CA LEAFY GREEN RESEARCH BOARD

2012-13 Project Report

Title: Developing risk-based preventive controls: synthesis from on-farm trials and research literature data-mining.

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ADMINISTRATOR'S NOTE: The CLGRB provides Dr. Suslow with Rapid Response funding. In the unlikely event that a grower learns of a significant contamination event on a ranch, the Suslow team can be called in on short notice to investigate the event. These studies provide the industry with more information about sources of contamination, pathogen survival, sampling methodology, and remediation procedures. These events represent a very difficult situation for a grower who has no choice but to destroy the crop. We greatly appreciate the grower's willingness to allow the industry to learn more about foodborne pathogens under actual field conditions.

Objective - Assessing Soil Sample Methodology for *Salmonella enterica* and Enterohemorrhagic *E. coli* Testing in Commercial Fields.

Introduction: Typical commercial, risk-based soil testing procedures to qualify a field for fresh produce production relies on 10 gram samples for screening. Practical evidence indicated this was inadequate. To improve preventive control standards, assessment of sample mass and pathogen detection protocols are necessary.

Purpose: To assess the effect of sample size on the reliability of pathogen detection schemes in agricultural soils.

Methods: Soil samples were screened for *Salmonella enterica* and enterohemorrhagic *E. coli* (EHEC), respectively. Thirteen fields from one ranch were monitored for persistence of a natural *S. enterica* contamination following application of inadequately managed compost. A single field affected by animal intrusion was screened for EHEC presence. Standard testing was performed using 10 g of soil, enrichment, and PCR screening. Soil samples from initially positive fields were comparatively analyzed

using 10 g and 100 g directly- enriched and 100 g soil extraction with sodium phosphate supplemented with 0.01% Tween 20 (NPT). Selected negative fields, based on 10 g samples, were retested with 100 g from the same retained soil sample.

Results: Detection using standard 10 g was achieved in 45 of 252 samples, from 5 of 13 fields. *S. enterica* detection using 10 g and 100 g directly enriched had 29.2% and 62.5% positives, respectively. Contrary to other applications of NPT to soil, pre-enrichment extraction on 100 g resulted in 4.2% *S. enterica* positives. All 10g positives tested at 100g sample size increased the frequency of positives among replicates and previously negative lots had positive outcomes among the replicate samples. Detection of EHEC in a different soil using 10 g direct, 100 g NPT extraction, and 100 g direct-enrichment had 20%, 25% and 35% positives, respectively.

Significance: As part of a valid soil sampling plan, sample mass is a significant determinant for field risk assessment and pre-planting standards. Increasing the standard sample size to 100 grams may increase the chance of detecting low level pathogen contamination. Natural contamination, attributed to a compost source, with Salmonella persisted in field soil for more than 135 days.

INTRODUCTION

Pathogen testing programs conducted on diverse raw and minimally-processed produce, especially since 2007, have demonstrated that contamination capable of or known to have reached market channels is present on fruits and vegetables intended for fresh consumption. Although the frequency and prevalence appears to be very low, these industry intercepts within pre-harvest and post-harvest monitoring programs have resulted in substantial loss to individual growers and handlers. Destruction of multiple fields per year, occasionally hundreds of acres, is damaging economically but also may negate initiatives of sustainability for the operation. While the decision to destroy a field or produce lot in cold storage is the responsible action when pathogens are detected, prevention of recurrence of contamination in rotational or replanted vegetable crops, originating from the prior contamination event, is an important component of a farm safety plan. Recently completed studies, within the Center for Produce Safety program (Koike et al. 2010 unpublished; https://cps.ucdavis.edu/grant_opportunities_awards.php), have demonstrated persistence of Salmonella in soil-crop residue following incorporation of more than 100 days. Prior to replanting, the applied Salmonella was detected only following a selective enrichment procedure signifying very low surviving populations. Transfer from soil to baby spinach seeded in the experimental plot, after this fallow interval, resulted in approximately 50% detection of the specific applied Salmonella on spinach leaves at the 2-3 and 5-6 leaf stages, simulating typical commercial harvest maturity.

