *E. coli* O157:H7 "super-shedders". These are individual cattle that are thought to excrete *E. coli* O157:H7 more frequently, more persistently and in greater numbers than most cattle. The presence of super-shedders in pens or herds is likely to have profound effects on the shedding parameters of other cattle in the herd/pen, as well as the degree of environmental contamination. The initial research project confirmed that a small number of cattle do appear to act as super-shedders, based on high RAJ colonization and fecal *E. coli* O157:H7 prevalences, counts and persistence. Furthermore, the presence of a super-shedder within a pen was associated with higher levels of *E. coli* O157:H7 excretion in cohorted cattle within that pen. Similarly, cattle that were consistently low or non-shedders of *E. coli* O157:H7 were associated with a lack of exposure to super-shedders.

Although the initial project supported the concept of super-shedders as an important factor in determining pen-level *E. coli* O157:H7 presence and linked this to RAJ colonization, it could not confirm that the super-shedders were the source of *E. coli* O157:H7 for co-penned cattle. That is, were the super-shedders the cause of high-level excretion of *E. coli* O157:H7 by other cattle in their pen, or the result of it? Furthermore, is pen-level environmental contamination with *E. coli* O157:H7 (which represents an important exposure source to cattle, as well as potentially to humans) linked similarly to the presence of a super-shedder? The current study was undertaken to explore these questions, as well as to further establish the role of super-shedders and associated RAJ colonization with pre-slaughter bovine transmission dynamics.

## VI.    Achievement of the specific objectives stated in your proposal.

Procedures and analyses have been performed necessary to address the stated objectives. Outcomes relating to each objective are briefly stated here. Further details of results and their implications in relation to the objectives are provided in section VIII below.

1. Removal of super-shedders from feedlot pens resulted in reduced prevalences, mean counts and durations of excretion of *E. coli* O157:H7 by other cattle in those pens. However, these reductions were not significant compared to control pens, which also demonstrated reductions in these parameters.
2. Addition of super-shedders to feedlot pens resulted in increases in the prevalences, mean counts and durations of excretion of *E. coli* O157:H7 by other cattle in those pens. This increase was significant for cattle prevalences when compared to control pens.
3. PFGE comparisons support the hypothesis that super-shedders are the principal source *E. coli* O157:H7 strains for other cattle in their pens, both prior to and after reassignment.
4. Associations were evident between the presence of super-shedders and the prevalence and identity of *E. coli* O157:H7 in the pen environment.

## VII.   Materials and Methods

Experimental design was that of a prospective longitudinal observational study, where natural levels of *E. coli* O157:H7 RAJ colonization among cattle in experimental feedlot pens were observed, and subsequent changes in colonization levels with introduction or

removal of a super-shedder determined. Changes in *E. coli* O157:H7 contamination levels for relevant environmental niches were similarly examined. Experimental cattle were housed and sampled at Lakeside Feeders' Research facility, Brooks, AB. Cattle were managed in a manner typical to commercial feedlot practice. They were randomly assigned to pens, with a total of eight head per pen for 20 pens. Throughout the course of the sampling, a total of three cattle were culled from the experimental program due to physical or behavioral problems in repeat handling. Pens were physically separated from each other by using alternate pens and by not sharing water or feed sources.

Sampling was conducted in two phases. The initial sampling phase was conducted over seven weeks, and was designed to identify super-shedders among experimental cattle. Cattle were individually sampled twice per week. Recto-anal samples were collected by vigorous mucosal surface swabbing of the rectal area 5-10 cm forward of the anus using sponge-tipped swabs, avoiding periods of defecation. Environmental samples were collected from each pen at each sampling day and comprised trough water, feeds (total mixed ration from feed bunks), and pen floor soil (avoiding obvious manure pats). All samples were transported immediately to the WSU laboratories. Although some sample delivery problems were encountered (logistics and customs problems with the freight company; Hurricane Katrina delays in freight handling – as described in the interim report), the vast majority of samples were received in a suitable condition. Some additional sampling days were added to the first and second phase of sampling to overcome potential problems associated with lost data. RAJ swabs were appropriately diluted and directly plated onto sorbitol MacConkey agar plates containing cefixime and potassium tellurite and *E. coli* O157:H7 counts were performed following incubation. Suspect colonies were confirmed as toxigenic *E. coli* O157:H7 for final count derivation, and prior to banking for later characterization. Feed and soil samples were enriched as 1:10 concentrations, and water as 1:1 concentrations in tryptic soy broth. *E. coli* O157:H7 were isolated from environmental sample enrichments using an automated immunomagnetic separation (IMS) technique, and similarly confirmed.

Super-shedders were nominated based on a combination of criteria determined during the first sampling phase. These criteria were: high sample prevalence; highe mean RAJ concentration; persistent colonization (as determined by consecutively positive RAJ samples); and presence within a high-*E. coli* O157:H7 level pen. High-level *E. coli* O157:H7 pens (generically referred to as "hot pens" within this study) were defined on the basis of a combination of mean pen prevalence, mean pen RAJ sample concentration, and the proportion of consecutively positive cattle within that pen. Five super-shedders were clearly identifiable on this basis. Similarly, based on Phase I sampling data, five non-shedders were nominated based on a total absence of RAJ colonization and presence within a low-level *E. coli* O157:H7 pens ("cold pens"). Following these classifications being made, super-shedders and non-shedders were switched, i.e. the five nominated super-shedders were reassigned to each of the five nominated cold pens, and the five non-shedders were reassigned to each of the nominated hot pens. Following a period of one week adjustment, Phase II of sampling was commenced. This was conducted for six weeks (12 sampling dates), using the same sampling plan and lab methods as described for Phase I. Of the remaining 10 pens that were neither nominated as hot or cold pens,

five were randomly chosen to represent control pens. Cattle within these pens were sampled using the same methods as per hot and cold pens, although at half the frequency, i.e. sampled once per week.

Data was analyzed using feedlot pens as the unit of study, not individual cattle, as a pen effect was evident. *E. coli* O157:H7 colonization parameter (prevalence, count, consecutive positives) differences from before to after pen reassignment were determined for cold, control and hot pens. These were evaluated for variance using Kruskal-Wallis tests. Pairwise comparisons of parameter differences were made using Wilcoxon rank sum tests. Data and statistical analyses were performed using SAS Version 8.05. A subset of isolates were subjected to pulsed field gel electrophoresis (PFGE) using standard methods in order to genotypically compare strains. The primary aims of analyses were to:
- Determine if the super-shedders were the principal source of pen-mates' RAJ and environmental strains in hot pens, both before and after super-shedder reassignment.
- Determine if super-shedders are the likely sources of pen-mates' RAJ and environmental sample strains in cold pens following their introduction into cold pens.
- Determine if non-shedders that become colonized with *E. coli* O157:H7 following reassignment are positive for the resident hot pen strains (presumably super-shedder strains – as tested above).
- Compare water to feed to soil strains: determine if all derive from a common source, presumably the super-shedder (as determined above), determine other potential transmission routes and roles as reservoirs of *E. coli* O157:H7.
- Use control pen isolate trends for comparative purposes to hot and cold pen analyses.

