“Multi-agency Collaborative Effort for the Study of *E. Coli* O157:H7 Prevalence in a Pre-harvest Produce Environment”

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Introduction

Consumption of fresh fruits and vegetables is growing in the U.S., however, this trend appears to correlate also with an increase in produce-associated outbreaks. Since 1995, 18 outbreaks of *Escherichia coli* O157:H7 (*Ec*O157) associated with fresh lettuce or spinach have occurred with five occurring since 2001; 9 of these outbreaks have been traced to, or near, the Salinas Valley region of California *(3, 4, 15)*. Recent evidence indicates that contamination of produce can occur pre-harvest, probably, by application of raw or poorly composted manure to the soil relatively close to crop harvest, or due to application of contaminated water during irrigation or from flooding *(9, 10, 12, 23)*. Raw produce is at risk also from direct application of raw manure applied as fertilizer, or deposited by livestock or wild animals.

This phase of the project started October 1, 2005 to determine the incidence of *Ec*O157 in selected sites in the Salinas region watershed for 1 year. In accordance with the sampling plan, sample sites were selected that represent several regions of the watershed. Particular regions were selected for sampling based on potential impact of farming, grazing, and accessibility. However, sample sites were also selected based on results from the first phase of this project which started January 1 2005. During that phase *Ec*O157 was found exclusively from the Gabilan, Tembladero and Old Salinas River. Sites on the Chular Creek and upstream on the Salinas River were added in phase 2, since they may be impacted also by farming and grazing. One site sampled in phase 1 on the Old Salinas River Estuary off Potrero Road was not included in phase 2 due to tidal influence and intrusion of salt water. A total of 21 sites were selected for regular monthly testing and the sites are illustrated on a map shown in Figure 1. However, for various reasons, not every site was regularly sampled. Chular Creek and one site on the Gabilan Creek (E) were sampled intermittently, because they were dry. Additionally, in January 2006, one site (R), became inaccessible because the owner of the land blocked access to the river. However, several sample sites were added during the course of the investigation in an attempt to discover the source(s) of contamination.

Additionally, samples were taken to determine coliform and generic E. coli as part of a Total Maximum Daily Load (TMDL) determination with the CCRWQCB. This data was also used to show correlation with the incidence of EcO157 where possible. Additionally, abiotic data was collected regularly at each site to determine if perhaps environmental parameters contribute to the survival of EcO157 in the watershed.

All EcO157 isolated from the watershed was typed by Multi-Locus Variable number tandem repeat Analysis (MLVA) and further characterized by Polymerase Chain Reaction (PCR) for virulence genes. MLVA has been shown to be more reproducible than Pulse Field Gel Electrophoresis (PFGE) and better at discriminating between closely related EcO157 isolates *(8, 13, 14, 17)*. Discrimination with MLVA relies on changes in hyper-mutable repeat elements located at various places in the genome. Very closely related isolates, for example, clinical isolates from the same outbreak, often are identical, or differ by only one or two tandem repeat (TR) changes at a single locus *(17)*. Selected isolates were also subjected to PFGE analysis.

Methods

Environmental sampling method. Samples of water (100 ml) were collected into sterile, disposable bottles. Collection point was as close to the center of the stream or pond as practical and was accomplished by attaching the bottle to a telescoping pole. In most cases 4 samples were collected at each location. Two samples were analyzed for coliform and generic *E. coli* and two samples were used to isolate *Ec*O157. Additionally, Moore swabs were deployed in the stream, anchored to a monofilament line 5 days prior to the collection date *(21)*. The water and swab samples were stored and transported on ice in marked Whirl-Pak bags (Nasco, Modesto, CA). The sample location and any observations regarding appearance of the sample or the surroundings were carefully recorded along with GPS location. Abiotic parameters were measured using a multifunctional, hand held meter (Quanta Hydrolab, Hach Environmental, Loveland, Colorado). The meter measures pH, conductivity, temperature, salinity, dissolved oxygen, oxidation/reduction potential (ORP) and turbidity. The meter was used whenever samples are obtained, except in a few instances when we could not get close enough to the water safely. Flooded roadways or excessive mud have prevented access to sample sites on a few occasions. Quantification of total coliforms and generic *E. coli* was determined by the Colilert® QuantiTray 2000 method per manufacturer's recommendations (Idexx Laboratories, Westbrook, ME).

Enhanced IMS method. A modified protocol, adapted from the Clay Center IMS method for isolation of EcO157 from feces was used *(1)*. Moore swabs were enriched in sterile 1 L flasks containing 250 ml TSB after briefly rinsing in tap water. The temperature of the incubator was then increased to 42 ^{o}C for 8 hr with shaking and then held at 4 ^{o}C without shaking until the following morning. Water samples were enriched by adding 11 ml 10x TSB (filter sterilized) to 100 ml sample and incubated as described above. One ml of the enrichment was tumbled with 20 µl of Dynal anti-O157 paramagnetic beads for 30 min, washed 1, 2 or 5 times with PBS-T (PBS + 0.05% TWEEN 20) and resuspended in 100 µl PBS *(18)*. Alternatively, the Dynal BeadRetriever was used with the EPEC/VTEC protocol. From the resuspended beads 50 µl each was spread on 2 plates, cefixime (0.05 µg/ml, Dynal), tellurite (2.5 µg/ml, Dynal) sorbitol Maconkey agar (CT-SMAC, Difco) and novobiocin (20 µg/ml, Sigma), tellurite (0.8 µg/ml, Dynal) Rainbow agar (NT-Rainbow, Biolog). Control plates were streaked with genuine *E. coli* O157:H7 pWM1029 (RM2315) for comparison *(5)*. Both plates were incubated at 37 ^{o}C overnight (approximately 18 hr) and colonies were selected which most closely resembled the control colonies. Selected colonies were patched twice onto duplicate Luria Broth (LB) agar (Fisher Scientific, PA) plates using a numbered grid with positive control RM2315. One set of the patched colonies was subsequently blotted onto Protran nitrocellulose membranes (BA 85, Schleicher & Schuell). The membrane was washed with TBS-Tween (25mM TRIS pH 7.4, 0.15 M NaCl, 0.1% Tween 20) and then blocked with casein blocker (0.5% casein, 0.01 M TRIS, 0.031 M sodium azide, 0.15 M NaCl pH 7.4) while shaking for 30 minutes at RT. The membrane was probed with anti-O157 MAb (13B3, *(26)*) in 10 ml Elisa Diluting Buffer (1x TBS-Tween, 1% BSA, 0.02% KCl, 0.1% sodium azide) at 1:2000 while shaking for 30 minutes RT. The membrane was washed four times for 1 min each with about 100 ml TBS-TWEEN, probed with the secondary antibody (Goat anti-Mouse IgG AP conjugate (Zymed) in Elisa Diluting Buffer at 1:2000 for 30 minutes with shaking at

RT and washed four times for 1 min each with TBS-TWEEN, then two times in distilled water. The blot was developed in 10 ml of alkaline phosphatase substrate (BCIP/NBT Sigma #B5655). The presence of genuine EcO157 antigen was distinguishable by monitoring the rate of the color reaction compared to RM2315.

Pathatrix isolation method. Alternately, the swabs were transferred to filter stomacher bags containing 250 ml of pre-warmed single-strength modified buffer peptone water supplemented with sodium pyruvate. Samples were incubated for 5 hours at 42ºC with shaking. Approximately 200-250 ml of the sample enrichment broth was circulated over anti-*E.coli* O157:H7-coated beads at 37ºC for 30 minutes using re-circulating immuno-magnetic separation (RIMS), in a Pathatrix™ instrument (Matrix Microscience Inc., Golden, CO). Following the circulation, the Pathatrix™, the beads were washed and resuspended in buffered peptone water and separated into 3 portions. One portion of the bead suspension was directly plated onto CT-SMAC and another portion was plated onto CHROMagar O157 (Paris, France). The third portion was used to perform a multiplex real-time PCR analysis for *stx*1, *stx*2 and a single base-pair mutation in the *uid*A gene *(11)*. Positive isolates were confirmed both by real-time PCR and by serological and biochemical procedures outlined in the US FDA Bacteriological Analytical Manual.

Putative *Ec*O157 colonies isolated by the enhanced IMS or Pathatrix procedures above were further analyzed by real-time PCR for the presence of the *rfb*E gene. A 20 µl reaction included 10 µl Stratagene Brilliant QPCR master mix, 0.3 µM each *rfb*E primer (5' TTTCACACTTATTGGATGGTCTCA3' and 5' TGAGTTTATCTGCAAGGTGATTCC3'), and 0.1 µM probe (5' 6-FAM-TTCTAACTAGGACCGCAGAGGAAAGAGAGGAATTA-BHQ-1 3', Biosearch Technologies, Inc., San Francisco, CA). Bacteria were transferred directly into the PCR tube using a sterile toothpick. No-template and RM2315 control colonies were included for the amplification in a Stratagene MX3000P Real-Time PCR machine for 95°C for 5 min, then 60 cycles of 95 ^{o}C for 15 sec and 60°C for 45 sec. Colonies shown to contain the *rfb*E gene were streaked for single colonies and further analyzed for *fli*C (H7) using the method of Fratamico et. al. *(7)*. Each 25 µl reaction contained: 1x ThermoPol buffer, 200 µM each dNTP, 0.25 µM each primer, 2.5 U TAQ, and 1 µl template. Thermal cycling parameters were 95 ^{o}C for 5 min, then 30 cycles of 94 ^{o}C for 30 sec, 58 ^{o}C for 30 sec, 72 ^{o}C for 30 sec, and a final extension at 72 ^{o}C for 5 min. DNA template was prepared by resuspending 1 µl of cells, scraped from a plate, into 100 µl of distilled water and boiled for 20 minutes. Boiled cells were centrifuged at 5000 RPM for 10 min and the resulting supernatant was used as template for PCR amplification. Other virulence genes (*stx*1, *stx*2, *eae*, *hly*) were detected by multiplex reaction using the method of Paton and Paton *(19)*. Each 15 µl reaction contains: 1x Qiagen multiplex PCR master mix (Qiagen #206143), 0.25 µM each primer, and 1 µl of template. Thermal cycling parameters were 95 ^{o}C for 15 min, then 35 cycles of 95 ^{o}C for 1 min, 65 ^{o}C for 2 min, 72 ^{o}C for 1.5 min. Annealing temperature decremented from 65 ^{o}C to 60 ^{o}C between cycles 10 and 15, and elongation time increments from 1.5 min to 2.5 min between cycles 25 and 35. All PCR ingredients were purchased from New England Biolabs. Reactions were done on a Tetrad thermal cycler (MJ Research) and run on a 2% agarose TAE gel using a 100bp ladder (New England Biolabs).