Whether on crop residue, from an irrigation source, the result of flooding, contaminated manure/compost or other acute contamination incident, persistent populations of human pathogens in soil can have devastating impacts for a grower or region This project's goal is to develop practices and supporting data that would apply to remediation and recovery of soil contaminated by Salmonella following intentional application of chicken manure/litter or compost during pre-plant and pre-harvest fertility management of vegetable crops.

Various US-based commodity-specific guidance documents, standards, and audit checklists describe the acceptance criteria for documentation of manure/compost/thermally-treated pellet supplier Certificate of Analysis, pathogen reduction prior to land application, microbiological testing, and pre-harvest intervals. An example is found in Commodity Specific Food Safety Guidelines for the Production, Harvest, Post-Harvest, and Processing Unit Operations of Fresh Culinary Herbs (15). Despite a best intent to work with suppliers to establish full continuity of programs to prevent re-growth or re-contamination,

Salmonella contaminated manure, litter, compost, or pellets applied to horticultural soils have resulted in contaminated crops and residually contaminated soils. Salmonella has been shown to be very persistent in horticultural crop soils, surviving on the surface for extended periods under summer conditions in the Sacramento and San Joaquin Valleys (18, 30).

A CLGRB supported Rapid Response effort during 2012-2013 was made available to the Suslow Lab which provided the opportunity to evaluate such natural contamination events involving large land areas cropped to lettuce and leafy greens. While full details are beyond the necessary scope of this proposal, improperly managed compost was involved in each situation.

1. MATERIAL AND METHODS

1.1. Environmental sampling of contaminated soil

1.1.1. Site A.

Thirteen fields from one ranch which had unknowingly been amended with inadequately managed/treated compost were assessed for *S. enterica* presence. The ranch was located within a major produce production region in California. The grower and handler became aware of this contamination event as a result of their standard preharvest testing programs. Following the initial confirmed recovery of Salmonella from the diverse mini-greens and leafy green crops, and subsequent to the destruction of these crops by discing and incorporation, the soil associated with the parcels was monitored for *S. enterica* for a period of four months, from June to September 2012, and separated into three sampling days. The irrigation source for the entire ranch was surface water and was applied to the fields by either sprinkler or drip irrigation. The number of soil samples per site varied from 6 to 29 and were collected into Whirl-pak bags (Whirl-Pak; Nasco, Modesto CA, USA) for transportation to UCD in coolers. Samples were analyzed for detection of Salmonella after standard non-selective and selective enrichment followed by PCR screening. Sampling consisted in five 50 g scoops from an area of 10 by 10 cm and 5 to 15 cm deep. Follow up sampling of positive fields and adjacent blocks occurred within the project timeframe. The last set of pre-plant samples occurred in October 2012. Samples were also collected and analyzed the following production season in 2013 for the fields that tested positive in Oct 2012 samplings.

1.1.2. Site B.

A single sprinkler irrigated spinach field was heavily impacted by bird droppings. At the time of sampling the impacted crop area was already disked down and reincorporated into the soil. Twenty representative soil samples of five 50 g scoops from an area of 10 by 10 cm and 5 to 15 cm deep were taken for shigatoxin-producing *E. coli* (STEC) screening. An earlier preharvest testing of the crop in these areas by a private lab had detected E. coli O157:H7 and therefore we restricted our analysis to this target group based on available personnel and funding resources. In addition to soil samples, 30 plant samples were taken from an adjacent non-disked area where multiple groups, that were judged to the best of our ability, of American Crow (*Corvus brachyrhynchos*) and Common Raven (*Corvus corax*) were observed during an initial assessment visit by Suslow and were still present in the standing crop area during the soil collection interval a few days later.