## VIIl. Summary of Results and Discussion.
### General Results
Patterns of *E. coli* O157:H7 colonization of the RAJ over the entire sampling period were similar to those determined in the previous NCBA-funded study on RAJ colonization (Figure 1). The prevalence curve for the current data set peaked in late summer-early fall, as has been noted in fecal prevalence studies by other researchers. The mean RAJ prevalence for the study was 11%, with a maximum of 25%. Considering the methods used were not the most sensitive available (e.g. no enrichment or IMS), this general prevalence was high, and satisfactory for the purposes of addressing the project objectives. Mean pen counts over the sampling period were also reasonably high (mean of 3.34 $\log_{10}$), and declined by around 1 $\log_{10}$ over the feeding period. This also matched previously recorded patterns of RAJ and fecal counts from the initial NCBA RAJ project.
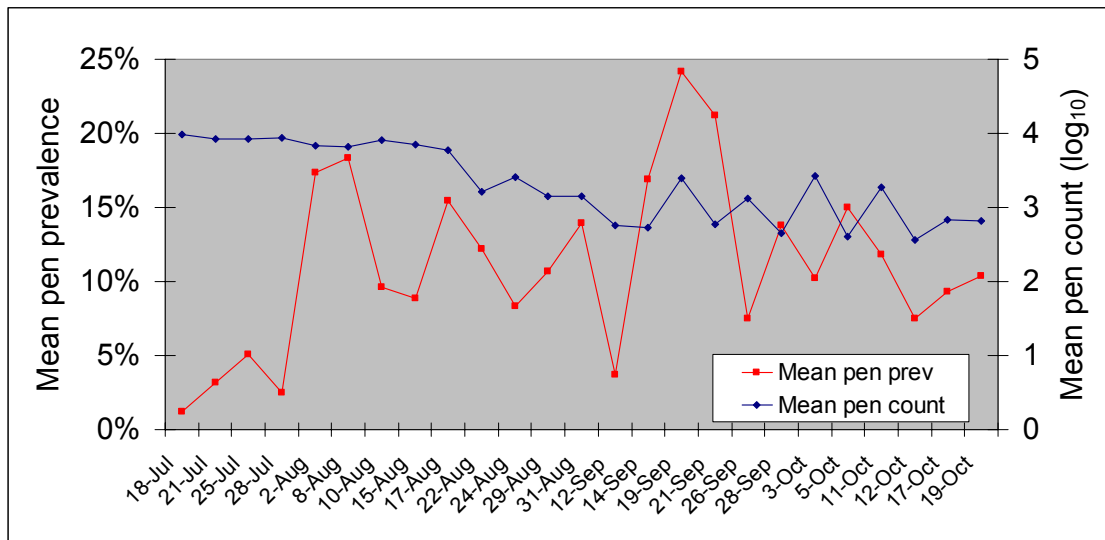
**Figure 1. Mean pen *E. coli* O157:H7 RAJ prevalence (red) and count (blue) over the entire sampling period.**

Large degrees of pen to pen variation were apparent with respect to mean RAJ prevalence and count (Figure 2). Again, this is in accordance with previous findings, and was a necessary prerequisite for the objectives to be met in the current study.

Five cattle (tag numbers 17, 26, 45, 63 and 119) were nominated as super-shedders based on: demonstrating a high RAJ prevalence (being positive ≥3 times); high mean RAJ count (>3 $log_{10}$); persistent shedding (≥3 consecutive positive samples); and location within a hot pen. Hot pens were nominated based on means of pen RAJ prevalence (>12%), count (>3 $log_{10}$) and number of consecutive positives (≥2). Non-shedders (tag numbers 21, 111, 74, 32 and 107) were identified as cattle whose RAJ samples were completely negative over Phase I of sampling and were located in low-level *E. coli* O157:H7 (cold) pens. Three cold pens were nominated on the basis of a complete lack of *E. coli* O157:H7 positive samples. The remaining two were identified based on the lowest combinations of mean pen RAJ prevalence, count and consecutive positives. Non-shedders were switched with each super-shedder as above, respectively.
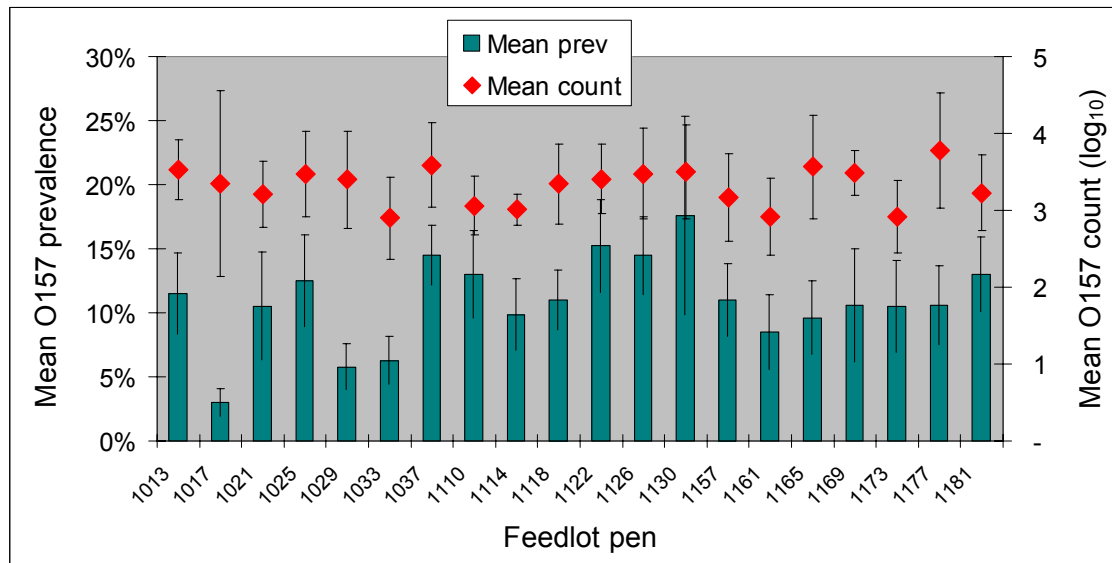
**Figure 2. Mean RAJ prevalence and count for each feedlot pen. Error bars represent standard errors of the mean.**

## Cattle Colonization Changes Associated with Super-shedder Reassignment

Differences in RAJ *E. coli* O157:H7 parameters for cattle (excluding super-shedder and non-shedder data) from before to after reassignment were compared between hot, cold and control pens – these are summarized in Figure 3. Cold pen prevalences increased significantly with the introduction of super-shedders, as compared to control pens. Hot pen prevalences decreased after super-shedders were removed, although this decrease was only significant compared to cold pens, not control pens. Changes in mean RAJ *E. coli* O157:H7 count with reassignment of super-shedders followed a similar trend (Figure 3). Cold pen mean RAJ concentrations increased, though this change was only significant compared with hot pens. The change compared to control pens was close to significance ($P = 0.06$). Hot pen counts decreased, but not significantly compared to control pens, which also decreased. A similar pattern of changes was noted for the number of consecutive positive *E. coli* O157:H7 RAJ samples (Figure 3). This parameter infers persistence of *E. coli* O157:H7 colonization. The persistence of colonization for cattle in cold pens increased significantly with the introduction of super-shedders. Hot pen persistent colonization decreased, but only significantly compared to cold pens.