MLVA typing. MLVA was performed using the capillary electrophoresis method of Linstedt et. al. or Hyytia-Trees et al. *(8, 14)*. Essentially, 10 loci are amplified in 3 multiplex reactions using fluorescent primers. Reaction 1 contains primers for VIC-Vhec1, NED-Vhec3, FAM-Vhec4, FAM-Vhec5 and reaction 2 contains primers for VIC-Vhec1, NED-Vhec2, VIC-Vhec6, FAM-Vhec7 *(14)*. Reaction 3 contains primers for FAM-O157-17, NED-O157-19, VIC-O157-37 *(8)*. Each 10 µL PCR reaction contains 1x multiplex PCR master mix (Qiagen), 0.2 µM each primer and 1 µl template. Thermal cycling parameters are: reactions 1 and 2: 95^{o}C for 15 min, then 25 cycles of 94^{o}C for 30 sec, 63^{o}C for 90 sec, 72^{o}C for 90 sec, and a final extension at 72^{o}C for 10 min; reaction 3: 95^{o}C for 15 min, then 35 cycles of 94^{o}C 20 sec, 65^{o}C 20 sec, 72^{o}C 20 sec, and a final extension of 72^{o}C for 5 min. The multiplex reactions were pooled and diluted 1:50 by adding 2 µL each into 94 µL distilled water. One µL of this dilution was added to 12 µL HiDi formamide (Applied Biosystems), and 0.08 µL ladder (ROX-labeled MapMarker 1000, Bioventures, Inc.) and heat denatured for 5 min at 95 ^{o}C, cooled on ice for 2 min, then loaded onto the ABI 3130xl Genetic Analyzer (Applied Biosystems) and run using the default settings for fragment analysis with Dye Set D in a 50 cm array and POP7 polymer for 2100 seconds. Fragments were sized using GeneMapper software (Applied Biosystems). If more than one fragment was detected for one locus, the fragment with the highest level of fluorescence was chosen. Fragment size was converted to number of tandem repeats by subtraction of the amplified, non-repeat sequences and division by the repeat size. Fractional repeat numbers were rounded to the nearest whole repeat number.

PFGE typing. Selected isolates were also typed using the standard PulseNet procedure with both *Xba*I and *Bln*I restriction enzymes *(2)*. Dendrograms comparing the isolates were constructed from both digests with BioNumerics using the Bandmatching and Phylogenetic Clustering Analysis methods (Applied Maths, Texas).

Phylogenic and statistical methods. Phylogenic comparison of the repeat structures of the MLVA types was done using Unweighted Pair-Group with Arithmetic Mean (UPGMA) algorithm in PAUP 4.0. Allelic diversity was based on Nei's diversity *(16)*, which is $1-\Sigma(\text{allele frequency})^2$. Precipitation totals were the average of daily accumulative data from 4 weather sites (northern Salinas, southern Salinas, Castroville and the Salinas airport) on the collection date and four days prior. Students t test was performed using the algorithm of SigmaStat version 3.0.

Results

Routine Watershed Sampling. Duplicate water samples were collected and analyzed for the presence of *Ec*O157 at 21 locations in the watershed (Figure 1, Table 2). At the same time, two additional samples were collected for determination of coliform and generic *E. coli* levels and abiotic measurements were taken. Moore swab samples were included also at several of the same locations starting in December 2005. *Ec*O157 was isolated 28 times from 600 water samples and 37 times from 213 swab samples at locations A-V (excluding I) over the 12 month sampling period (Table 1). More than 70% of the positive samples were collected from the Gabilan or its tributaries.

Generic *E. coli* levels varied considerably during the 12 month period, from <10 MPN/100 ml to 30,440 MPN/100 ml (April 06, CHU CCR). Initially, generic *E. coli* levels were confirmed by plating the individual Colilert wells on Chromagar. Seventy five percent of the wells were confirmed to be *E. coli*. However, in many cases the presence of large numbers of uncharacterized bacteria from the Colilert test prevented confirmation on Chromagar plates. As a result, confirmation of the generic *E. coli* results was abandoned after only a few months. Sampling was also done after rain events, since rain and potential runoff have been deemed an important potential parameter associated with increased incidence of fecal bacteria. However, due to increased sampling difficulties under very wet conditions, not all sites were sampled. Using information from USGS and NOAA, we monitored rainfall and river levels in Salinas. Initially, any rainfall accumulation of 0.5 inches in 5 days was considered a rain event. However, later during this project, that criteria was adjusted upwards to 1.0 inches rainfall in 5 days. This adjustment was necessary since the lower limit would have overwhelmed our capacity to process the samples. As a result, we sampled sites after five rain events from November 2005 through April 2006 (Nov 30-05, Dec 19-05, Jan 4-06, Mar 7-06, Apr 5-06). This in addition to the same sites sampled monthly.

The water sampling results are summarized in Table 3, which indicates that the average generic *E. coli* levels increased after rain events (5.4 fold increase), this despite the fact that one of the monthly samples fell on a rain event (Mar 20-06). However, overall, there was a poor correlation between generic *E. coli* levels at any location and the recovery of *Ec*O157, primarily because there are many instances of high generic *E. coli* levels where *Ec*O157 was not recovered (Table 1). Therefore, it was necessary to restrict the analysis to regions of highest EcO157 incidence, namely Gabilan Creek.

Flow measurements were available from USGS equipment on the Gabilan Creek at location D. During the rainy season (November through April), the flow in the Gabilan Creek can increase more than 1000 fold. The average generic *E. coli* levels at locations A-D (Table 1, Figure 2) increased five fold as flow increased from 0.17 to 272 cu ft per min. Likewise, the incidence of *Ec*O157 increased from undetectable to more than 85% of the samples positive. Generally throughout the watershed, high levels of generic *E. coli* were detected at the same time as frequent isolation of *Ec*O157 occurred (Table 1). Nevertheless, generic *E. coli* levels and incidence of *Ec*O157 correlated poorly when individual sampling sites were compared. Generic *E. coli* levels and flowrates were often high where *Ec*O157 was not recovered. Additionally, it is noteworthy that *Ec*O157 was also found during times of relatively low flow (Figure 2). Several samples from the Gabilan taken in dry months exhibited low genetic *E. coli* and were positive for *Ec*O157 (Table 1). This would suggest that factors other than rain are important. Importantly, stalker cattle arrive from a variety of locations during the winter to feed on the abundant grass prior to transport to feedlots or other locations.

Usually, generic *E. coli* levels were similar (+/-15%) between duplicate samples at the same time and location (Table 1). However, the correlation between duplicate samples with the incidence of *Ec*O157 was only 40%, i.e., where *Ec*O157 was detectable in either sample, *Ec*O157 was recovered from the duplicate sample only 40% of the time (Table 1). Furthermore, in duplicate samples where *Ec*O157 was recovered from both samples, the same MLVA type was found in both only 39% of the time. These data suggested that contamination of the watershed was either in a dynamic state or the level is very close to (and most often below) the sensitivity of our isolation procedure.

Experimental samples. Moore swabs were included as an experimental sampling technique starting in December 2005. These are composed of individual strips of gauze-type fabric tied together and attached to a line. The swabs are suspended in water for approximately five days before they are obtained for testing. Swabs were not deployed at all sample sites due to lack of a solid support to attach the line or difficulty in recovering the swab once deployed. In some cases the swabs were lost due to breakage of the line or vandalism. Additionally, swabs could not be used for rain event sampling because swabs were not continually deployed and would need to be deployed when the rain started. During the 10 months, 213 swabs were recovered. Prior to March 2006, isolation of *Ec*O157 strains from Moore swabs was achieved using the modified IMS isolation method. In March 2006, California Department of Health Services, Food and Drug Branch isolated *Ec*O157 from Moore swabs using the Pathatrix recovery system (Table 1). CDHS analyzed 103 swabs using both multiplex PCR and culture following the Pathatrix method. PCR indicated the presence of *Ec*O157 in 23 swabs, but the pathogen could not be cultured 11 times. Furthermore, PCR failed to detect *Ec*O157 5 times when the pathogen was recovered by culture. USDA ARS analyzed 110 swabs and recovered the pathogen 21 times. Nevertheless, recovery of *Ec*O157 from the swabs was quite efficient, relative to the water samples. Where both water samples and swabs were used together there was only one instance where the swab failed to find contamination when the corresponding water sample was positive for *Ec*O157 (Table 1, Jul 18-06, site C). Conversely, *Ec*O157 was recovered from swabs 15 times when water samples obtained at the same location were negative (Table 1). Additionally, the number of MLVA types recovered from swabs is nearly as high as the number recovered from water samples (45 vs 50) and yet fewer swab samples were processed.

Additional samples were collected at several new locations upstream where contamination was found in an attempt to track the source of the contamination (Table 1). Since the Gabilan Creek and Towne Creek were frequently positive, significant investigative effort concentrated in this region of the watershed. Added sites included those in the upper regions of Towne Creek; TOW OSR 1.5, 2, 3, 4 and tributaries north of the Gabilan; GAB SAN 1,2,3. Sample sites SRC HEB, SRC RUS and SRC 101 were also added along the Santa Rita Creek in an effort to investigate the initial contamination in sediment discovered in Jul-04. Additionally, sample sites were added on the El Toro River (ORO END, ELT 68), a tributary upstream of site J, in response to contamination at this location. Most of these additional sites were not productive, possibly due to the transient nature of the contamination. Towne Creek was the only region where the *Ec*O157 source was sufficient to make source tracking possible. In the upper region of Towne Creek, *Ec*O157 was isolated frequently. At one location (TOW OSR3) the headwaters of the creek moved through a corral containing approximately eight head of cattle. *Ec*O157 isolates from samples at this location were very closely related by MLVA possibly indicating a point source.

Abiotic parameters. Correlation between the recovery of *Ec*O157 and any of the seven abiotic parameters was determined initially only for site A. Site A was selected for this analysis because it is the only site where *Ec*O157 has been isolated routinely.

Statistical analysis by a Students t-test indicated the lack of any significant correlation between recovery of *Ec*O157 and six of the parameters (temperature, P=0.774; conductivity, P=0.570; salinity, P=0.461; pH, P=0.393; ORP, P=0.974; turbidity, P=0.214). However, significant correlation was found between recovery of *Ec*O157 (by water sample or swab) and dissolved oxygen (P=0.05). Furthermore, when all 21 sample sites were considered in the analysis the correlation improved to P=0.012.

MLVA data. Sixty five samples (water or swab) were positive for *Ec*O157 and from these 722 separate CFU's were subcultured for MLVA, resulting in 41 different MLVA types. Each of the 722 isolates was analyzed also by PCR for the presence of four virulence genes and *fli*C (H7). All *Ec*O157 isolates contained *fli*C, *eae*, *hly* and *stx*2. Strains typed as MLVA types 90, 91, 93, 97, 98, 100, 101, 102, 103, 105, 160 or 161 were negative for *stx*1. Strains of the same MLVA type also had the same profile of virulence genes by PCR analysis. Thirteen of the 21 total sample sites yielded at least one *Ec*O157 isolate. Only four sites (K, M, N, O) yielded just one MLVA type during the 12 months of this study. One of these sites (M) was sampled only once in 12 months, due to lack of water flow. It is noteworthy that site A, yielded 11 different MLVA types; this was the largest number of MLVA types identified for a single site.

Identical MLVA types were isolated at multiple sample locations as shown in Table 4. However, regions of the watershed generally yielded MLVA types specific to that region. Ninety-five percent (39 types) were recovered exclusively from specific drainages. Nevertheless, on two separate sampling dates identical MLVA types were recovered simultaneously from locations separated by relatively large distances, although they were isolated from sites in the same drainage path. MLVA type 93 was isolated at sites on the Gabilan (B) and the Tembladero (G) on April 5, 2006, a distance of 18.5 miles. Likewise, MLVA type 100 strains were recovered from a site on the Alisal Creek (N) and Tembladero Slough (G) (15.5 miles) on April 18, 2006. Waterflow in the rivers was relatively high at the time of sampling compared to other sampling days.

