Food Research International 51 (2013) 654–662

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Modified Moore swab optimization and validation in capturing *E. coli* O157:H7 and *Salmonella enterica* in large volume field samples of irrigation water

Adrian Sbodio, Sharyn Maeda, Gabriela Lopez-Velasco, Trevor V. Suslow *

Department of Plant Sciences, University of California, One Shields Avenue, 124 Mann Laboratory Davis, CA, 95616, USA

ARTICLE INFO

Article history:
Received 21 September 2012
Accepted 5 January 2013

Keywords:
Irrigation
Modified Moore swab
Filtration
E. coli O157:H7
Salmonella enterica

ABSTRACT

Knowledge of the risk potential of an irrigation source during routine use or in 'root-cause' investigation of recall and outbreak events is a fundamental expectation in produce food safety management. Current water filtration techniques, while effective in the concentration of human pathogens, are generally unable to process large volumes of water or require access to expensive equipment. In this study, a modified Moore swab (MMS) was evaluated for practical efficacy in capture-filtration of low concentrations of human pathogens from volumes of irrigation water larger than are commonly used for analysis. Water samples were artificially inoculated with isolates of either commensal *E. coli*, *E. coli* O157:H7 or *Salmonella enterica* expressing Green fluorescent protein (Gfp) and antibiotic resistance at concentrations between 10^0 and 10^2 CFU/10 L. Detection of the three mock-contaminant bacterial isolates was performed after enrichment with incubation times that varied from 4 to 24 h, depending on the initial inoculation dose. Detection of commensal *E. coli* in artificially inoculated nanopure water samples was confirmed after 4 h of enrichment incubation. Pathogen detection in artificially inoculated complex irrigation water samples such as canal, reservoir and irrigation runoff water was possible during a time course of 7 h to 18 h. Verification of MMS functionality was performed by analyzing non-inoculated environmental samples that led to the identification of *E. coli* O157:H7 presence in 4 of 114 irrigation water samples. This field acquired data demonstrated the MMS system approach was successful in trapping target microorganism in large water volumes, across a range of natural water turbidity and densities of indigenous total coliform and *E. coli* populations. The outcomes of these studies will likely be useful in improving testing for routine monitoring water quality and an economic approach for assessment of viable pathogen presence in irrigation water by assessing larger volumes of collected water in the laboratory or on-site.

1. Introduction

It is well established that human pathogens can be present and survive in surface and groundwater (Macler & Merkle, 2000; Meays, Broersma, Nordin, & Mazumder, 2004) and be ingested from contaminated recreational water and inadequately treated drinking water and, therefore cause waterborne outbreaks (Al-Qadiri, Lu, Al-Alami, & Rasco, 2011; Craun et al., 2010; Wang & Doyle, 1998). Illness and outbreaks from consumption of fresh whole and minimally processed vegetables and fruits due to irrigation or foliar crop management applications with water contaminated by diverse human pathogens has equally been a long recognized reality (Gelting, Baloch, Zarate-Bermudez, & Selman, 2011; Greene et al., 2008; Olsen et al., 2002; Pachepsky, Shelton, McLain, Patel, & Mandrell, 2011; Soderstrom et al., 2008; Steele & Odumeru, 2004; Suslow, 2010; Thurston-Enriquez et al., 2002). Regular programs for characterizing potential sources of contamination in irrigation water sources and conveyance systems combined with monitoring for presumptive fecal indicator presence have become a prominent feature of Best Practice Guidance and Audit Standards towards minimizing risk and preventing crop contamination (Solomon, Yaron, & Matthews, 2002; Solomon, Potenski, & Matthews, 2002; Suslow, 2010; US FDA, 1998). Good Agricultural Practices (GAP's) framework documents emphasize the importance of adequate microbiological quality of production water to reduce the burden of foodborne illness attributed to raw agricultural commodities (US FDA, 1998). In a recent comprehensive review Pachepsky et al. (2011) expansively detailed the body of knowledge and public health rationale that formed the foundation for these standards with specific risk categories based on culturable indicator organisms. An alternative criterion to establish adequate water quality used for irrigation could be the direct detection and quantification of key human pathogens of concern (Pachepsky et al., 2011; Suslow, 2010). As population densities of pathogens in irrigation sources, in developed nations, are typically low and non-uniformly distributed, concentration or capture-filtration is generally applied (Koster et al., 2003). However, the challenge encountered when filtering large

* Corresponding author. Tel.: +1 530 754 8313; fax: +1 530 752 4554.
E-mail addresses: aosbodio@ucdavis.edu (A. Sbodio), shmaeda@ucdavis.edu (S. Maeda), glopezvelasco@ucdavis.edu (G. Lopez-Velasco), tvsuslow@ucdavis.edu (T.V. Suslow).

0963-9969/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.foodres.2013.01.011

volumes of environmental water resides within the detection limit capabilities, in particular with water samples of high turbidity and suspended solids. Loge, Thompson, and Call (2002) reported that factors influencing pathogen detection limit of pathogens in water were the limited amount of volume filtered, which is often less than 100 mL, as well as the presence of inhibitors in applications attempting to use direct detection methods such as PCR assays. Thus, larger volumes of water should be analyzed in order to effectively assess the potential for an acute or chronic contamination of an irrigation source based on specific pathogen presence. While diverse equipment using highly advanced engineering and membrane technologies have been developed, methods that are low cost and employ simple technology must be accessible to the agricultural farming sector which is challenged to characterize the acceptability of their water sources for fresh produce production.