These findings indicate that addition of a super-shedder to pens naïve to *E. coli* O157:H7 or with relatively low levels of *E. coli* O157:H7 increases the prevalence, count and persistence of colonization of cattle within those pens. Therefore, recognition and control of super-shedders has the potential to reduce the number of pens positive for *E. coli* O157:H7, and the levels of *E. coli* O157:H7 excretion within those pens.
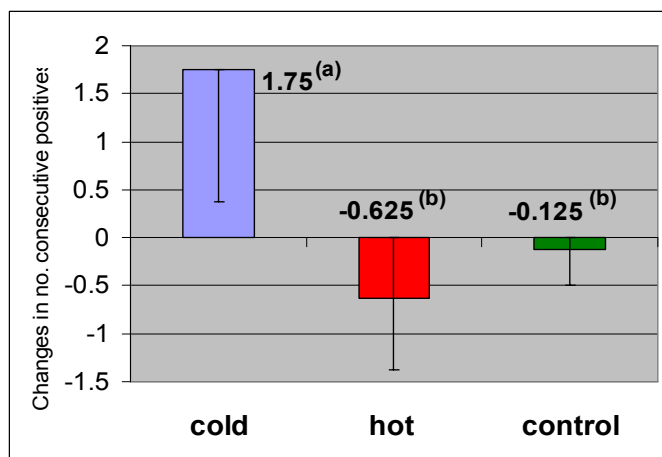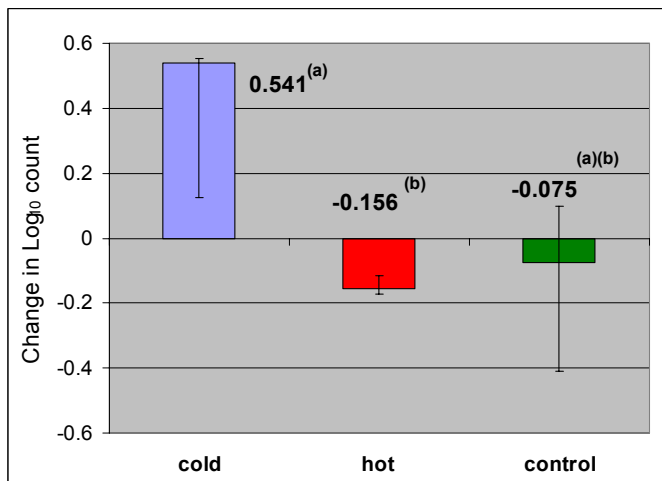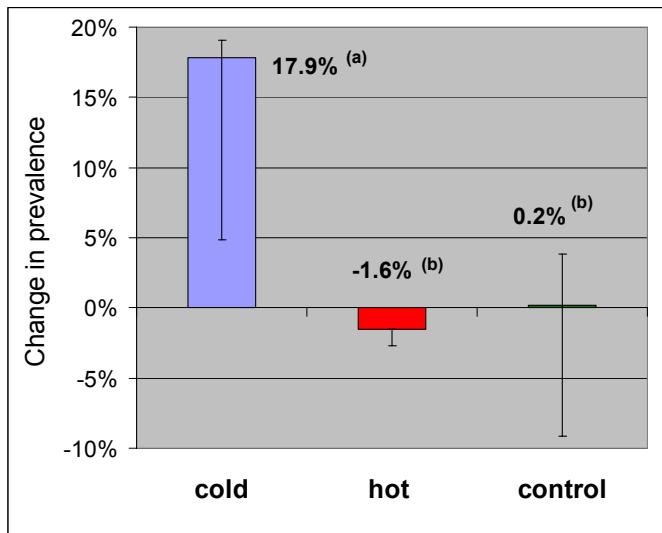
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**Figure 3. Changes in RAJ *E. coli* O157:H7 prevalence, count (log$_{10}$) and number of consecutive positives (persistence) in cold, hot and control pens after reassignment. Columns represent medians. Error bars are 25$^{th}$ and 75$^{th}$ percentiles. Letters in parentheses represent pair-wise statistical comparisons – different letters indicate significant differences ($P \leq 0.05$).**

## Changes in Colonization of Super-shedders and Non-shedders after Reassignment

The colonization parameters of super-shedders and non-shedders were also examined following reassignment of each to respective pens (Figure 4). Non-shedders' prevalence and counts increased significantly (compared to control cattle) when introduced into the hot pens. This is likely to be due to exposure of non-shedders to residual strains in hot pens excreted by the remaining cattle and within the pen environment. Super-shedders' prevalence and count declined after placement into the cold pens, though this was a non-significant change compared to controls. Although a super-shedder is likely to be the predominant shedder of *E. coli* O157:H7 in any given pen, it seems evident from this data that the super-shedders' degree of colonization and excretion also relies on its continued exposure to high levels of *E. coli* O157:H7 – hence the moderate decrease in RAJ prevalence and count with transfer to cold pens.
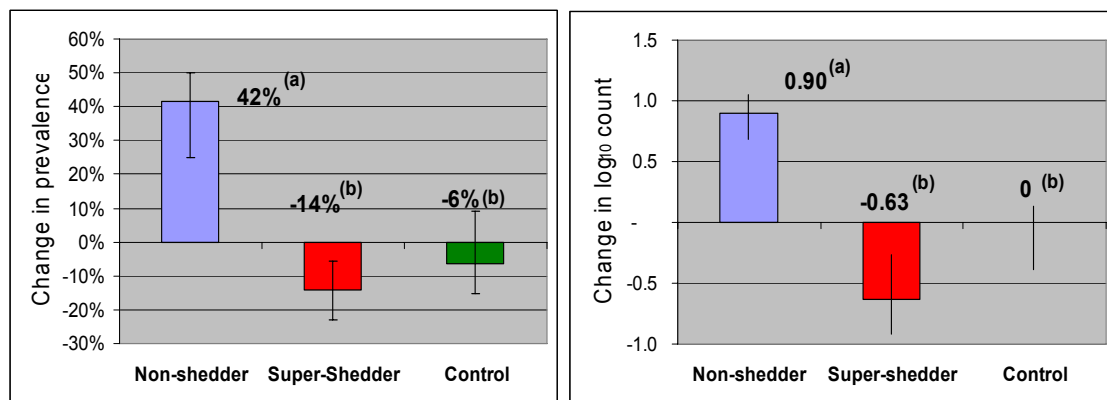