A set of 105 human clinical strains, associated predominately with foodborne sporadic illnesses in California, but also outbreak strains isolated in other states, were typed by MLVA also for comparison to environmental isolates. Strains representing multiple outbreaks associated with lettuce or spinach, taco meat and apple juice were included. Also included were 16 environmental isolates from phase 1 of this project. None of the clinical isolates were identical by MLVA to any of the 57 environmental isolates. The environmental and clinical isolates had a similar mean allelic diversity at the 10 MLVA loci of 0.656 and 0.682, respectively. Nevertheless, phylogenetic analysis of all of the strains indicated that nearly 85% of the environmental isolates clustered into six groups (Figure 3). The MLVA types within two of the groups were related also to one another spatially, since with only a few exceptions, all the strains within the group were isolated from samples from the same watershed location. For example, strains in the largest group (Figure 3, group 3) were isolated exclusively from the upper portion of the Gabilan Creek. Group 2 strains were isolated from samples from the Salinas River. Group 1 strains were isolated at distant sites on a watershed, however, the sites were all on a specific drainage extending from Towne Creek to Tembladero Slough. It is noteworthy that four sporadic human illness isolates were related by MLVA to some environmental isolates and two of the human strains MLVA types 60 and 86) were within Groups 3 and 5. Group 4 represents a loose cluster of both environmental and sporadic isolates that nevertheless had similar PFGE profiles (see below). Other human strains, typed as MLVA types 112 and 129, were not linked to any MLVA clusters, but to single environmental isolates typed as closely related MLVA types 90 and 4, respectively. The four human sporadic illness strains were from California.

The relationships between a selected set of strains were investigated further by PFGE analysis. Several isolates related closely by MLVA were identical by PFGE. In general, the strains related by MLVA also appeared to be consistent with clusters of strains identified by PFGE (Figure 3). Nevertheless, several differences were noted. Cluster 6 formed two PFGE clusters; one with MLVA 155, 156 and 157 and another with MLVA 8, 9, 10, 12, 14, 104 and 154. Additionally, two isolates of MLVA 142 were split between the two PFGE clusters. PFGE cluster 3 contained two clinical isolates (MLVA types 18 and 19) not present in the corresponding MLVA cluster. Similarly, linkage relationships between other clinical isolates were less conserved between MLVA and PFGE, even among closely related isolates. For example, MLVA types 56 and 57, which differ only at a single MLVA locus, appeared highly divergent by PFGE profile differences. Additionally, a cattle fecal isolate (strain RM5038) and a water isolate (strain RM5036) were both typed as MLVA 15, but were divergent by PFGE. Furthermore, strain RM5038 was >94% similar by PFGE to human isolate RM5184 (MLVA type 56), but these two strains were different by MLVA at 7 of the10 loci. Additionally, MLVA type 93 strains were isolated from two physically separate drainage locations, the Salinas River and the Gabilan/Tembladero. PFGE analysis indicated that the isolate from the Salinas River (RM5606) was similar, but not identical, by PFGE (96% similarity) to the other MLVA type 93 strains.

Conclusions.

Multiple strains of *Ec*O157 were isolated from samples obtained over a 12-month period from the Salinas Valley watershed. *Ec*O157 was isolated more frequently from elevated watershed locations near grazing land compared to other locations, and during periods of increased waterflow following heavy rain events. These results have implications for identifying the sources of *Ec*O157 contamination of fresh produce associated with continued outbreaks traced to this growing region. Repeated recovery of *Ec*O157 from a variety of distant sites in the Salinas watershed, either by direct sampling and/or with swabs, indicated that contamination may be more prevalent than assumed previously, and stimulates questions regarding the mechanisms of survival, growth and prevalence of *Ec*O157 in these environments. Duplicate water samples were analyzed also for coliform and generic *E. coli*, to assess the total maximum daily load (TMDL) in the watershed. Results from these sampling studies indicated that the Salinas Valley watershed was “impaired” since >10% of the samples exceeded coliform levels of 400 MPN/100ml, and many individual samples exceeded generic *E. coli* levels of 409 MPN/100ml, based on the US EPA recommended levels for water contact recreation *(20)*. Isolation of many strains of *Ec*O157 at multiple times of the year and multiple sites is consistent with this conclusion. The necessity for an enrichment step in the method of isolation of *Ec*O157 precluded determination of the concentration of *Ec*O157 contamination.

Frequent isolation in the elevated regions of the Gabilan suggests there may be a higher incidence of the pathogen in this region. However, 45% of duplicate samples collected seconds apart from a watershed site produced different results, i.e., *Ec*O157 isolated from one sample, but not the other sample. We speculate that the concentration of *Ec*O157 in the water at certain times is relatively high compared to baseline levels at most times, and that the incidence and concentration are dynamic events. Alternatively, the level of *Ec*O157 is uniform, but at a concentration close to the sensitivity of our isolation method. During the rainy season, it is anticipated that large volumes of run-off water would collect, dilute and disseminate pathogens from point sources (e.g. animal feces containing high concentrations), but, at least for *Ec*O157, at levels close to or below the level of sensitivity of our method of detection. Our isolation method was adapted from a successful isolation procedure developed for isolation of *Ec*O157 from cattle feces and hides *(1)*. However, water and other environmental samples often yielded many non-target bacterial CFU on the selection plates that interfered with identification and selection of authentic *Ec*O157 CFU. These background flora may have decreased the sensitivity of our method, indicating an incidence of *Ec*O157 lower than may exist.

Of the 41 MLVA types isolated from the Salinas watershed during this project, 39 types were recovered exclusively from specific drainages. Twenty seven of the 41 types (65%) were recovered exclusively from the Gabilan or its tributaries. Eight types were recovered from the Salinas River and 1 type each was recovered from the Tembladero Slough, the Chular and Alisal Creek. Three MLVA types were recovered from multiple locations. One type (MLVA97) was recovered from both the Gabilan and the Tembladero Slough. Indeed the Gabilan drains into the Tembladero Slough, so it is possible MLVA97 originated in the Gabilan. MLVA 93 and 100 were recovered both from the Gabilan, as well as the Salinas River and Alisal Creek, respectively (Table 1, Table 4). Discovery of identical MLVA types at multiple locations suggests that the pathogen can be transported long distances from a point source in the watershed, but it is also possible that pathogens are being transported and disseminated from multiple point sources to multiple locations in the watershed.

Identical, or closely related, MLVA types were isolated also from the same location, days or months apart. For example, MLVA 16 was isolated from a single cattle feces sample near Towne Creek in August 2005 and then eight months later from water samples in April 2006 at sites A and E (Table 1, Table 4). Additionally, MLVA type 89 was isolated multiple times from the same location (site A) over a six-month period. The most likely explanation for this result is re-introduction from one or more sources in the vicinity. However, long-term survival of the pathogen in the environment is also possible.

Abiotic parameters were measured in conjunction with sampling. However, measurements were not taken on all sample dates due to problems with the Quanta meter. Additionally, selected sites were not measured on occasion due to difficulties with deploying the meter in very wet conditions. Nevertheless, the meter was deployed for 173 sampling events. Statistical analysis comparing the incidence of *Ec*O157 isolation and each of the parameters indicated significance only with dissolved oxygen ($P=0.05$) when considering data only at site A, where the incidence of *Ec*O157 was the greatest. Interestingly, when all samples sites were included in the analysis , significance increased to P=0.012. Survival of *E. coli* in water systems is know to be positively effected by dissolved oxygen *(6)*.

The incidence of *Ec*O157 increased following heavy rains that produced a significant rise in stream flow. However, it is not known if rainfall (and subsequent flooding) releases bacteria already existing in the creek sediments or washes contamination into the creek by run-off. It is probable that both events occur. Despite the high incidence of *Ec*O157 during flooding, it is important to note that *Ec*O157 was also isolated during periods of drought. These results may reflect the rate of deposition due to the prevalence of fecal shedding during these months *(22, 24, 25)*. Importantly, any detectable incidence of *Ec*O157 during the growing season may be critically important due to its potential impact on produce production in this region.

Figure Legends

Figure 1. Map of the Salinas watershed with sampling locations.
A map of Salinas, CA region showing locations and dates of sampling from Oct 25, 2005 to Oct 10, 2006. The circle marked with "X" is coded by position 1-17 representing the sampling dates show in the list below. The accumulated rainfall (inches) for the day of sampling and 4 days prior is shown in the list. Dates indicated in red text indicate at least one EcO157 strain was isolated on that date. Circles on the map "A" through "V" (excluding I) correspond to the locations where samples were obtained. Open circles within the "location circles" designate that no sample was obtained on that date; blue circles designate that a sample was obtained, but no EcO157 strain was isolated; and red circles designate that a sample was obtained, and at least one strain of EcO157 was isolated.

Figure 2. Incidence of EcO157 and generic E. coli with flow in Gabilan Creek
Correlation of water flow, the concentration of generic *E. coli* and incidence of EcO157 in the Gabilan Creek, Salinas, CA. EcO157 incidence is calculated as the fraction of the number of positive samples compared to the total samples obtained at locations A-D. The flow rate was obtained from data from the USGS sampling station #11152600 located at position D (see Figure 1).

Figure 3. Phylogenetic tree of MLVA data.
A comparison of the phylogenetic data of strains by MLVA and PFGE analysis. The grouped environmental strains are highlighted in yellow fill with significant clusters of related strains labeled "1 through 6". Outbreak isolates are designated in red text. Sporadic clinical isolates are designated in regular or blue text within the environmental groups.