1.2. Soil recovery methodology comparison

1.2.1. Soil Extraction

Following homogenization of the composite soil samples, 100 g replicates were removed an placed in a new Whirl-Pak® bag (Nasco , Salida, CA); 200 mL of 0.01 M sodium phosphate supplemented with 0.05% Tween 20 were added (Fisher, Fair Lawn, NJ)(NPT). The suspension was gently shaken and allowed to settle for 20 min (Gutierrez-Rodriguez et al., 2011; G. Lopez-Velasco et al., 2012). In addition, 50 mL of the soil-extraction supernatant were transferred into 100 mL of double strength (2X) Universal Pre-enrichment Broth (UPB; Difco, Sparks, MD) and incubated at 37 °C for 12-14 h.

1.2.2. Direct Soil Enrichment

For comparison of detection and recovery efficiencies, direct soil enrichment was also conducted on each soil composite per ranch block. Homogenized composite soil bags were weighed out in replicates of 10 g and 100 g and placed in Whirl-pak bags to which 90 mL of UPB and 200 mL of 2X UPB were added, respectively. Samples were gently massaged and incubated at 37 °C for 12-14 h.

For *S. enterica* recovery in all three methodologies, 10 mL of UPB enrichments were transferred to 90 mL of tetrathionate broth (TBB; Difco, Sparks, MD) incubated for 6 h at 42 °C. After incubation, 20 mL of the enriched TBB were transferred to 180 mL of Bacto M broth (Bacto, Sparks, MD) and further incubated at 37 °C for 18 h. For EHEC/STEC recovery, 10 mL of UPB enrichment were transferred to 90 mL of modified EHEC (Enterohemorrhagic E. coli) media (mEHEC, Biocontrol; Bellevue, WA) and incubated at 42 °C for 24 h.

1.3. Plant and cow manure procedure

Thirty spinach samples from site B were harvested from four 150 m long beds closest to non-disked area. Approximately 200 g of plant material were harvested into Whirl-pak bags and placed in cooler for transportation to UC Davis. Upon arrival, 25 g of plant material were weighed out into Whirl-pak bags and enriched with 100 mL of mEHEC for 18 h at 37 °C.

Two fresh cow manure pats found in proximity to the field (within 10 m) were collected in Whirl-pak bags and placed in cooler for transportation. Upon arrival at UC Davis, samples were homogenized and 10 g replicates per source were placed in stand-up Whirl-pak bag and enriched with 90 mL mEHEC for 18 h at 37 °C.

1.4. Pathogen detection and culture confirmation

1.4.1. Sample lysis

For DNA extraction of final enrichment of both mBroth (for S. enterica) and mEHEC (for EHEC), 1 mL of enrichment broth was transferred to 1.5 mL Eppendorf tube and pelleted by means of centrifugation at 1500 ×g for 3 min. The pellet was re-suspended and washed three times in 1 mL Butterfield's phosphate buffer (Whatman Inc., Piscataway, NJ, USA)(BPB) and finally re-suspended in 500 µL of BPB. To obtain a lysate for PCR screening, an aliquot of 200 µL was transferred to another tube and placed in a heating block for 10 min at 95 °C for 10 min.

1.4.2. Probe-based quantitative real time PCR

Soil enrichments were screened to detect virulence markers of *Salmonella* and EHEC using Taqman® probe-based quantitative real-time PCR (qrt-PCR). Probes and primers used for each gene are reported in Table 1. Each reaction was composed of 10 µL of a 2× Taqman® Gene expression master mix (Applied Biosystems Inc., Foster City, CA, USA), 0.5 µM of forward and reverse primers, 2.5 pmol of probe targeting genes (Table 1) and 2 µL of washed enrichment (either mBroth or mEHEC) for a final volume of 20 µL. Each reaction including amplification of selected genes was obtained by thermocycler (7300 Real Time PCR System, Applied Biosystems Inc., Foster City, CA, USA) protocol consisting of one cycle of 50 °C for 5 min, one cycle of denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s and annealing at 60 °C for 1 min.