**Figure 4. Changes in RAJ *E. coli* O157:H7 prevalence and count for super-shedders, non-shedders and control cattle after reassignment. Columns represent medians. Error bars are 25th and 75th percentiles. Letters in parentheses represent pair-wise statistical comparisons – different letters indicate significant differences ($P \leq 0.05$).**

## Environmental Contamination with *E. coli* O157:H7

Substantial *E. coli* O157:H7 prevalences for environmental samples were noted throughout the sampling period (Figure 5). Peaks of environmental prevalence were evident, with different types (soil, water, feed) of sample frequently peaking together. This likely relates to common and varying sources of contamination, i.e. cattle (most likely super-shedders). Soil sample prevalences were generally the highest. This probably simply reflects the much greater fecal load in feedlot pen soil samples (where often the "soil" is simply dried manure). Although the feed and water samples were not as frequently positive, they represent important sources of transmission and maintenance of *E. coli* O157:H7 due to the larger volumes of these samples that cattle ingest.
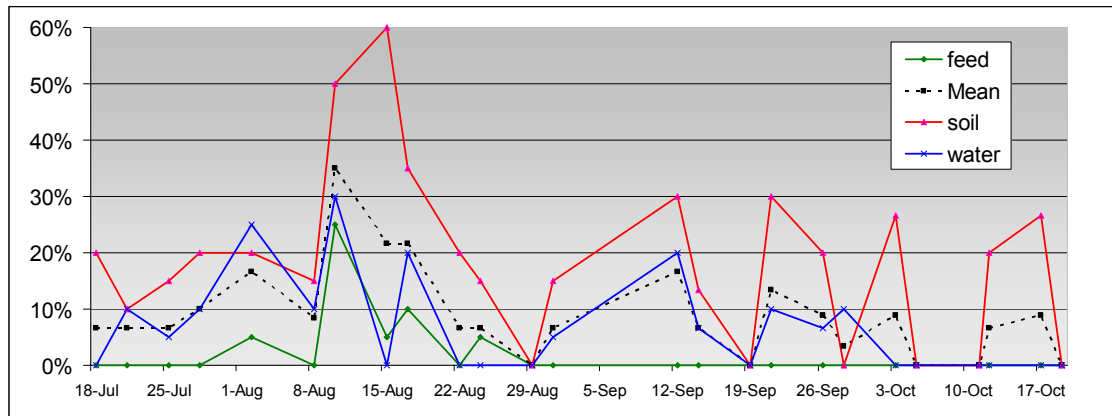
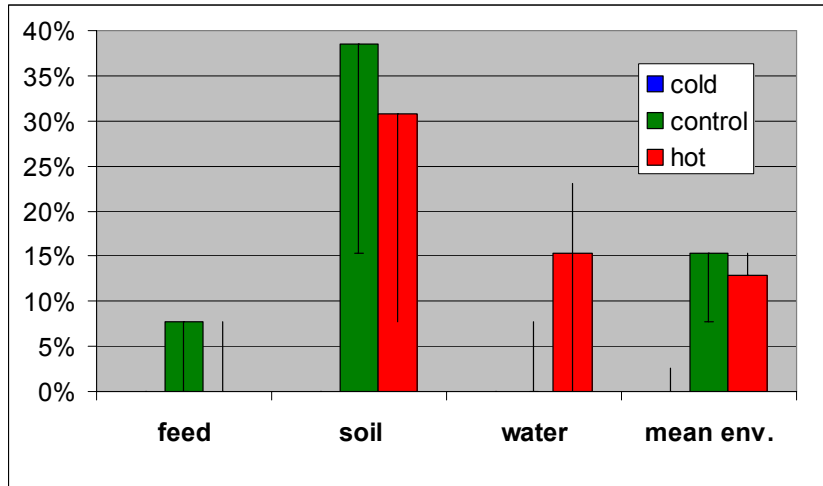**Figure 5. Mean pen environmental sample prevalences for *E. coli* O157:H7 over the sampling period.**

Moderate correlations were evident between mean RAJ prevalences and counts and environmental sample prevalences in pre-reassignment pens (Figure 6). This suggests that RAJ and environmental contamination levels are linked, presumably representing fecal excretion contaminating environmental niches within pens.
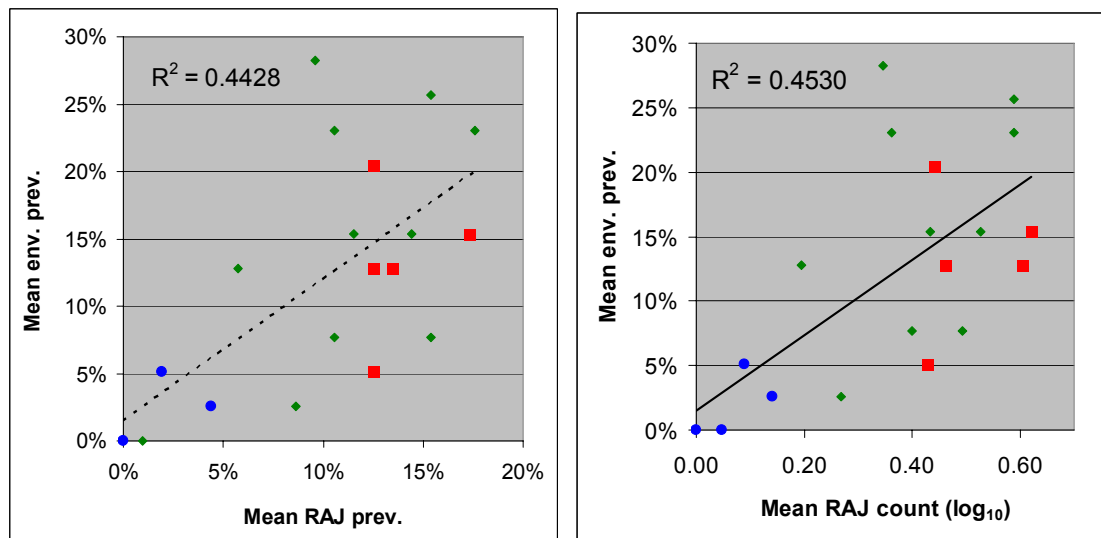


**Figure 6. Scatterplots comparing mean pen environmental prevalence with mean pen RAJ prevalence (left chart) and mean RAJ count (right chart). All data are from Phase I of sampling only (i.e. prior to reassignment). Red squares and blue circles represent data points for hot and cold pens respectively.**

Prior to reassignment, prevalences of *E. coli* O157:H7 in environmental samples were considerably lower in the cold pens than in either hot or control pens (Figure 7). This is likely to simply reflect the respective RAJ prevalences and counts in each of the pen types, i.e. lower levels of contamination from bovine fecess. This is confirmed by the

finding that pen type (cold, hot or control) had no significant association with environmental prevalence when RAJ prevalence and RAJ count were disregarded from modeling analysis (GLM with environmental prevalence as the dependent variable).