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Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
Oct-05 Monthly samples														
102505ALI AIR	>2419,>2419	142,102	-,-	ND[11],ND,ND	None	15.3	ND	ND	ND	ND	ND	ND	ND	
102505BLA COO	>2419,>2419	48,29	-,-	ND,ND,ND	None	15.0	ND	ND	ND	ND	ND	ND	ND	
102505CHU CCR	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Dry
102505CHU CRR	>2419,>2419	154,204	-,-	ND,ND,ND	None	14	ND	ND	ND	ND	ND	ND	ND	
102505GAB CRA	870,>2419	416,488	-,-	ND,ND,ND	None	13.7	ND	ND	ND	ND	ND	ND	ND	
102505GAB HER	>2419,>2419	2419,1203	-,-	ND,ND,ND	None	13.3	ND	ND	ND	ND	ND	ND	ND	
102505GAB NAT	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Dry
102505GAB OSR	1733,2419	1120,980	-,-	ND,ND,ND	None	12.9	ND	ND	ND	ND	ND	ND	ND	
102505GAB VET	1553,2419	17,28	-,-	ND,ND,ND	None	15.3	ND	ND	ND	ND	ND	ND	ND	
102505OLS MON	>24190,24190	170,250	-,-	ND,ND,ND	None	18.2	ND	ND	ND	ND	ND	ND	ND	
102505REC VIC	>2419,>2419	2419,2419	-,-	ND,ND,ND	None	15.8	ND	ND	ND	ND	ND	ND	ND	deep channel
102505SAL BLA	>2419,>2419	42,81	-,-	ND,ND,ND	None	16.2	ND	ND	ND	ND	ND	ND	ND	
102505SAL CHU	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
102505SAL DAV	2419,>2419	10,15	-,-	ND,ND,ND	None	16.4	ND	ND	ND	ND	ND	ND	ND	good flow
102505SAL GON	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
102505SAL MON	>2419,>2419	<1,17	-,-	ND,ND,ND	None	17	ND	ND	ND	ND	ND	ND	ND	
102505SDR PUM	>2419,>2419	199,238	-,-	ND,ND,ND	None	19.4	ND	ND	ND	ND	ND	ND	ND	
102505SRC COR	2610,2220	30,30	-,-	ND,ND,ND	None	15.7	ND	ND	ND	ND	ND	ND	ND	
102505TEM MOL	>24190,>24190	110,60	-,ND	ND,ND,ND	None	18.3	ND	ND	ND	ND	ND	ND	ND	
102505TEM PRE	2419,>2419	74,59	-,-	ND,ND,ND	None	16.6	ND	ND	ND	ND	ND	ND	ND	
102505TOW OSR	2419,2419	1120,1203	-,-	ND,ND,ND	None	13.6	ND	ND	ND	ND	ND	ND	ND	
		Total	33 (0)	0 (0)										
Nov-05 Monthly samples														
111505ALI AIR	4200,6130	20,310	-,-	ND,ND,ND	None	12.73	7.48	1.87	0.94	38.7	4.07	275	22.6	
111505BLA COO	6870,6490	10,30	-,-	ND,ND,ND	None	12.9	7.45	2.92	1.5	63.6	6.65	266	27.9	
111505CHU CCR	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Dry
111505CHU CRR	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Dry
111505GAB CRA	8660,6488	280,345	-,-	ND,ND,ND	None	12.88	8.33	0.7	0.34	101	10.6	316	33.7	
111505GAB HER	5794,5475	631,657	-,-	ND,ND,ND	None	11.39	8.43	0.7	0.34	100	10.9	328	18.6	

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
111505GAB NAT	ND,ND	ND,ND	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Dry
111505GAB OSR	657,985	231,169	-,-	ND,ND,ND	None	12.04	8.19	0.69	0.33	97.2	10.4	286	10.7	good flow_ evidence of flooding onto the adjacent field
111505GAB VET	2489,2382	31,31	-,-	ND,ND,ND	None	12.08	8.16	1.15	0.57	82.2	8.81	289	7	
111505OLS MON	19863,15531	185,146	-,-	ND,ND,ND	None	15.42	8.02	11.7	6.59	63.6	6.08	300	72.5	
111505REC VIC	24190,24190	602,663	-,-	ND,ND,ND	None	13.81	8.2	1.53	0.76	93.8	9.65	280	107	
111505SAL BLA	5794,1989	63,63	-,-	ND,ND,ND	None	15.09	7.94	1.91	0.96	106	10.6	305	16.8	
111505SAL CHU	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	flooded
111505SAL DAV	24196,19863	169,74	-,-	ND,ND,ND	None	15.44	8.52	1.49	0.74	150	14.9	250	29.2	
111505SAL GON	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
111505SAL MON	1789,1658	10,20	-,-	ND,ND,ND	None	15.94	8.26	2.85	1.47	66.1	6.47	299	24	
111505SDR PUM	>24200,>24200	676,865	-,-	ND,ND,ND	None	14.98	7.72	1.07	0.53	14.9	1.5	175	13.4	
111505SRC COR	>24200,>24200	206,226	-,-	ND,ND,ND	None	10.84	7.57	0.84	0.4	20.9	2.34	170	303	
111505TEM MOL	12033,5172	122,169	-,-	ND,ND,ND	None	15.83	8.12	13.5	7.69	61.1	5.74	301	65.4	
111505TEM PRE	7556,7701	161,175	-,-	ND,ND,ND	None	14.5	8.14	1.93	0.97	53.8	5.44	297	107	
111505TOW OSR	2603,2014	1119,1153	-,-	ND,ND,ND	None	13.37	8.02	0.67	0.32	96.3	10	187	11.9	
		Total	34 (0)	0 (0)										
Nov-05 Rain samples														
113005GAB CRA	ND,ND	ND,ND	-,-	ND,ND,ND	None	12.38	8.32	0.18	0.08	103	11.1	229	18.4	barbed wire, cattle NE of the bridge with access to the stream
113005GAB HER	ND,ND	ND,ND	-,-	ND,ND,ND	None	11.72	8.4	0.7	0.34	100	10.8	206	14.3	no increased flow due to the rain
113005GAB OSR	ND,ND	ND,ND	-,-	ND,ND,ND	None	11.65	8.27	0.68	0.33	102	11	202	7.6	clear, no increased flow due to the rain
113005SRC COR	ND,ND	ND,ND	-,-	ND,ND,ND	None	12.04	7.74	0.57	0.27	42.9	4.61	203	224	no flow

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
113005TOW OSR	ND,ND	ND,ND	+,-	ND,ND,ND	89	12.5	7.89	0.67	0.32	101	10.7	182	13	clear, no increased flow due to the rain, smell of manure
		Total[12]	10(1)	0 (0)										
Dec-05 Monthly samples														
121305ALI AIR	6840,4083	41,21	-,-	ND,ND, -	None	9.61	7.53	1.28	0.63	17.9	2.03	320	97.5	very slow flow
121305BLA COO	6590,3811	31,52	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
121305CHU CCR	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Dry
121305CHU CRR	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Dry
121305GAB CRA	3100,1970	960,650	-,-	ND,ND, -	None	10.74	8.52	0.74	0.36	91.9	10.2	244	164	
121305GAB HER	6500,2500	1310,780	-,-	-,ND,-	None	9.89	8.85	0.69	0.33	97.5	11	207	13	
121305GAB NAT	ND,ND	ND,ND	-,-	ND,ND,ND	None	9.78	7.79	0	0.01	36.8	4.18	237	45.1	
121305GAB OSR	703,573	246,218	-,-	ND,ND, -	None	10.19	8.38	0.68	0.33	88.2	9.89	237	10.7	clear 1'/sec
121305GAB VET	1440,1288	20,23	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
121305OLS MON	9900,9900	600,500	-,-	ND,-, -	None	11.17	8.11	1.52	0.75	73.3	8.01	291	55.8	
121305REC VIC	3450,2110	76,52	-,-	ND,ND,ND	None	11.95	8.07	1.71	0.85	120	12.9	274	130	2 ducks, very turbid
121305SAL BLA	1674,1370	98,120	-,-	ND,ND,ND	None	12.16	7.92	1	0.49	96.3	10.3	304	21.4	
121305SAL CHU	910,1296	36,31	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	C. Rose picked up no meter
121305SAL DAV	1450,1270	36,31	-,-	ND,ND,ND	None	10.84	8.35	0.58	0.28	111	12.2	257	20.1	
121305SAL GON	1486,2419	32,31	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	C. Rose picked up no meter
121305SAL MON	ND,ND	ND,ND	-,-	ND,ND,ND	None	12.32	8.16	2.77	1.42	91.4	9.68	306	27.9	
121305SDR PUM	4083,5000	630,520	-,-	ND,ND,ND	None	12.35	7.8	1.41	0.7	9.8	1.04	211	12.5	
121305SRC COR	4083,5940	201,231	-,-	ND,-, -	None	9.78	7.79	0	0.01	36.8	4.18	237	45.1	minimal flow <0.01CFS Swabs on surface not submerged

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
121305TEM MOL	4080,4870	630,630	-,-	ND,ND, -	None	11.17	8.03	1.76	0.88	66	7.19	300	206	
121305TEM PRE	3430,2140	84,52	-,-	ND,ND,ND	None	11.19	8.21	2.08	1.05	68.9	7.52	250	102	
121305TOW OSR	1480,1870	750,630	-,-	ND,ND, -	None	11.33	8.23	0.67	0.32	86.4	9.43	186	30.9	shallow ~ 5-7 " Clear
Dec-05 Rain samples		Total	38 (0)	10 (0)										
121905ALI AIR	ND,ND	ND,ND	-,-	ND,ND,ND	None	12.53	7.14	0.48	0.23	76.7	8.15	336	2000	muddy and flowing
121905CHU CRR	ND,ND	ND,ND	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	too muddy, couldn't collect meter data, small amount of flow
121905GAB CRA	ND,ND	ND,ND	-,-	ND,ND,ND	None	13.68	8.26	0.01	0.01	97.9	10.2	279	38.3	much higher flow compared to previous rain even
121905GAB HER	ND,ND	ND,ND	-,-	ND,ND,ND	None	13.57	8.34	0.66	0.32	94.6	9.81	269	57.3	new batteries, much higher flow compared to previous rain event
121905GAB OSR	ND,ND	ND,ND	-,-	ND,ND,ND	None	12.93	8.05	0.67	0.32	92.7	9.76	287	14	good clarity
121905GAB VET	ND,ND	ND,ND	-,-	ND,ND,ND	None	13.94	7.59	0.87	0.42	65.9	6.81	287	24.9	a little flow
121905OLS MON	ND,ND	ND,ND	-,-	ND,ND,ND	None	12.43	8.08	3.6	1.87	90.7	9.54	294	91.2	good flow, some flooding beyond the riverbank
121905SAL DAV	ND,ND	ND,ND	-,-	ND,ND,ND	None	13.28	8.48	0.46	0.22	118	12.3	270	45.4	low flow
121905SAL MON	ND,ND	ND,ND	-,-	ND,ND,ND	None	11.83	8.15	0.99	0.48	91.1	9.82	296	31.4	higher water levels
121905SRC COR	ND,ND	ND,ND	-,-	ND,ND,ND	None	13.64	7.6	0.51	0.25	82.7	8.58	262	2000	small amount of flow

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
121905TEM MOL	ND,ND	ND,ND	-,-	ND,ND,ND	None	13	7.91	0.62	0.3	75.8	7.94	284	2000	good flow
121905TOW OSR	ND,ND	ND,ND	-,-	ND,ND,ND	None	13.44	7.81	0.69	0.33	94.8	9.87	276	27.1	not much increase in flow, cattle and horses directly up stream
		Total	24 (0)	0 (0)										
Jan-06 Rain samples														
010406ALI AIR	20500,22400	2000,2000	-,-	ND,ND,ND	None	9.71	7.59	0	0	97.2	11.1	296	1382	Flowing and dirty
010406BLA COO	2510,3760	120,60	-,-	ND,ND,ND	None	14.88	7.88	3.06	1.58	92.9	9.28	286	105	good flow
010406CHU CRR	8910,4120	840,1020	-,-	ND,ND,ND	None	8.84	7.87	1.01	0.49	103	11.9	307	410	Flowing and dirty
010406GAB CRA	2580,3540	820,540	-,-	ND,ND,ND	None	11.5	8.46	0.47	0.22	102	11.1	205	67.8	Flowing and cleaner
010406GAB HER	3760,5000	760,960	-,-	ND,ND,ND	None	11.43	8.4	0.46	0.22	104	11.3	213	125	good flow
010406GAB NAT	6170,4830	400,550	-,-	ND,ND,ND	None	11.21	8.16	0.43	0.2	102	11.1	288	217	Flowing and dirty
010406GAB OSR	1540,1170	360,210	-,-	ND,ND,ND	None	11.01	8.07	0.48	0.23	103	11.3	271	31.9	Flowing and cleaner _ cattle on a tributary to the east of GAB
010406GAB VET	3760,4250	710,560	-,-	ND,ND,ND	None	12.61	7.99	0.53	0.25	99.2	10.5	276	177	good flow
010406OLS MON	17700,21400	1900,2100	-,-	ND,ND,ND	None	15.11	7.82	1.17	0.58	96.7	9.69	271	365	good flow, flowing backwards
010406SAL DAV	20400,18400	2700,2700	+,+	ND,ND,ND	90,91,92	10.89	8.56	0.48	0.23	111	12.3	211	2000	flooded over the roadway
010406SRC COR	20600,19700	1000,1000	-,-	ND,ND,ND	None	12.22	7.97	0.44	0.21	100	10.7	261	2000	good flow, dirty
010406TEM MOL	14100,16700	3100,3100	-,-	ND,ND,ND	None	11.74	7.8	0.87	0.42	90.3	9.78	281	901	good flow
010406TEM PRE	20100,25000	3800,3300	-,-	ND,ND,ND	None	11.87	8	0.74	0.36	89.7	9.66	230	992	good flow_ evidence of