1.4.3. Pathogen isolation and culture confirmation

EHEC culture isolation from qrt-PCR positive enrichments was done after plating GDS®-O157 immunomagnetic beads onto CHROMagar™-STEC (DRG International Inc. Mountainside, NJ) followed by 24 h incubation at 37 °C. Mauve chromogenic and non-chromogenic colonies were considered as presumptive EHEC/STEC positives and were further purified on same media. Colony lysis was performed by resuspending one colony in 200 µl of BPB followed by boiling for 10 min at 95 °C. Confirmation of EHEC isolates was performed by multiplex PCR (mPCR) to detect five genes: *eae*A, *stx*1, *stx*2, *flic*A and *rfbE* as previously described by Haack et al. (2009).

S. enterica culture isolation was obtained by plating GDS®-Salmonella immunomagnetic beads onto CHROMagar™-Salmonella Plus (ChromSalP) and Xylose Lactose Tergitol 4 (XLT-4)(Difco, Sparks, MD) which was amended with 1 g/L sodium pyruvate (Fisher, Fair Lawn, NJ) (XLT4-pyr) followed by 24 h incubation at 37 °C. Mauve and black colonies on ChromSalP and XLT4-pyr, respectively, were considered as presumptive positives. Isolated colonies were purified on the same media and cell lysis was performed as described above. Confirmation was done with mPCR targeting the *inv*A gene (Ziemer & Steadham, 2003). PCR confirmed isolates were submitted to the California Animal Health & Food Safety Laboratory System (CAHFS, San Bernardino, CA) for serotyping.

Additionally, a total of 43 colonies recovered from selective media were randomly collected and analyzed by pulse field gel electrophoresis (PFGE) to identify their individual band patterns and to determine diversity and identity among selected strains within all tested positive samples. The protocol for PFGE analysis was followed according to the PulseNet USA protocol (Ribot et al. 2006) using *Xba*I as the sole restriction enzyme.

RESULTS

S. enterica detection in site A using the standard 10 g sample size was achieved in 45 of 252 samples, from 5 of 13 fields. All 45 positive samples came from five fields: G (n=20), H (n=9), B (n=8), M (n=5).and F (n=3) (Table 2). When comparing soil pathogen recovery methodologies, *S. enterica* detection using 10 g and 100 g directly enriched had 29.2% and 62.5% positives, respectively. Contrary to other applications of NPT to soil in similar studies, pre-enrichment extraction on 100 g resulted in a lower than expected frequency of *S. enterica* positives, only 4.2% recovery (Table 4). All 10 g positives tested at 100g sample size increased the frequency of positives among replicates and previously negative lots had positive outcomes among the replicate samples when assessed as 100g units (Table 3). Follow-up soil samples (n=37) from five original positives fields (F, G, H and M) were tested before the land preparation of 2013 production season, using 100 g direct enrichment methodology. All 36 samples tested negative with qrt-PCR for the presence of *inv*A gene (data not shown).

Preliminary study in site B for EHEC detection in spinach (petiole and leaf blade) soil and cow manure was screened with qrt-PCR in composites of 5 samples each. Screening resulted in positive detection in 16.7%, 75% and 0% positives for spinach (petiole and leaf blade), soil (for 10 g and 100 g) and cow manure (from both sources), respectively. Plant sample positives for STEC and *eae*A (EHEC positive), were also positive for *stx*2. Soil EHEC positive samples were *stx*1 and *stx*2 positive (Table 5). PFGE results on 43 isolated colonies showed three distinctive Salmonella strains belonging to two serotypes: *Salmonella* serovar *Senftenberg* and *Salmonella* serovar *Cubana* (Figure 1 and 2).