**Figure 7.** *E. coli* **O157:H7 prevalences for environmental samples prior to reassignment of super-shedders. Columns represent medians. Error bars are 25ᵗʰ and 75ᵗʰ percentiles.**

Changes in environmental sample prevalence were evident with reassignment of super-shedders and non-shedders (Figure 8). Mean environmental sample (pooled data for soil, water and feed samples) prevalence in cold pens increased by 3% with addition of super-shedders. Hot pen mean environmental sample prevalence decreased by 7.3% after removal of the super-shedders. Although neither of these changes were significant as compared to control pen prevalence changes (decrease of 4.3%), they were significantly different to each other.
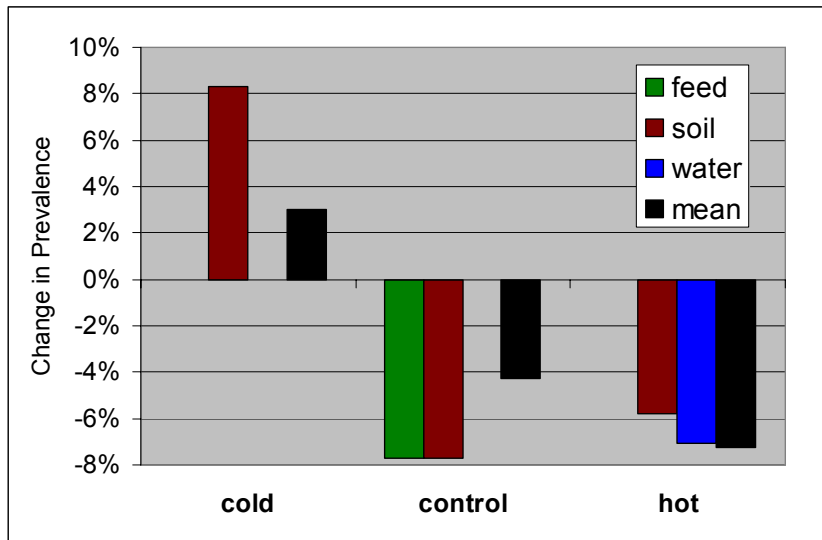
**Figure 8. Changes in *E. coli* O157:H7 prevalence for environmental samples following super-shedder reassignment. Columns represent median changes in the percent of samples positive. Mean column represents pooled prevalence data for each sample type (soil, feed, water).**

## PFGE Comparisons of *E. coli* O157:H7 Isolates

A total of 169 isolates were subjected to PFGE analysis to compare genetic identity between strains, as required for Objective 3. A total of 60 individual PFGE restriction types (RT) were identified (Figure 9). Some RTs were over-represented (e.g. RTs 13 and 23), with >50% of isolates represented by only seven RTs. This is compatible with other studies, which found *E. coli* O157:H7 PFGE types to be relatively conserved compared to other bacteria, resulting in closely related RT's among isolates. However, strain diversity was evident, with a number of less common RTs being represented by only a single strain. Some of this is due to anomalies in with the bioinformatics program, whereby visually similar patterns can be designated as different by the software algorithm. Problems also arise with poor banding of isolates (due to imperfect enzymatic restriction or electrophoresis) or where isolates run on different gels are compared (imperfect normalization between gels). Tolerance and optimization parameters were both set to relatively stringent levels (1%) for this analysis, which also results in enhanced discrimination of PFGE patterns. To overcome these commonly encountered limitations, comparative analyses were based on RTs having ≤1 band difference between them being considered highly related. Further analyses of isolates can be performed at an improved level of discrimination by combining them on the same gel and re-electrophoresing from stored gel plugs. This will be done for select isolates from this study for manuscript preparation.
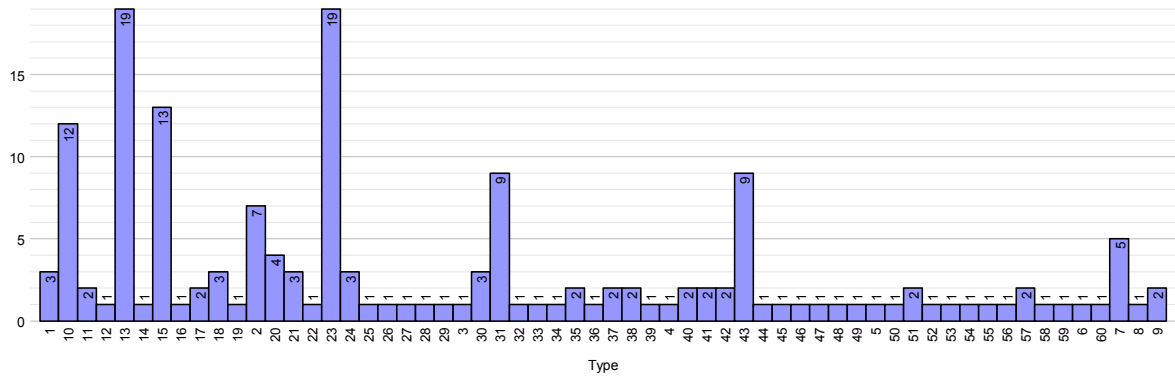
**Figure 9. Frequencies of isolates within each PFGE restriction type (RT).**