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
														flooding onto the adjacent field
010406TOW OSR	2030,3760	440,860	-,-	ND,ND,ND	None	11.98	7.98	0.6	0.29	101	10.9	259	29.6	Flowing and cleaner _ cattle upstream
		Total	28 (2)	0 (0)										
Jan-06 Monthly samples														
011706ALI AIR	27780,25000	1450,1220	-,-	ND,ND,ND	None	8.32	7.72	0.46	0.22	70.1	8.21	300	824	
011706BLA COO	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Road flooded, unable to access
011706CHU CCR	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
011706CHU CRR	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
011706GAB CRA	1541,1354	305,216	-,-	-,-,-	None	9.96	8.28	0.59	0.28	91.9	10.4	278	18.1	
011706GAB HER	1835,2400	213,216	-,-	ND,ND,ND	None	10.06	8.43	0	0.01	93.4	10.5	305	99	
011706GAB NAT	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Dry
011706GAB OSR	1153,1354	171,189	-,-	-,-,-	None	9.52	8.23	0.6	0.29	92.5	10.5	228	10.6	
011706GAB VET	1777,2224	20,72	-,-	ND,ND,ND	None	14.52	7.72	1.18	0.58	80.5	8.15	321	15.1	
011706OLS MON	21780,22540	2590,4040	-,-	-,-,-	None	11.36	7.61	6.17	3.31	72	7.69	261	117	
011706REC VIC	23100,19040	860,1080	-,-	+,-,-	None	9.93	7.83	0.66	0.32	79.3	8.95	269	230	
011706SAL BLA	1892,1726	41,20	-,-	ND,ND,ND	None	9.67	8.3	0	0	92.8	10.6	238	101	
011706SAL CHU	1483,1892	10,31	-,-	ND,ND,ND	None	8.67	8.5	0.45	0.21	94.4	11	248	115	
011706SAL DAV	1301,886	63,20	-,-	-,-,+	93, 94, 95, 96	9.86	8.52	0.45	0.21	95.6	10.8	221	97.9	
011706SAL GON	1576,1317	20,74	-,-	ND,ND,ND	None	8.63	8.2	0.45	0.21	80.8	9.41	275	70.6	
011706SAL MON	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	Private Property, no access
011706SDR PUM	14670,17800	100,200	-,-	ND,ND,ND	None	11.73	7.24	0.34	0.16	16.5	1.77	247	23.6	
011706SRC COR	11370,12260	410,1090	-,-	ND,ND,ND	None	14.85	8.04	0.65	0.31	86.1	8.69	293	57.2	
011706TEM MOL	21050,16180	3360,1710	-,-	ND,ND,ND	None	10.87	7.7	11	6.14	74.7	7.78	322	131	
011706TEM PRE	12460,15650	400,630	-,-	ND,ND,ND	None	9.86	8.07	1.63	0.81	85.2	9.59	303	115	

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
011706TOW OSR	1670,1664	689,670	-,-	-,-,-	None	10.4	8.24	0.65	0.31	91.7	10.2	212	13.8	
		Total	32 (0)	12 (1)										
Feb-06 Monthly samples														
022206 ALI AIR	25950,29240	200,310	-,-	ND,ND,ND	None	8.5	8.08	0	0	31.9	3.75	224	204	
022206 BLA COO	426,556	20,63	-,-	ND,ND,ND	None	17.34	8.41	0	0.01	148	13.9	270	49.6	
022206 CHU CRR	345,231	<10,<10	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	bank too steep and muddy to access water
022206 GAB CRA	571,591	109,175	-,-	-,-,-	None	8.69	8.75	0.12	0.06	93.3	10.9	226	15.4	
022206 GAB HER	657,833	148,146	-,-	-,-,-	None	10.09	8.57	0.67	0.32	95.8	10.8	235	22.8	
022206 GAB OSR	317,317	98,75	-,-	-,-,-	None	8.38	8.39	0.65	0.31	91.6	10.7	257	14.1	
022206 GAB VET	1430,1439	31,41	-,-	ND,ND,ND	None	16.57	8.01	0	0.01	100	9.79	256	18.7	
022206 OLS MON	1354,2198	218,199	-,-	-,-,-	None	19.07	7.66	33.2	20.6	64.8	5.25	283	264	
022206 REC VIC	19360,19040	100,<100	-,-	-,-,-	None	15.01	8.31	1.11	0.55	127	12.8	272	41.8	
022206 SAL BLA	145,85	<10,41	-,-	ND,ND,ND	None	12.28	8.36	1.01	0.49	99.6	10.6	277	21.1	
022206 SAL CHU	158,146	10,10	-,-	ND,ND,ND	None	7.96	8.65	0.99	0.48	103	12.2	227	24.7	
022206 SAL DAV	73,537	<10,<10	-,-	-,-,-	None	12.66	8.88	0.95	0.46	122	12.9	248	20.9	
022206 SAL GON	199,110	20,41	-,-	ND,ND,ND	None	7.54	8.4	0.99	0.48	95.2	11.4	189	25.9	
022206 SDR PUM	1725,2700	41,52	-,-	ND,ND,ND	None	11.01	7.81	0.97	0.47	43.5	4.78	279	242	
022206 SRC COR	45690,39680	410,630	-,-	-,-,-	None	10.78	8.65	0	0.01	123	13.6	243	63.8	
022206 TEM MOL	3555,3333	62,410	-,-	-,-,-	None	15.42	8.3	2.26	1.15	81.6	8.09	257	548	
022206 TEM PRE	1679,2254	20,52	-,-	ND,ND,ND	None	14.07	8.38	2.07	1.05	101	10.4	279	120	
022206 TOW OSR	1178,934	364,373	-,-	-,-,+	97,98	10.02	8.38	0	0.01	94.1	10.6	212	19	
		Total	36 (0)	18 (1)										
Mar-06 Rain samples														
030706ALI AIR	57170,87040	12590,11910	-,-	ND,ND,ND	None	9.78	7.36	0.45	0.21	67.7	7.67	160	852	
030706BLA COO	3169,2352	131,96	-,-	ND,ND,ND	None	16.95	8.06	2.8	1.44	98.1	9.4	222	124	
030706CHU CRR	20480,20980	520,410	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
030706GAB CRA	14010,18600	2620,3050	-,-	ND,ND,ND	None	10.96	8.37	0	0.01	89.7	9.9	214	218	
030706GAB HER	86640,72700	4730,4350	-,-	ND,ND,ND	None	10.75	8.21	0.32	0.15	94.8	10.5	228	1954	

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
030706GAB NAT	34360,24150	2750,1690	-,-	ND,ND,ND	None	10.6	8.48	0.35	0.16	103	11.4	177	5999	
030706GAB OSR	1267,1169	323,259	-,-	ND,ND,ND	None	10.51	8.34	0.48	0.23	95.9	10.7	212	34.6	
030706GAB VET	38730,34480	5630,3680	-,-	ND,ND,ND	None	12.96	7.63	0.29	0.14	90.4	9.52	259	2000	
030706OLS MON	16070,13340	410,200	-,-	ND,ND,ND	None	15.38	7.61	11.1	6.21	69.2	6.64	243	201	
030706SAL BLA	5012,3578	95,74	-,-	ND,ND,ND	None	12.6	8.34	0.5	0.24	91.5	9.71	246	209	
030706SAL DAV	3436,3169	323,275	-,-	ND,ND,ND	None	12.41	8.27	0.41	0.2	91.5	9.76	253	214	
030706SRC COR	53350,64050	1710,1730	-,-	ND,ND,ND	None	11.78	7.86	0.23	0.11	93.3	10.1	234	5999	
030706TEM MOL	141360,86640	1600,1350	-,-	ND,ND,ND	None	13.85	8.04	0.94	0.46	77.2	7.97	233	1121	
030706TEM PRE	21780,31440	1610,1220	-,-	ND,ND,ND	None	13.73	7.9	0.87	0.42	80.8	8.33	226	1081	
030706TOW OSR	14390,15970	4040,3550	+,-	ND,ND,ND	99	11.75	8.22	0.59	0.28	96.2	10.4	205	206	
		Total	30 (1)	0 (0)										
Mar-06 Monthly samples														
032006 ALI AIR	3300,2667	120,86	-,-	ND,ND,ND	None	10.62	8.18	0.46	0.22	80.3	8.92	284	855	Heavy rains the entire day
032006 CHU CRR	3088,3314	146,98	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
032006 GAB CRA	1785,1246	249,299	-,-	+,+,+	93,102	11.06	8.2	0.46	0.22	80.9	8.89	304	45.3	
032006 GAB HER	1439,1296	121,241	-,+	+,+,-	99?	11.04	8.38	0.45	0.21	84.8	9.33	303	71.8	
032006 GAB NAT	27230,35780	520,520	-,-	+,+,+	93,101, 103	10.57	8.39	0.28	0.13	78.8	8.76	298	2000	
032006 GAB OSR	285,422	86,160	-,-	ND,ND,ND	None	10.55	8.24	0.43	0.2	88	9.78	292	35.7	
032006 GAB VET	34300,38110	2530,1580	-,-	ND,ND,ND	None	12.11	7.89	0.21	0.1	82.6	8.88	308	783	
032006 OLS MON	3877,4541	393,573	-,-	+,-,-	None	12.95	7.99	1.48	0.74	83.8	8.79	339	212	
032006 REC VIC	19680,14390	960,750	-,-	-,-,-	None	12.2	7.88	0.3	0.14	82.9	8.89	338	493	
032006 SAL BLA	1616,1145	52,10	-,-	ND,ND,ND	None	12.33	8.25	0.58	0.28	86.2	9.2	317	136	
032006 SAL CHU	471,697	120,75	-,-	ND,ND,ND	None	11.37	8.24	0.56	0.27	87.2	9.52	244	241	
032006 SAL DAV	1354,1664	168,265	-,-	-,-,-	None	12.3	8.33	0.56	0.27	85.6	9.15	305	134	
032006 SAL GON	743,909	135,185	-,-	ND,ND,ND	None	10.8	8.12	0.56	0.27	89.8	9.92	303	151	
032006 SDR PUM	38110,57480	14550,9590	-,-	ND,ND,ND	None	12.71	7.72	0.05	0.03	81.7	8.67	303	143	
032006 SRC COR	33140,43710	3270,1990	-,-	-,-,-	None	11.73	8.06	0	0.01	88	9.54	320	1253	
032006 SRC HOR	28780,14390	1220,620	-,-	ND,ND,ND	None	11.44	8.04	0.01	0.01	84.7	9.26	285	5999	
032006 TEM MOL	3282,5099	246,350	-,-	-,-,-	None	12.72	8.03	1.25	0.62	76.8	8.1	319	364	
032006 TEM PRE	28090,27780	1450,980	-,-	-,-,+	100	12.85	8.01	1.37	0.68	77.6	8.17	294	436	