Comparison of soil pathogen recovery methodologies were tested individually. EHEC detection for 10 g and 100 g soil directly enriched had 20% and 35% positives, respectively. NPT soil extraction enrichment on 100 g resulted EHEC positive in 25% of samples (Table 4).

Only directly enriched 100 g soil samples were screened for *stx*1 and *stx*2, achieving 25% positives. The target gene *rfb*E, specific for *E. coli* O157:H7 was not amplified in any of the different samples, neither in composites or individually tested (Table 4 and 5). Regrettably, permission to continue our analysis to determine both soil and crop persistence of STEC and whether birds were a

likely vector for the contamination incidents was withdrawn by the owner.

DISCUSSION

Producers of fresh fruits and vegetables need practical methods to minimize the survival of human pathogens, such as Salmonella, in production soil following any source of known contamination. Contaminated soil has resulted in hundreds of acres of abandoned crop due to pathogens such as *Salmonella enterica*, especially with lettuce and salad greens. Destruction of multiple fields per year, occasional hundreds of acres, is damaging economically but also may negate initiatives of sustainability for the operation. Predicting both inherent characteristic survival and options for remediation of soils contaminated Salmonella requires appropriate sampling design and sample processing protocols. The potential outcome we recognized in the primary Rapid response opportunity was to directly compare methods which our lab had used in artificially inoculated field trials in the Salinas region on natural contamination unlikely to be homogeneously distributed. We feel this effort has shown that, as part of a valid soil sampling plan, sample mass is a significant determinant for field risk assessment and pre-planting standards. Increasing the standard sample size to 100 grams may increase the chance of detecting low level pathogen contamination and better inform the parties involved in crop scheduling. This investigation also identified a potential issue with the previous standard method develop using larger soil mass and the soil extraction buffer that had been very successful in recovery of low levels of EHEC and Salmonella for diverse soils. Preliminarily, we conclude that the association of Salmonella in this incident was predominantly with organic matter from the compost. In this case, the sodium phosphate and surfactant, designed to release cells from clay particles would not be effective. We plan to conform this in future trials. In addition, this protocol will be applied future assessments of sequential remediation strategies i in the practical elimination of residual *Salmonella enterica* contamination.

TABLES

Table 1. Taqman® probes and primers utilized in this study for pathogen detection

Target	Probe/primer	Sequence (5'- 3')	Reference
TPEC (Total pathogenic *E. coli*)[a]	TPEC (probe)	6FAM-TGCTTCTGTGTATCAGGG-MGBNFQ	Sbodio, et al. 2013
	TPEC(forward)	TGATCACTGGCGGCGATA	
	TPEC(reverse)	TATGATGTCCTCATCTTCAGAGAGAAC	
Pathogenic *E. coli*	*eaeA* (probe)	FAM-AAAACGCTGACCCGCAC-MGBNFQ	Modified from Yoshitomi, et al. 2006
	eaeA (forward)	CCAACATGTTTGCAGGAAGGA	
	eaeA (reverse)	CCCGCTTTACGGCAAATTTA	
E. coli O157:H7	$rfbE_{O157}$ (probe)	NED-CAAAAGCACCCTATAGCT-MGBNFQ	Bertrand and Roig 2007
	$rfbE_{O157}$ (forward)	GATGCCAATGTACTCGGAAAAAT	
	$rfbE_{O157}$ (reverse)	CCACGCCAACCAAGATCCT	
Shigatoxin producing *E. coli*	*stx1* (probe)	FAM-TGATGAGTTTCCTTCTATGTGTC- MGBNFQ	Modified from Yoshitomi, et al. 2006
	stx1 (forward)	GTGGCATTAATACTGAATTGTCATCA	
	stx1 (reverse)	GAAGAGTCCGTGGGATTACGC	
	stx2 (probe)	FAM-CCGCCATTGCATTAACAGA- MGBNFQ	Modified from Yoshitomi, et al. 2006
	stx2 (forward)	TGGAAAACTCAATTTTACCTTTAGCA	
	stx2 (reverse)	GCAAATAAAACCGCCATAAACATC	
Salmonella enterica[b]	*invA* (probe)	FAM-CAATGGTCAGCATGGTATA-MGBNFQ	Ziemer et al., 2003
	invA (forward)	TGGGCGACAAGACCATCA-	
	invA (reverse)	TTGTCCTCCGCCCTGTCTAC	