Analysis revealed a great deal of RT diversity within each pen (Figure 10). The Shannon Weiner index indicates the diversity of RTs based on the number of overall isolates examined, the number of RTs, and the number of isolates within each RT. An increasing index rating indicates a higher degree of diversity, i.e. a greater amount of strain heterogeneity. While some pen-based clustering was evident, i.e. isolates within pens (both RAJ and environmental) were generally more similar than those between pens, this was not a strong association, and many RTs were shared across pens. Some of this effect is explained by the relative homology of *E. coli* O157:H7 RTs. While every effort was made to segregate pens (e.g. empty pens between experimental pens, no shared water or feed sources), other mechanisms of pen to pen transmission, e.g. wild animals, airborne (dust), animal handlers, are also likely to explain this phenomenon.
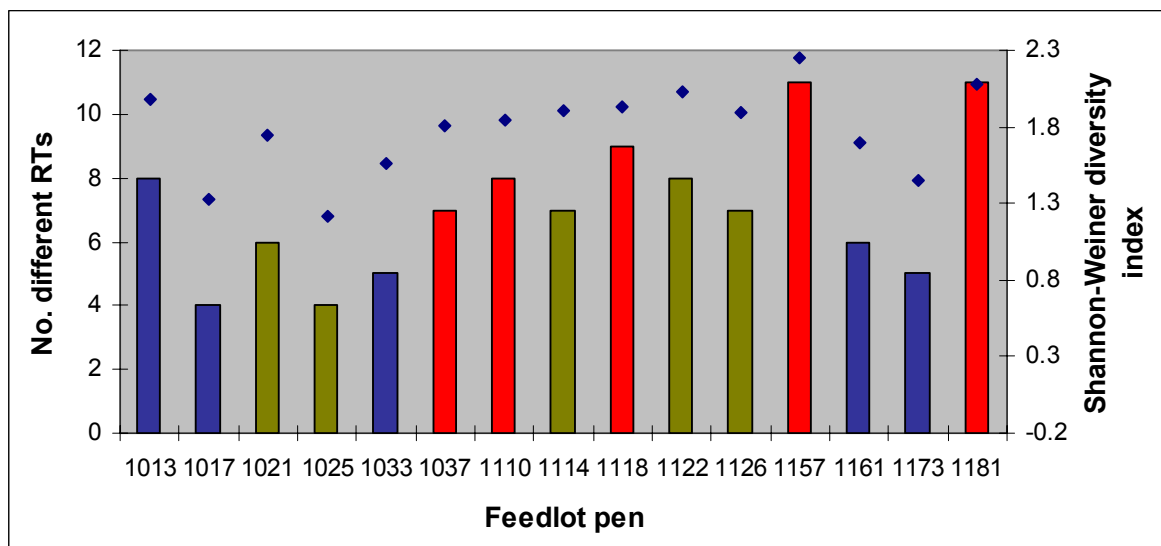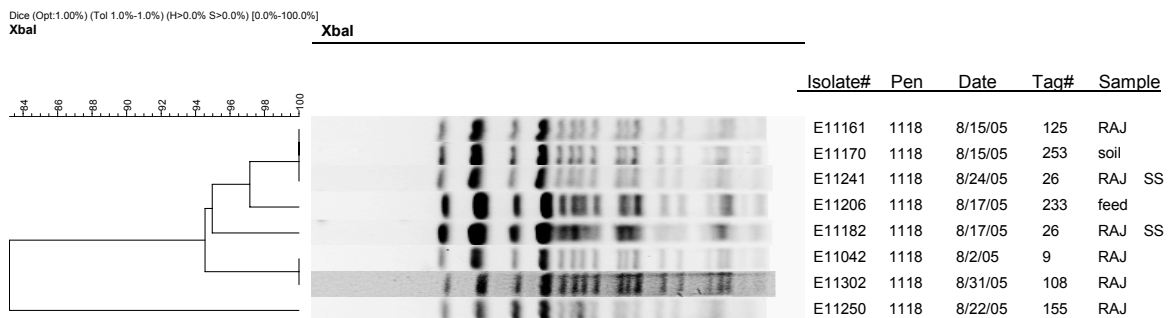


**Figure 10. Pen to pen variation in *E. coli* O157:H7 PFGE RTs. Bars represent the number of RTs within each pen: red, green and blue bars indicating hot, control or cold pens, respectively. Blue diamonds indicate the diversity (as measured by the Shannon-Weiner index) of RTs within each pen.**

Temporal trends in RT diversity were evident (Figure 11). The number and diversity of RTs on a given sampling date successively waxed and waned over the feeding period. Variation in the range of STEC RTs in feedlot cattle have been examined before, with the general finding being one of initially high strain diversity, followed by increasing levels of homogeneity with time. The findings of the current study differ from this in that waves of diversity appear to occur over time. This phenomenon, coupled with the findings of changing *E. coli* O157:H7 count and *E. coli* O157:H7:*E. coli* ratio over the feeding period (as described in the preceding sections of the final report) are interesting, and warrant further investigation.
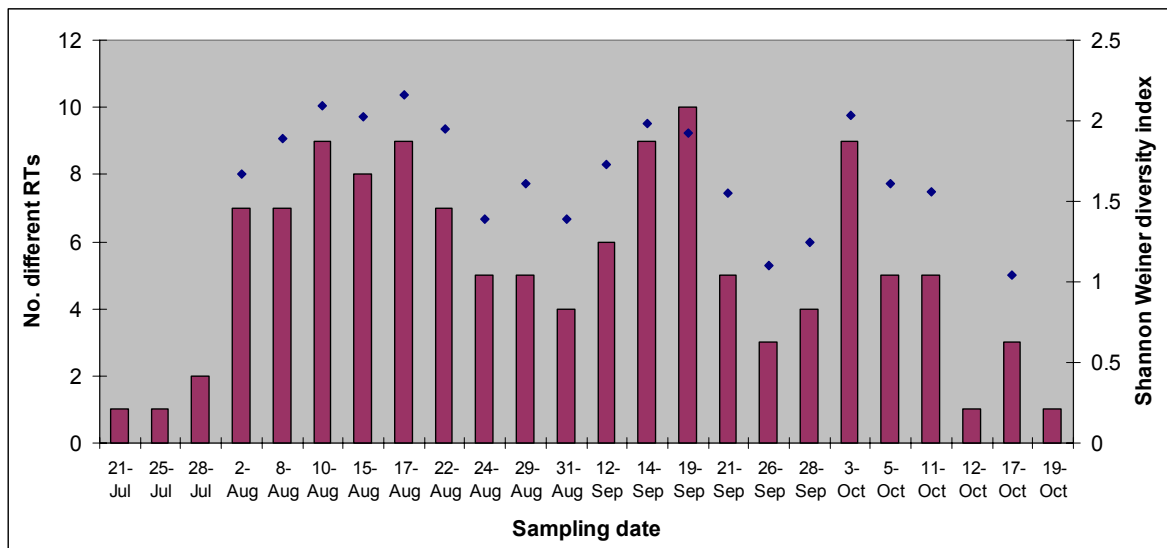


**Figure 11. Temporal trends in *E. coli* O157:H7 PFGE RTs. Bars represent the number of RTs identified at each sampling date. Blue diamonds indicate the diversity of RTs within each date.**

Comparisons of super-shedder RTs with those of pen-mates suggest substantial sharing of isolates between cattle within pens (Figure 12). Prior to re-assignment, 69% of isolates from cattle within hot pens were closely related (≤1 band difference) to super-shedder isolates. This varied between pens, ranging from 38% isolates being related to 100%. Based on the high level shedding of the super-shedders within each pen, it would be reasonable to infer that they were the principal source of the predominant strains within each pen, though transfection between all of the pen members would naturally occur too.

**Xbal**                                              **Xbal**

| Isolate# | Pen | Date | Tag# | Sample | |
|----------|-----|------|------|--------|---|
| E11161 | 1118 | 8/15/05 | 125 | RAJ | |
| E11170 | 1118 | 8/15/05 | 253 | soil | |
| E11241 | 1118 | 8/24/05 | 26 | RAJ | SS |
| E11206 | 1118 | 8/17/05 | 233 | feed | |
| E11182 | 1118 | 8/17/05 | 26 | RAJ | SS |
| E11042 | 1118 | 8/2/05 | 9 | RAJ | |
| E11302 | 1118 | 8/31/05 | 108 | RAJ | |
| E11250 | 1118 | 8/22/05 | 155 | RAJ | |

**Figure 12. Dendrogram of *E. coli* O157:H7 PFGE RTs demonstrating matching between isolates from super-shedders (SS), other cattle in pen 1118 (RAJ), and environmental samples prior to reassignment.**

After re-assignment, an average of 48% (ranging from 17%-86% for different pens) of isolates matched those for the pre-reassignment super-shedder, suggesting that these likely super-shedder-derived predominant isolates remained present in hot pens once the super-shedder was removed. Figure 13 provides an example of this for pen 1037. In this pen, as well as in all pens, isolates that were different to predominant pre-reassignment isolates arose in the post-reassignment period. This is likely due to mutations in the predominant strain over time (resulting in modifications in restriction loci within the *E. coli* O157:H7 genome), or introduction of truly novel strains into pens. Isolates from environmental samples in the post-reassignment period matched that for the predominant isolates, indicating that feedlot water, soil and feed sources represent important potential reservoirs of *E. coli* O157:H7.