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
032006 TOW OSR	1782,1576	384,364	-,-	+,-,+	97	11.78	8.17	0.59	0.28	88.5	9.55	256	37.8	
		Total	38 (1)	20 (7)										
Apr-06 Rain samples														
040506CHU CCR	55040,66530	30440,29240	+,-	ND,ND,ND	104	8.84	8.15	0.28	0.13	93.8	10.9	274	2000	
040506CHU CRR	53350,53350	6020,6770	-,-	ND,ND,ND	None	9.66	7.25	0.34	0.16	89.6	10.2	330	5999	Heavy flow
040506ELT 68	17800,15700	5040,3320	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
040506GAB CRA	12340,10760	6570,4870	+,+	ND,ND,ND	89,93	11.36	7.89	0.01	0.01	91.6	9.99	263	624	strong flow from Crazy Horse creek
040506GAB HER	16740,17800	6200,6200	+,-	ND,ND,ND	89	11.42	8.14	0.02	0.01	95.4	10.4	208	817	heard rooster crowing
040506GAB NAT	20120,17270	7590,4800	+,+	ND,ND,ND	16,89,93	11.57	7.95	0.23	0.11	94.1	10.2	251	1568	
040506GAB OSR	9080,5910	2280,1580	+,+	ND,ND,ND	93, 102	10.3	7.93	0.25	0.12	94.2	10.5	275	537	
040506ORO END	22090,27780	11780,12460	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
040506SAL CHU	7270,5910	2310,1310	-,-	ND,ND,ND	None	11.55	7.72	0.3	0.14	90.3	9.82	317	1572	
040506SAL DAV	10710,9340	2460,2230	-,-	ND,ND,ND	None	12.59	8.22	0.33	0.15	90.4	9.6	212	2000	flooded over the roadway
040506TEM PRE	33140,48330	8820,7590	+,-	ND,ND,ND	93	12.44	7.85	0.37	0.17	83.8	8.95	250	2000	flooded
040506TOW OSR	11120,15290	4870,5480	+,+	ND,ND,ND	89	11.3	7.92	0.31	0.15	97.8	10.7	272	245	
		Total	24 (11)	0 (0)										
Apr-06 Monthly samples														
041806 ALI AIR	173290,>241,960	4640,13740	+,-	ND,ND,ND	100, 105	12.38	8.27	0.62	0.3	123	13.2	251	26.3	
041806 ARR GOR	243,41	10,20	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
041806 BLA COO	7630,23340	100,100	-,-	ND,ND,ND	None	19	8.5	2.77	1.43	190	17.5	239	40.9	
041806 ELT 68	12340,15850	1610,970	-,-	-,-,-	None	17.46	8.07	0.76	0.37	117	11.2	229	106	
041806 GAB CRA	1561,2841	275,201	-,-	+,+,+	89, 109	11.8	8.6	0.47	0.22	127	13.8	190	40.6	
041806 GAB HER	1529,1727	189,168	-,-	-,-,+	89	12.48	8.38	0	0.01	117	12.5	224	159	
041806 GAB NAT	43710,39450	2410,1970	-,-	-,-,+	89	15.36	8.41	0.48	0.23	116	11.6	249	341	
041806 GAB OSR	591,414	41,31	-,-	-,-,-	None	10.73	8.25	0.43	0.2	119	13.2	236	40.3	
041806 GAB SAN 1	862,697	<10,<10	-,-	ND,ND,ND	None	15.13	7.91	0.3	0.14	86.4	8.68	195	18.9	
041806 GAB SAN 2	3945,4635	31,20	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
041806 GAB SAN 3	1882,1391	253,359	-,-	ND,ND,ND	None	14.32	8.21	0.51	0.24	119	12.3	259	36.3	

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
051506 SAL GON	1887,2025	52,20	-,-	ND,ND,ND	None	19.68	7.99	0.66	0.32	76.8	7.01	271	43.8	
051506 SCR COR	13740,10760	200,100	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
051506 SDR PUM	13360,21780	100,100	-,-	ND,ND,ND	None	15.47	7.93	1.68	0.84	84.5	8.38	240	15.3	
051506 SRC 101	ND,ND	ND,ND	ND,ND	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
051506 SRC HEB sediment	ND,ND	ND,ND	ND,ND	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
051506 SRC HOR	30880,27530	4570,4080	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
051506 SRC RUS	43600,30880	2160,1200	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
051506 TEM MOL	7440,4220	520,310	-,-	-,-,-	None	22.47	8.54	2.76	1.42	158	13.6	269	222	
051506 TEM PRE	4950,3880	200,200	-,-	ND,ND,ND	None	21.87	8.46	1.91	0.97	153	13.3	275	224	
051506 TEM SJR	1374,1374	52,52	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
051506 TOW OSR	3640,4810	1310,2110	-,-	-,-,-	None	15.98	8.04	0.65	0.31	101	9.93	238	16.8	
051506 TOW OSR 2	7800,7850	1560,1220	-,-	ND,ND,ND	None	19.5	7.79	0	0.01	93.7	8.61	276	37.6	
051506 TOW OSR 3	13910,14140	8860,8820	+,+	+,+,+	143, 144, 145, 146	18.72	8.4	0.64	0.31	102	9.53	247	50.8	
		Total	50 (3)	30 (7)										
Jun-06 Monthly samples														
061906 ALI AIR	>24196,10112	631,591	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 BLA COO	>24196,24196	75,120	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 GAB CRA	3448,2755	1250,1274	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 GAB HER	>24196,>24196	703,832	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 GAB OSR	1281,1354	345,441	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 GAB VET	15531,11197	228,135	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 OLS MON	>24196,5335	105,51	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 REC VIC	10462,17330	504,310	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 SAL BLA	6167,4366	121,228	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 SAL CHU	1296,1187	74,85	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 SAL DAV	2924,2603	107,148	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 SAL GON	1414,1296	63,86	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 SCR COR	10112,10112	3436,3968	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 SDR PUM	4674,15531	191,262	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 SRC 101	>24196,6910	310,520	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
061906 SRC RUS	10112,10112	1071,1789	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 TEM MOL	10112,>24196	41,30	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 TEM PRE	3877,2933	185,243	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 TEM SJR	12033,7701	279,292	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 TOW OSR	5170,3784	1153,697	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 TOW OSR 1.5	2700,4884	836,959	-,-	+,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 TOW OSR 2	2481,3130	691,624	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 TOW OSR 4	5475,5172	2247,1918	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
061906 TOW OSR sed-A	ND,ND	ND,ND	ND,ND	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
		Total	46 (0)	30 (0)										
Jul-06 Monthly samples														
071806 ALI AIR	65860,54930	410,740	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 BLA COO	26020,29870	209,199	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 GAB CRA	8840,4640	1350,410	+,+	-,-,-	142	ND	ND	ND	ND	ND	ND	ND	ND	
071806 GAB HER	57480,43600	410,410	-,-	-,+,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 GAB OSR	318,983	31,364	-,-	+,+,+	142, 155, 156, 157	ND	ND	ND	ND	ND	ND	ND	ND	
071806 GAB VET	15760,9870	74,51	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 OLS MON	79150,79150	74,61	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 REC VIC	8600,10140	520,980	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 SAL BLA	6970,7120	63,98	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 SAL CHU	5910,4130	85,10	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 SAL DAV	1616,2075	<10,52	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 SAL GON	5540,6240	62,63	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 SCR COR	15760,11870	1100,740	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 SDR PUM	54930,43600	1340,1090	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 SRC 101	ND,ND	ND,ND	-,ND	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 SRC RUS	26020,36090	200,200	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 TEM MOL	39680,43000	<10,41	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 TEM PRE	24890,22820	74,134	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 TEM SJR	3840,4870	411,410	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 TOW OSR	6500,5630	2030,2110	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
071806 TOW OSR 1.5	13540,17850	11370,10760	-,-	+,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 TOW OSR 2	11300,12230	2130,1100	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
071806 TOW OSR 4	12810,14500	8010,10500	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
		Total	45 (1)	32 (3)										
Aug-06 Monthly samples														
082206 ALI AIR	>241960,>241960	1090,520	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 ARR GON	2333,3654	<10,<10	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 BLA COO	24810,27550	75,98	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 GAB CRA	1565,1835	521,645	-,-	+,-,+	158,162	ND	ND	ND	ND	ND	ND	ND	ND	
082206 GAB HER	5650,6380	1210,860	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 GAB OSR	1374,1317	241,259	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 GAB VET	15650,14670	52,52	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 OLS MON	241960,>241960	710,830	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 REC VIC	19350,18600	410,200	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 SAL BLA	6910,4500	563,464	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 SAL CHU	145,2841	10,134	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 SAL DAV	14390,17890	20,20	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 SAL GON	2750,6760	740,310	+,+	ND,ND,ND	160,161	ND	ND	ND	ND	ND	ND	ND	ND	
082206 SCR COR	3436,6867	30,52	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 SDR PUM	27550,51720	630,364	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 SRC 101	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 SRC RUS	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 TEM MOL	26030,21870	300,510	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 TEM PRE	34410,23820	410,310	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 TEM SJR	26130,20980	630,100	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 TOW OSR	6270,9590	970,1320	-,-	+,+,+	162	ND	ND	ND	ND	ND	ND	ND	ND	
082206 TOW OSR 1.5	4800,2950	1100,410	-,+	+,+,+	159, 162	ND	ND	ND	ND	ND	ND	ND	ND	
082206 TOW OSR 2	4950,2920	860,410	-,-	+,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
082206 TOW OSR 4	4480,6570	1220,1350	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
		Total	44 (3)	24 (5)										
Sep-06 Monthly sample (delayed)														
101006 ALI AIR	54750,72700	4480,4870	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
101006 ARR GON	657,789	<10,<10	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 BLA COO	12590,8360	20,41	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 GAB CRA	6440,3930	2750,1990	-,-	+,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 GAB HER	7890,7330	327,341	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 GAB OSR	3990,545	160,108	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 GAB VET	15530,10700	63,107	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 OLS MON	21430,20260	20,41	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 REC VIC	9880,26460	262,327	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 SAL BLA	1835,2489	75,86	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 SAL CHU	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 SAL DAV	46110,57940	345,428	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 SAL GON	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 SDR PUM	38730,29090	1950,1480	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 SRC 101	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	No flow, water pooled
101006 SRC COR	ND,ND	ND,ND	ND,ND	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	No flow, water pooled
101006 SRC RUS	ND,ND	ND,ND	ND,ND	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	no flow
101006 TEM MOL	34480,32550	41,84	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	minimal flow <0.01CFS Swabs on surface not submerged
101006 TEM PRE	41060,81640	520,520	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	small amount of flow
101006 TEM SJR	27230,27230	134,122	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	good flow, dirty
101006 TOW OSR	6500,7330	1560,2280	-,-	ND,ND,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 TOW OSR 1.5	1552,2750	459,481	-,-	+,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 TOW OSR 2	3640,4080	980,970	-,-	+,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
101006 TOW OSR 4	2352,1993	350,345	-,-	+,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
		Total	38 (0)	15 (0)										
		Grand total	600 (28)	213 (37)										

1 Coliform and generic *E. coli* levels from duplicate water samples (separated by a comma) determined by Colilert test (MPN/100ml)
2 Incidence of EcO157 from duplicate water samples (separated by a comma); +,EcO157 detected; -,EcO157 not detected

3 Incidence of EcO157 from duplicate Moore swab samples (separated by commas) by real-time PCR detection after Pathetrix, culture after Pathetrix, enhanced IMS
4 MLVA types recovered from the sample. MLVA type designations were assigned sequentially.
5 Specific Conductivity (mS/cm)
6 Salinity (PSS)
7 Dissolved Oxygen (percent of saturation)
8 Dissolved Oxygen (mg/L)
9 Oxidative Reduction Potential (mV)
10 Turbidity (NTU)
11. ND, not determined
12. Total number of samples analyzed for each sampling date (number of positive samples). Swab totals do not include real-time PCR positives unless verified by culture from the same swab.