[a]TPEC primers target a negative regulator protein (L0044), on the locus of enterocyte effacement (LEE) with no other known homologues. This is a 372 bp region, which is located outside of the five major operons of LEE, and thus is required for expression of the LEE genes in both EHEC and EPEC. The primers for *L0044* were redesigned from those previously reported by Lio and Syu (2004) to adapt it to a real time PCR platform.

[b]For *S. enterica*, detection was based in the amplification of *inv*A, which is a member of the genetic locus, inv, which allows *Salmonella* spp. to enter the epithelial cells (Galan, Ginocchio, & Costeas, 1992).

Table 2. Distribution of presumptive positive *S. enterica* soil samples around different fields within site A

Fields	Presumptive positive samples /total samples analyzed[1] (% Positive Enrichments)							
	1st sampling		2nd sampling		3rd sampling		Total samples per Field	
A			0/11	*(0)*			0/11	*(0)*
B					8/14	*(57.1)*	8/14	*(57.1)*
C			0/12	*(0)*			0/12	*(0)*
D			0/14	*(0)*			0/14	*(0)*
E			0/12	*(0)*			0/12	*(0)*
F			3/16	*(18.8)*			3/16	*(18.8)*
G	14/48	*(29.2)*	6/27	*(22.2)*			20/75	*(26.7)*
H			9/12	*(75)*			9/12	*(75)*
I					0/24	*(0)*	0/24	*(0)*
J			0/12	*(0)*			0/12	*(0)*
K					0/29	*(0)*	0/29	*(0)*
L					0/12	*(0)*	0/12	*(0)*
M			5/12	*(41.7)*			5/12	*(41.7)*
Total	**14/48**	***(29.2)***	**23/128**	***(18)***	**8/79**	***(10.1)***	**45/255**	***(17.8)***

[1] Results represent the ratio of positive samples out of the total number of analyzed samples after enrichment followed by detection of *invA* gene through qRT-PCR analyzed utilizing 10 g of soil.

Table 3. Preliminary study to determine the effect of sample mass and bacterial extraction methods on the detection of *Salmonella* in soil samples.

	Presumptive positive samples /total samples analyzed[1] (% Positive Enrichments)							
Site A Field G	NPT Soil Extraction and Enrichment				Direct Soil Enrichment			
	Supernatant		Pellet		10g		100g	
Sample # 28[2]	0/5	*(0)*	0/3	*(0)*	0/10	*(0)*	2/3	*(66.7)*
Sample # 15[2]	3/5	*(60)*	2/3	*(66.7)*	4/10	*(40)*	3/3	*(100)*

[1] Results represent the ratio of positive samples out of the total number of analyzed samples after enrichment followed by detection of *invA* gene through qRT-PCR.

[2] Sample 28 and 15 had been initially classified as a presumptive negative and positive, respectively after testing using 10 g of sample.

Table 4. Effect of sample mass and bacterial extraction on the detection of *Salmonella* and EHEC on sites A and B.

		Presumptive positive samples /total samples analyzed[1] (% Positive Enrichments)		
Location	Target gene	NTP Soil Extraction and Enrichment	Direct Soil Enrichment	
		Supernatant	10 g	100 g
Site A[2]	***invA***	2/48 *(4.2)*	14/48 *(29.2)*	30/48 *(62.5)*
Site B	**STEC**	5/20 *(25)*	4/20 *(20)*	7/20 *(35)*
	eaeA	5/20 *(25)*	4/20 *(20)*	7/20 *(35)*
	rfbE	0/20 *(0)*	0/20 *(0)*	0/20 *(0)*
	stx1	nd	nd	5/20 *(25)*
	stx2	nd	nd	5/20 *(25)*

[1] Results represent the ratio of positive samples out of the total number of analyzed samples after enrichment followed by detection of *invA* gene through qRT-PCR.