**Xbal**                                              **Xbal**

| Isolate# | Pen | Date | Tag# | Sample | |
|----------|-----|------|------|--------|---|
| E11386 | 1037 | 9/14/05 | 31 | RAJ | |
| E11476 | 1037 | 9/28/05 | 72 | RAJ | |
| E11304 | 1037 | 8/31/05 | 119 | RAJ | SS |
| E11477 | 1037 | 9/28/05 | 83 | RAJ | |
| E11519 | 1037 | 10/5/05 | 145 | RAJ | |
| E11231 | 1037 | 8/22/05 | 119 | RAJ | SS |
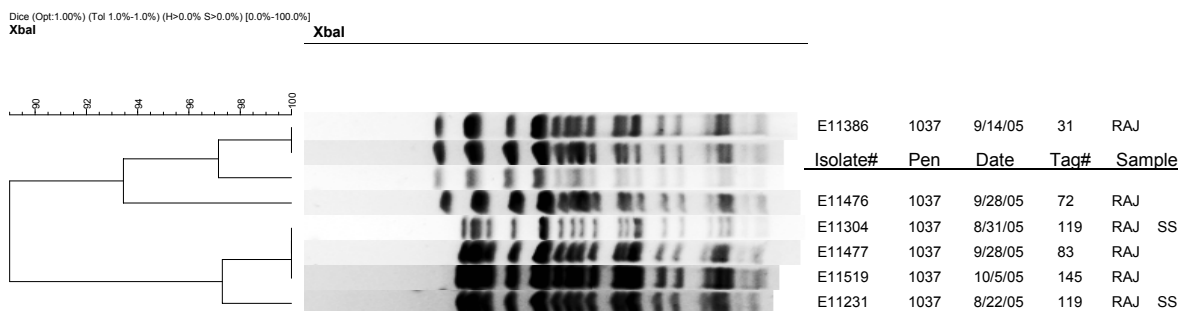
**Figure 13. Dendrogram of *E. coli* O157:H7 isolates demonstrating matching of PFGE RTs between super-shedders (SS), other cattle in pen 1037 (RAJ), and environmental samples after reassignment.**

The principal goal of PFGE analysis for Objective 3 was to assess the effect of super-shedder reassignment on cold pen *E. coli* O157:H7 isolate identity. Table 1 summarizes comparisons of RTs for isolates from each cold pen after reassignment to the introduced super-shedder, and to cold pen isolates prior to reassignment. Averaging across pens,

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100% of post-reassignment cold pen isolates were closely related (≤1 band difference) to the super-shedder isolates, while only 11% of cold pen isolates after reassignment matched isolates from the same pen before reassignment. This suggests that cattle in the cold pens were more frequently excreting isolates derived from the newly introduced super-shedder rather than isolates that were already present in the cold pens prior to introduction of the super-shedder, and highlights the role of super-shedders in disseminating *E. coli* O157:H7 among groups of cattle. The dendrogram presented in Figure 14 graphically demonstrates this phenomenon for pen 1013, where comparisons between post-reassignment isolates could be directly made with both super-shedder and pre-reassignment isolates. Note the closer identity of the post-reassignment isolates to the super-shedder RTs, as compared to the RTs from the pen prior to reassignment.

**Table 1. Comparisons of *E. coli* O157:H7 PFGE RTs of cold pen isolates after reassignment with super-shedder isolates (SS) and isolates from the cold pen before reassignment (pre cold). NA indicates no isolates were available for comparison.**

| Cold pen | 1013 | | 1173 | | 1161 | | 1017 | | 1033 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Comparison with | SS | pre cold | SS | pre cold | SS | pre cold | SS | pre cold | SS | pre cold |
| No. isolates compared | 6 | | 10 | | 8 | | 5 | | 3 | |
| % identical | 50% | 0% | 90% | | 25% | | | 0% | | 33% |
| % 1 band different | 50% | 0% | 10% | | 75% | | | 0% | | 0% |
| **% ≤ 1 band different** | **100%** | **0%** | **100%** | **NA** | **100%** | **NA** | **NA** | **0%** | **NA** | **33%** |
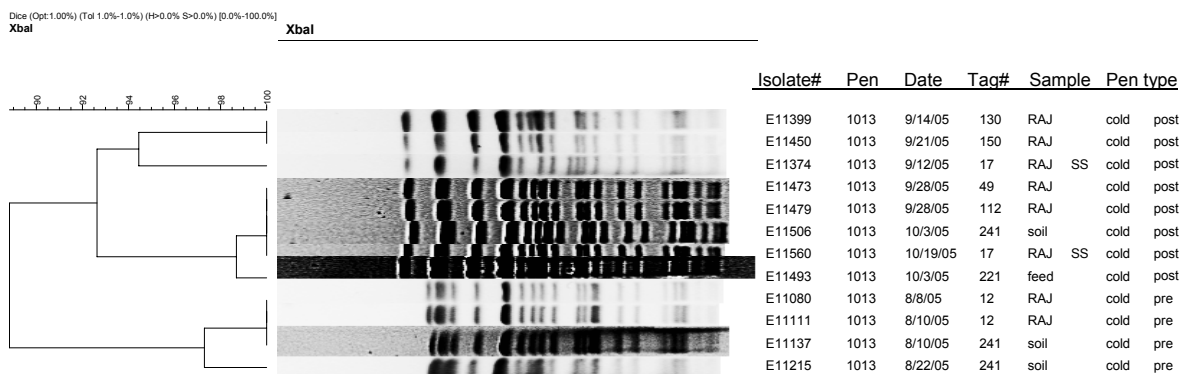


**Figure 14. Dendrogram of *E. coli* O157:H7 RTs from pen 1013. "SS" indicates super-shedder isolates. "Pre" and "post" indicate isolates from before and after super-shedder reassignment, respectively.**