Table 2. Location and description of sample sites

Site Name	Code	Latitude	Longitude	Description
TOW OSR	A	36.79557	-121.57503	Towne Creek at Old Stage Rd
GAB OSR	B	36.78047	-121.58543	Gabilan Creek at Old Stage Rd.
GAB CRA	C	36.77144	-121.60209	Gabilan Creek at Crazy Horse
GAB HER	D	36.75583	-121.61037	Gabilan Creek at Hebert Rd
GAB NAT	E	36.73122	-121.61250	Gabilan Creek at Natividad Rd.
GAB VET	F	36.69405	-121.62724	Gabilan Creek at Veteran's Park
TEM PRE	G	36.76512	-121.75960	Tembladero Slough at Preston Rd
TEM MOL	H	36.77165	-121.78659	Tembladero Slough at Molera Rd
SAL DAV	J	36.64687	-121.70209	Salinas River at Davis Rd
OLS MON	K	36.77165	-121.79008	Old Salinas River at Monterey Dunes Way
REC VIC	L	36.68481	-121.66828	Reclamation Ditch at Victor St.
CHU CCR	M	36.602126	-121.430729	Chular River at Chular Canyon Rd
ALI AIR	N	36.66138	-121.62101	Alisal Creek at the Airport
SAL GON	O	36.48722	-121.46917	Salinas River Gonzolas
SAL CHU	P	36.55560	-121.54860	Salinas River at Chular River Rd.
CHU CRR	Q	36.56177	-121.54233	Chular River at Chular River Rd
SAL MON	R	36.73185	-121.78199	Salinas River at Monte Rd
SAL BLA	S	36.67805	-121.74528	Salinas River at Blanco Rd.
BLA COO	T	36.69855	-121.73498	Blanco Drains at Cooper Rd.
SRC COR	U	36.73439	-121.64204	Santa Rita Creek at Cornwall St.
SDR PUM	V	36.66038	-121.68340	Storm Drains at the pump station
		Experimental Sites		
ARR GOR		36.280521	-121.322708	Arroyo Seco at gorge in park
TEM SJR		36.70496	-121.7052	Tembladero Slough at San Jon Rd
TOW OSR 1.5		36.797692	-121.570233	Towne Creek 0.5 km upstream of A
TOW OSR 2		36.79969	-121.56857	Towne Creek 1.0 km upstream of A
TOW OSR 3		36.800183	-121.566389	Towne Creek 1.7 km upstream of A
TOW OSR 4		36.808597	-121.553353	Towne Creek 2.7 km upstream of A
SRC 101		36.725486	-121.658422	Santa Rita Creek at Hwy 101
SRC RUS		36.730549	-121.642489	Santa Rita Creek at Russell Rd.
ELT 68		36.580836	-121.719244	El Toro Creek at Hwy 68
ORO END		36.612086	-121.689675	Eastern entrance, Toro Regional Park
GAB SAN 1		36.773564	-121.603033	Tributary to Gabilan, San Juan Grade Rd.
GAB SAN 2		36.789101	-121.589060	Tributary to Gabilan, San Juan Grade Rd.
GAB SAN 3		36.801131	-121.579741	Tributary to Gabilan, San Juan Grade Rd.
SRC HOR		36.727338	-121.673372	Santa Rita Creek at horse ranch
SRC HEB		36.762458	-121.620823	Santa Rita Creek at Hebert Rd.

Table 3. Water samples summary

Date	Reason sampled	Rainfall (inches)[a]	No. of samples	Average *E. coli* (SE)[b]	EcO157 incidence[c]	*Ec*O157-positive location[d]
Oct 25-05	Monthly	0.05	33	467 (123)	0.0	None
Nov 15-05	Monthly	0.01	34	300 (53)	0.0	None
Nov 30-05	Rain	0.38	10	ND	0.1	A
Dec 13-05	Monthly	0.00	38	304 (58)	0.0	None
Dec 19-05	Rain	1.07	24	ND	0.0	None
Jan 4-06	Rain	2.25	28	1553 (223)	0.071	J
Jan 17-06	Monthly	0.45	32	693 (175)	0.0	None
Feb 22-06	Monthly	0.43	36	124 (25)	0.0	None
Mar 7-06	Rain	1.39	30	2430 (567)	0.033	A
Mar 20-06	Monthly	1.08	38	1202 (447)	0.026	D
April 5-06	Rain	1.79	24	7510 (1523)	0.458	A,B,C,D,E,G,M
April 18-06	Monthly	0.2	50	1293 (440)	0.08	A,G,H,N
May 15-06	Monthly	0.00	50	899 (249)	0.02	C
Jun 19-06	Monthly	0.00	46	458 (136)	0.0	None
Jul 18-06	Monthly	0.00	45	1440 (444)	0.044	C
Aug 22-06	Monthly	0.00	44	487 (58)	0.045	O
Oct 10-06[e]	Monthly	0.00	38	768 (189)	0.0	None
		Total =	600		9 dates positive	

[a] Rainfall was computed as a five day accumulation prior to and including the sample date and is the average from four gauges in the Salinas area.

[b] SE, standard error of the mean; ND; not determined.

[c] Number of positive samples/total number of samples.

[d] Location codes are shown in Figure 1.

[e] September sampling was delayed due to spinach outbreak investigation.

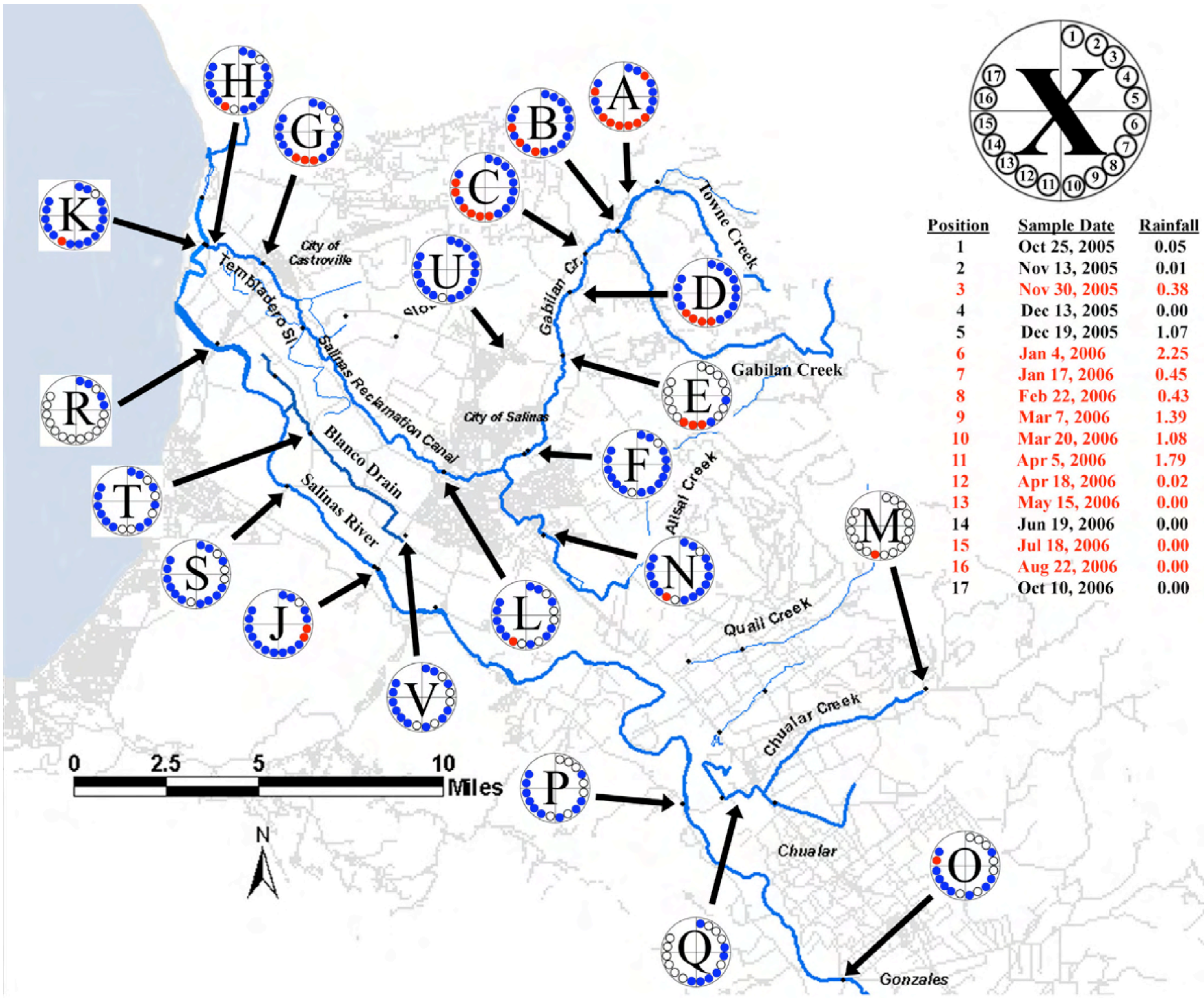
Table 4. Temporal and spatial relationships between MLVA types recovered from water and swab samples.