[2]Site A refers to samples collected only within field G. Nd; not done

Table 5. Distribution of EHEC presumptive positive samples among soil, plant and cow manure within site B

Target EHEC gene	Presumptive positive samples /total samples analyzed[1] (% Positive composites)[2]											
	Spinach				Direct Soil Enrichment				Cow Manure			
	Petiole		Leaf blade		10g		100g		Source A		Source B	
STEC	1/6	*(16.7)*	1/6	(16.7)	3/4	(75)	3/4	(75)	0/5	(0)	0/5	(0)
***eae*A**	1/6	*(16.7)*	1/6	*(16.7)*	3/4	*(75)*	3/4	*(75)*	0/5	*(0)*	0/5	*(0)*
***rfb*E**	0/6	*(0)*	0/6	*(0)*	0/4	*(0)*	0/4	*(0)*	0/5	*(0)*	0/5	*(0)*
***stx*1** [3]	0/1	*(0)*	0/1	*(0)*	3/4	*(75)*	3/4	*(75)*	nd	nd	nd	nd
***stx*2** [3]	1/1	*(100)*	1/1	*(100)*	3/4	*(75)*	3/4	*(75)*	0/5	*(0)*	0/5	*(0)*

[1] Results represent the ratio of positive samples out of the total number of analyzed samples after enrichment followed by detection of target EHEC genes through qRT-PCR

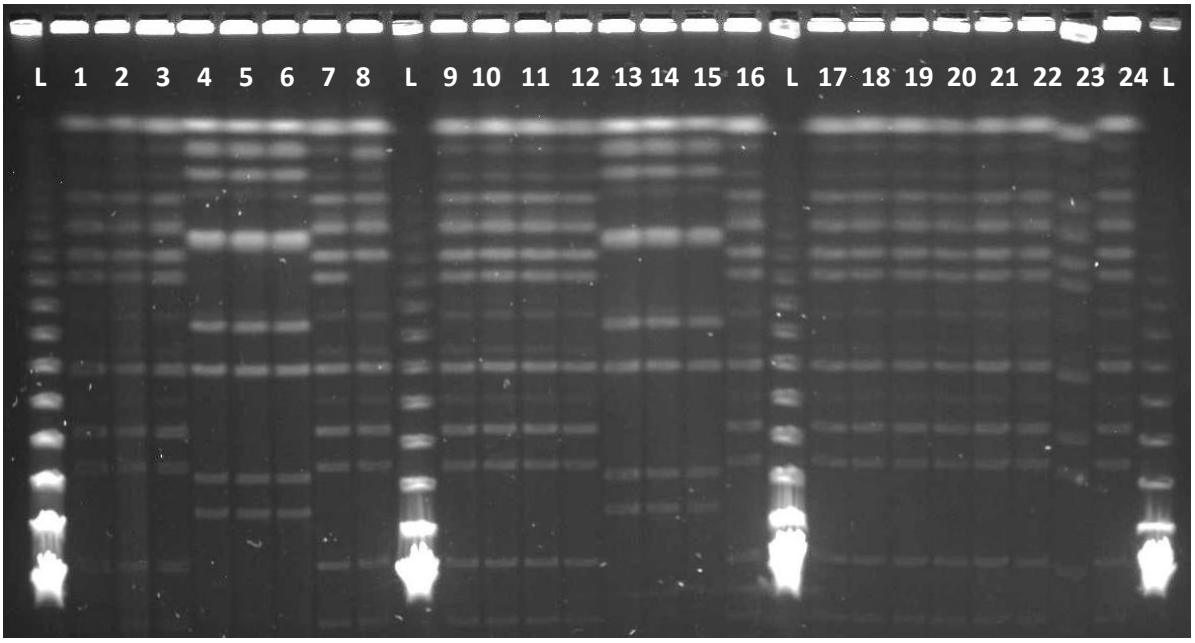
[2]Samples composited every 5: petiole (n=30), leaf blade (n=30), soil enrichments (n=20 for both 10g and 100g) and cow manure (n=25 for both manure sources A and B).