Isolates from non-shedder cattle (*E. coli* O157:H7-negative cattle that were pen-switched with super-shedders during reassignment) were compared to those of the hot pens after reassignment. On average, 65% (17-86% across pens) of non-shedder isolates matched those of the post-reassignment hot pen isolates. Unfortunately, no pre-reassignment non-shedder isolates were available for comparison, but these findings are consistent with the

suggestion that the introduced non-shedders acquired the predominant isolate type of the hot pen. Figure 15 demonstrates such an association. On some occasions, the RTs for non-shedder isolates more closely matched those for environmental isolates, indicating again the important role of environmental niches for harboring and disseminating *E. coli* O157:H7 to cattle. Many other comparisons demonstrated matching RTs between RAJ and environmental isolates, further confirming the link between cattle colonization and environmental contamination. RT diversity was higher for the RAJ isolates (mean Shannon-Weiner index of 3.42) than for soil (2.87), feed (2.36) or water (1.83) isolates. This indicates that the RAJ represents a niche for a wide variety of different *E. coli* O157:H7 strains, and that the environmental niches are more restricted in terms of inhabitant *E. coli* O157:H7. Environmental niches could remain positive for the same RT of *E. coli* O157:H7 for prolonged periods of time, suggesting continual re-contamination from cattle or persistence in that niche.
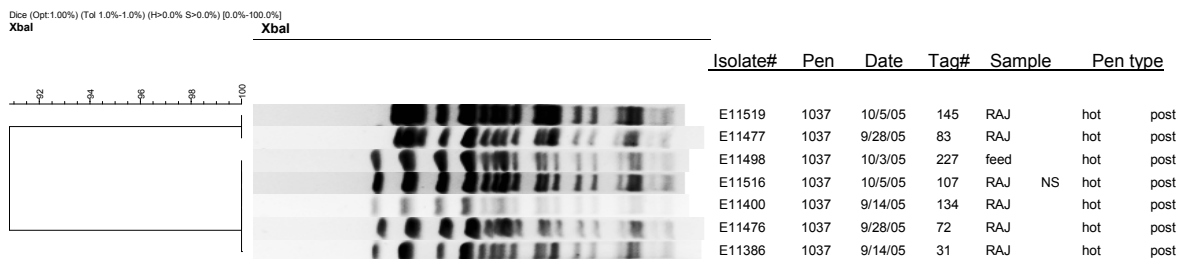


**Figure 15. Dendrogram comparing *E. coli* O157:H7 RTs from a non-shedder (NS) to other isolates in pen 1037 after reassignment.**

## General Discussion Points

This project further confirms the role of RAJ colonization and super-shedders in influencing the epidemiology of *E. coli* O157:H7 in feedlot cattle. From the previous project, higher pen prevalences and counts among cattle were associated with the presence of a super-shedder in their pen. Data derived in this project suggests a causal link between presence of a super-shedder and pen *E. coli* O157:H7 parameters, i.e. that the super-shedder is responsible for higher pen parameters. Introduction of a super-shedder into a pen that has relatively low levels of *E. coli* O157:H7 activity results in significant increases in *E. coli* O157:H7 prevalence and persistence among cattle, and moderate (though non-significant) increases in colonized count and environmental prevalence. Interventions that aim to identify super-shedders and prevent their entry into pens or dissemination among other cattle are warranted. Logistical consideration needs to be taken into account to determine where in the beef production process this is best done; i.e. on entry into the feedlot, at saleyards, during transport, during lairage, at a number of these points. It certainly indicates that mixing of cattle from different pens or herds has the potential to allow co-housing of super-shedders and naïve/low-shedding cattle, which would result in the latter becoming more likely to carry and or shed *E. coli* O157:H7, in turn making them greater potential on-going sources of *E. coli* O157:H7.

Removal of super-shedders from hot pens resulted in reductions in *E. coli* O157:H7 parameters for cattle colonization and environmental contamination. However, these reductions were no greater than those seen in control pens, where general reductions in *E. coli* O157:H7 prevalence, count and persistence also occurred over the course of sampling. Based on this, it seems that while it is optimal to minimize the presence of super-shedders within various populations (pens, herds) of cattle, removal of super-shedders from already high-level *E. coli* O157:H7 populations will not necessarily or immediately improve the *E. coli* O157:H7 status. Based on the hot pen parameter changes noted from before to after reassignment, it seems that residual colonization of cattle and environmental contamination in hot pens will maintain a significant *E. coli* O157:H7 presence, at least in the short term. Earlier removal of super-shedders or other interventions (e.g. removal of other high-colonized/shedding cattle, cleaning of feed bunks/water troughs) combined with this may be indicated.

Aspects of experimental design and analysis provide limitations to the findings and interpretations in this current study. Lack of significant differences for many of the parameter comparisons is likely to be simply due to low sample numbers (i.e. only five super-shedders and hot pens being compared to similar numbers of non-shedders, cold and control pens). Similarly, with a limited time period available for Phase I sampling, greater errors in identifying super-shedders are possible. With more optimal and/or real-time identification of super-shedders, it is possible that changes as a result of reassignment would have been more profound. All data analyzed in this project was done so using pen as the unit of measure, rather than individual cattle. Non-parametric statistical methods were utilized, and super-shedder and non-shedder data were excluded from analyses. Because of this, these results are likely to be conservative.

## IX.   Publications, abstracts, manuscripts in progress, thesis or presentations that resulted from this research.

Portions of the findings from the current research project were recently (27th June 2006) presented at a scientific update meeting for Australian Quarantine and Inspection Services On-plant Veterinarians (equivalent to US FSIS Veterinary Inspectors). Only summary findings were presented, and were done so in context of broad descriptions of RAJ colonization and super-shedder hypotheses. Feedback and discussion on practical aspects and potential in-plant interventions that may arise from these findings were solicited. An abstract describing the findings of the current project has been submitted for the VTEC2006 (6th International Symposium on Shiga Toxin (Verocytotoxin) - Producing Escherichia coli Infections) meeting. This is the premier international conference relating to *E. coli* O157:H7 and other enterohaemorrhagic *E. coli*, and will be held in Melbourne, Australia this quadrennium. Manuscripts for this and the previous project relating to RAJ colonization are in preparation, and are anticipated to be submitted to international peer-reviewed journals within the next 12 months.

## X.   Additional funding secured as a result of beef industry support of this project.

None at this stage. Funding to continue this line of research is anticipated to be sought in the near future.

## Xl.   Brief Lay Interpretation of Results suitable for public release (maximum 200 words as a separate report).

Certain cattle within feedlots are colonized by *E. coli* O157:H7 more frequently, persistently and in greater numbers than other cattle. These "super-shedders" appear to influence the degree of *E. coli* O157:H7 colonization of other cattle in their pens. When super-shedders are moved to pens with low levels of *E. coli* O157:H7 activity, the prevalence and persistence of *E. coli* O157:H7 colonization increases among other cattle in that pen. Similarly, levels of environmental contamination appear to increase as well. Presuming these effects can result in greater levels of general *E. coli* O157:H7 carriage, excretion and hide contamination, this represents a risk for greater introduction of *E. coli* O157:H7 into the slaughterhouse. Interventions designed to identify and negate the effects of super-shedders at strategic points in beef production may help mitigate the food safety risk of *E. coli* O157:H7. The site of *E. coli* O157:H7 colonization in cattle appears to be the recto-anal junction. An improved knowledge of how *E. coli* O157:H7 specifically reside at this site and become excreted in feces will also allow development of interventions designed to reduce *E. coli* O157:H7 carriage and transmission to other cattle or the food chain.