MLVA type (location[a])												
2005		**2006**										
8/15	11/30	1/4	1/17	2/22	3/7	3/20	4/5	4/18	5/15	5/23	7/18	8/22
16(A)[c]		90(J)					16(E)	16(A)	142(CD)	150(A)	155(B)	158(C)
15(A)[c]	89(A)	91(J)					89(ACDE)	89(ACDE)	143(AC)	143(A)	156(B)	159(A)
		92(J)	93(J)			93(CE)	93(BCEG)	105(N)	144(A)	148(A)	157(B)	160(O)
			94(J)	97(A)		97(A)		97(G)	145(A)	145(A)		161(O)
			95(J)	98(A)	99(A)	99(D)		106(A)	146(A)	146(A)		162(AC)
			96(J)			100(G)		100(GHKLN)	154(B)	147(A)		
						101(E)		107(A)		149(A)		
						102(C)	102(B)	108(A)		151(A)		
						103(CE)	104(M)	109(AC)		152(A)		
								122(A)				

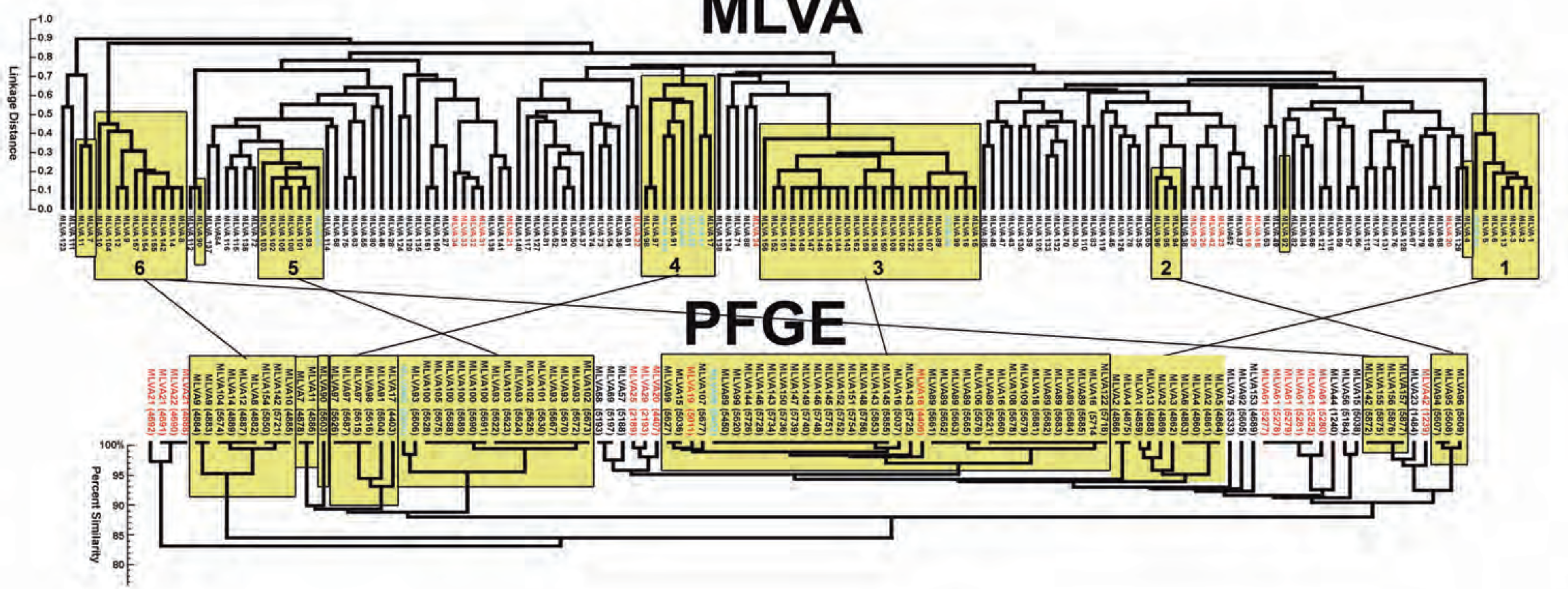
[a] Locations in the Salinas watershed refer to Figure 1 and Table 2
[b] Colors indicate MLVA types showing temporal displacement.
[c] Cattle feces isolates collected near Towne Creek.

Table 1 Combined sample data

Sample	Coliform[1]	E. coli[1]	Water sample[2]	Moore Swab[3]	MLVA Types[4]	Temp	pH	SpC[5]	Salin[6]	DO%-Sat[7]	DO-mg/L[8]	ORP[9]	Turb[10]	Notes
041806 GAB VET	12540,8200	100,100	-,-	ND,ND,ND	None	17.74	8.14	0.61	0.3	126	12.2	256	224	
041806 OLS MON	9340,8160	310,730	-,-	-,+,+	100	17.05	7.85	1.19	0.59	123	11.8	225	191	
041806 REC VIC	57170,51630	3090,16240	-,-	-,+,+	100	17.77	7.81	0.64	0.31	101	9.62	262	173	
041806 SAL BLA	1374,1191	31,<10	-,-	ND,ND,ND	None	15.91	8.11	0	0.01	115	11.4	248	121	
041806 SAL CHU	1430,2367	30,41	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
041806 SAL DAV	1674,2981	52,52	-,-	ND,ND,ND	None	15.78	8.38	0.51	0.24	124	12.2	199	110	
041806 SAL GON	368,708	41,20	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
041806 SDR PUM	>241,960,>241,960	3320,3310	-,-	-,-,-	None	13.82	7.59	0	0.01	63.9	6.65	182	57	
041806 SRC COR	14390,14970	200,750	-,-	ND,ND,ND	None	17.85	8.16	0.65	0.32	155	14.7	239	112	
041806 TEM MOL	8820,8330	850,310	+,-	-,+,+	100	16.69	7.95	1.16	0.57	146	13.8	247	197	
041806 TEM PRE	15290,14390	970,630	+,-	-,-,+	97, 100	17.47	7.74	1.12	0.55	106	10.1	258	230	
041806 TOW OSR	1679,1835	754,573	+,-	+,+,+	16,89,106,107,108,109,122	12.41	8.03	0.62	0.3	101	10.7	257	37.3	
041806 TOW OSR 2	1145,985	295,384	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
041806 TOW OSR 3	1124,1552	201,231	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
		Total	50 (4)	22 (13)										
May-06 Monthly samples														
051506 ALI AIR	53350,241960	850,1090	-,-	ND,ND,ND	None	17.66	7.61	0	0.01	50.4	4.81	275	205	
051506 ARR GOR	365,649	11,9	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
051506 BLA COO	1145,1414	31,10	-,-	ND,ND,ND	None	23.08	8.24	2.73	1.41	159	13.5	271	41.6	
051506 ELT 68	3410,2130	860,310	-,-	-,-,-	None	ND	ND	ND	ND	ND	ND	ND	ND	
051506 GAB CRA	2280,2090	860,950	-,+	+,+,+	142, 143	15.74	8.12	0	0.01	109	10.8	241	22	
051506 GAB HER	1920,13760	300,2330	-,-	-,+,+	143	17.3	8.24	0	0.01	109	10.5	248	71.8	
051506 GAB NAT	ND,ND	ND,ND	-,-	ND,ND,ND	None	ND	ND	ND	ND	ND	ND	ND	ND	
051506 GAB OSR	1012,759	156,246	-,-	+,+,-	154	14.66	8.11	0.56	0.27	100	10.2	265	16.9	
051506 GAB VET	10580,5940	300,310	-,-	ND,ND,ND	None	23.44	7.67	1.24	0.62	84	7.11	259	23.9	
051506 OLS MON	3050,3180	520,410	-,-	-,-,-	None	23.46	8.62	4.55	2.42	207	17.3	265	330	
051506 REC VIC	2584,3026	189,122	-,-	-,-,-	None	23.15	8.51	1.16	0.57	154	13.1	230	71.1	
051506 SAL BLA	2098,1421	31,31	-,-	ND,ND,ND	None	23.08	8.38	0.65	0.32	107	9.13	244	91.9	
051506 SAL CHU	2392,573	144,86	-,-	ND,ND,ND	None	20.01	8.2	0.59	0.29	72.8	6.61	229	51.1	
051506 SAL DAV	1470,1169	20,<10	-,-	-,-,-	None	23.01	8.37	0.66	0.32	107	9.17	261	64.2	



Position	Sample Date	Rainfall
1	Oct 25, 2005	0.05
2	Nov 13, 2005	0.01
3	Nov 30, 2005	0.38
4	Dec 13, 2005	0.00
5	Dec 19, 2005	1.07
6	Jan 4, 2006	2.25
7	Jan 17, 2006	0.45
8	Feb 22, 2006	0.43
9	Mar 7, 2006	1.39
10	Mar 20, 2006	1.08
11	Apr 5, 2006	1.79
12	Apr 18, 2006	0.02
13	May 15, 2006	0.00
14	Jun 19, 2006	0.00
15	Jul 18, 2006	0.00
16	Aug 22, 2006	0.00
17	Oct 10, 2006	0.00


MLVA
Linkage Distance
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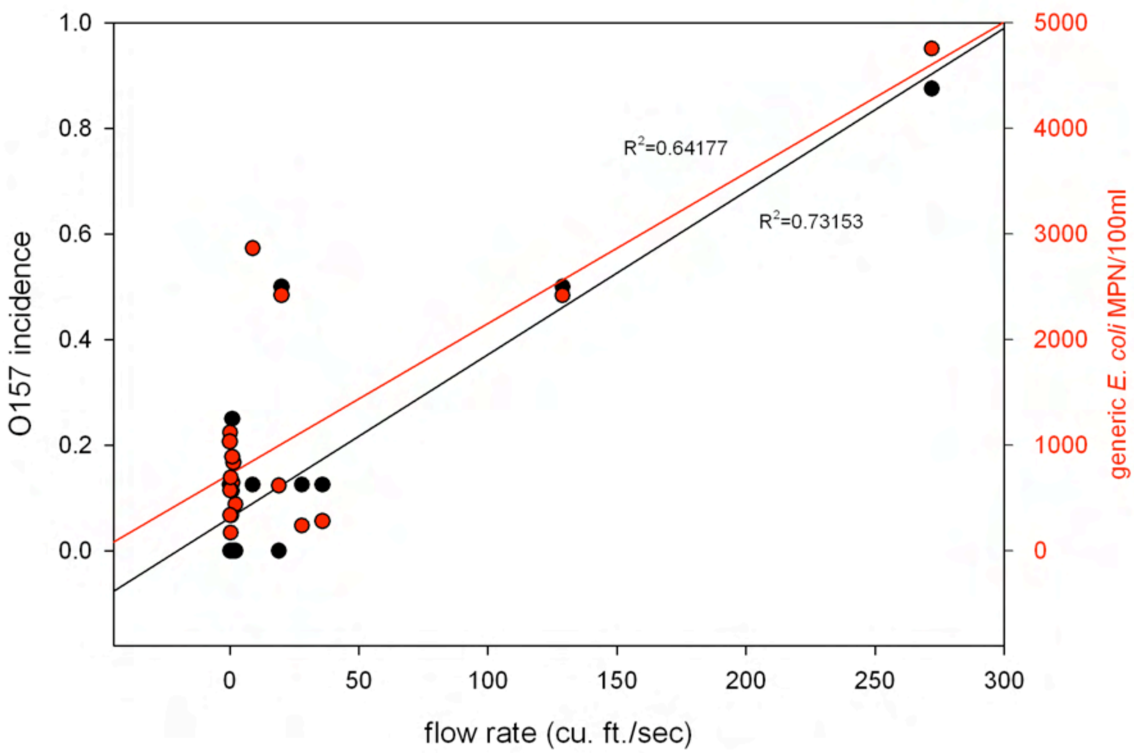
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
6
5
4
3
2
1
PFGE
100%
95
90
85
80
Percent Similarity



O157 incidence
1.0
0.8
0.6
0.4
0.2
0.0
R2=0.64177
R2=0.73153
0
50
100
150
200
250
300
flow rate (cu. ft./sec)
5000
4000
3000
2000
1000
0
generic E. coli MPN/100ml