[3] Samples tested positives for TPEC and *eae*A were only screened for *stx*1 and *stx*2.

Nd; not done

FIGURES

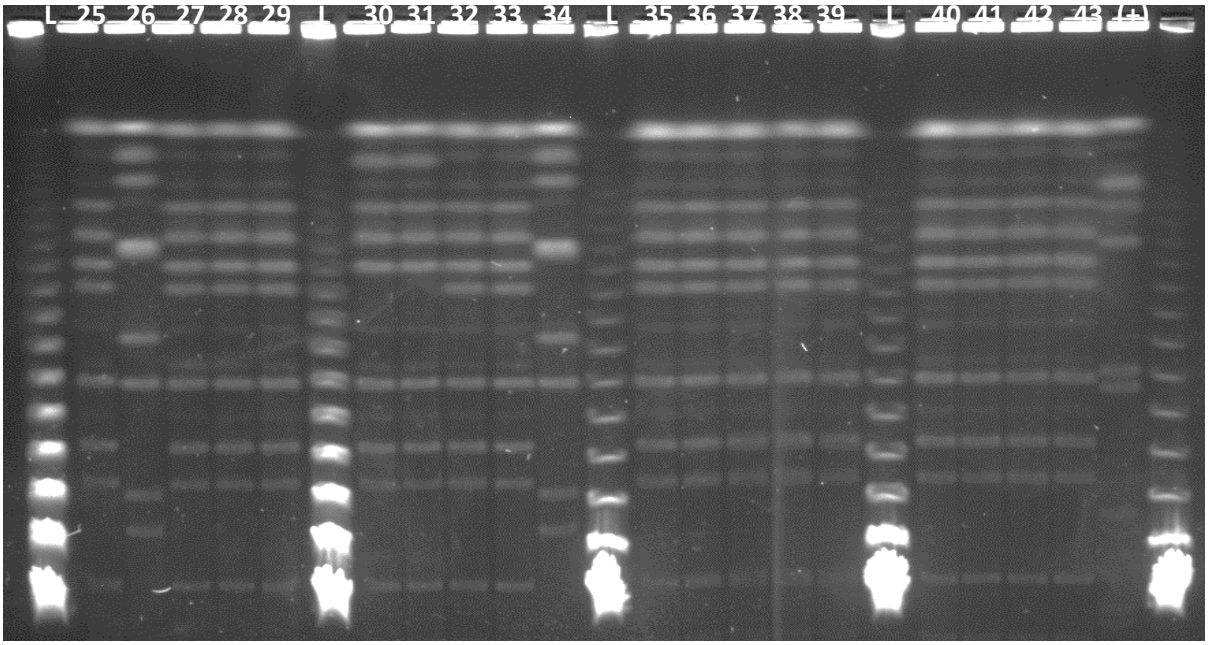
Figure 1. *Distribution of Salmonella* enterica PFGE patterns of colony isolates from soil samples.



Salmonella serovar *Cubana* strains: samples 4, 5, 6, 13, 14, and 15.

Salmonella serovar *Senftenberg*: all other strains.

Figure 2. *Distribution of Salmonella* enterica PFGE patterns of colony isolates from soil samples.



Salmonella serovar *Cubana* strains: samples 26, and 34.

Salmonella serovar *Senftenberg*: all other strains

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