Moore swabs have already been used to increase the frequency of detection of *E. coli* O157:H7 in watershed run-off in the Salinas region in relation to studying environmental risk factors for leafy greens (Cooley et al., 2007). Cooley et al. (2007) retained the prior practice of deploying swabs in a flowing environmental surface water for one to several days before retrieval and microbiological testing. While effective, this is labor and travel intensive and may not always be practical. Bisha, Perez-Mendez, Danyluk, and Goodridge (2011) reported comparative information for modified Moore swabs (MMS) concentration efficiencies, as related to a continuous flow centrifuge. However, these comparisons were limited to applications using de-chlorinated municipal water sources inoculated with target pathogens as a demonstration of quantitative recovery. For their purpose, the residual water in the MMS swab was manually expressed, cheesecloth swab discarded, and up to 35 mL filtered with a presumed 0.45 μm filter (though not specified) to allow assessment of pre and post-concentrated water in both systems. Direct microbiological filtration of this volume from diverse irrigation sources, as compared to municipal water, is not always possible due to the high inherent suspended solids content.

The main objective of this study was to evaluate MMS system efficiency and detection limit when analyzing agricultural water samples of different complexity and pathogen concentrations and further application in complex environmental samples of surface water used in fresh produce production. The anticipated outcome was to qualify MMS as a practical, inexpensive and broadly accessible method, without the requirement of placement and retrieval from remote locations, to assess risk associated with irrigation or environmental water as a source of crop contamination.

2. Material and methods

2.1. Bacterial strains and inoculum preparation

For preliminary experiments commensal generic *E. coli* isolated from an irrigation water source in the Salinas region of California and transformed to produce Green fluorescent protein (Gfp) and resistant to 50 mg/L of Kanamycin (Gfp+/Kan+; strain ID TVS 356), *E. coli* O157:H7 (Gfp+/Kan+; strain ID PTVS81) and *Salmonella enterica* serovar Michigan (Gfp+/Kan+; strain ID PTVS 055) were used (D'Lima & Suslow, 2009; Miller, Leveau, & Lindow, 2000). For inoculum preparation, bacterial strains were revived from −80 °C storage freezer stock to Petri dish plates of Trypic Soy Agar (TSA; Difco, Sparks, MD) and incubated for 24 h at 37 °C. After the incubation period, single colonies were selected and transferred to 5 mL of tryptic soy broth (TSB; Difco, Sparks, MD) and incubated further 18 h at 37 °C. Cell cultures were centrifuged at 2500 ×*g* for 5 min; pellets were washed three times in Butterfield's phosphate buffer (BPB; Whatman, Florham Park, NJ) and resuspended in BPB. Final bacterial pellet suspension was adjusted in spectrophotometer (GeneQuantpro UV/Vis Spectrophotometer, Amersham Biosciences) by determining optical density of 0.750, measured at 600 nm, which corresponds to concentration of 10^9 CFU/mL for generic *E. coli*, *E. coli* O157:H7 and *S. enterica*. Serial dilutions were prepared with BPB from 10^9 to 10^0 CFU/mL. Dilutions were plated on TSA supplemented with Kanamycin (50 mg/L) and 1% (w/v) of sodium pyruvate (Fisher, Bioreagents, Fair Lawn, NJ) (TSA+Kan+Pyr) to verify inoculum concentration.

2.2. Optimized assembly of modified Moore swab (MMS)

A 92 by 95 cm^2 cheesecloth grade #90 (44×36 Weave; Chesapeake Wiper & Supply, Inc.) was folded horizontally in half 3 times and tightly rolled resulting in a cylindrical shape-swab 12 cm long and 4.4 cm in diameter. This procedure was determined to be optimal for capturing planktonic cells and fine environmental silt-clay cohesive sediments (typically 8 to 24 μm) that may contain adhering target pathogens. On one end, a 10 cm long by 3.81 cm wide PVC pipe was used to join on one end a 3.81 cm PVC connector to a 3.81 cm PVC Male-to-Male coupler, and on the other end a second 3.81 cm PVC D-2466 connector (Fig. 1). Connections were first primed with Purple Primer (Oatey 31901) and then glued with PVC cement (Oatey 31841) allowing cement to cure for 4 h. This model of MMS-cassette can be constructed at a low cost and used multiple times by sterilizing all parts in 20% bleach for 18–24 h followed by rinsing in deionized water (DI) to later be washed with soap and rinsed again with DI water. Prior to use for filtration, MMS was sterilized in an autoclave to later be placed inside a PVC pipe to create a filtration cassette unit. After irrigation water collection, MMS units were capped and placed in cooler with gel-ice packs. Shipment of MMS was performed by extracting from the cassette into a Whirl-pak bag and placed with gel-ice packs for overnight delivery. Processing occurred within 24 h from sample collection.

2.3. MMS water filtration

For capture filtration, a MMS cassette was connected to sterile tubing (Masterflex platinum-cured silicone tubing, L/S® 18; Cole Parmer, USA) at each end-cap nipple. One end of the tubing was placed into the influent artificially inoculated water sample and the other end into the rotating head of a peristaltic pump set between the MMS and an effluent collection receptacle. Pump speed was set at a flow rate of 0.5 L/min. Filtration time for 10 L varied from 10 to 30 min depending on the water turbidity.

2.4. MMS capture of generic E. coli, E. coli O157:H7 and S. enterica in inoculated water

Water from two irrigation sources used for vegetable crop production, an irrigation canal and an on-farm reservoir, and nanopure water were utilized to test the effectiveness of MMS to trap targeted bacteria (Fig. 2). Water turbidity was measured using Hach Colorimeter DR/850 protocol setting # 95 (Hach Company; Loveland, CO) prior to inoculation and capture-filtration with MMS. Water volumes of 10 L were inoculated with 100 μL of a washed cell suspension containing 10^0, 10^1 and 10^2 CFU/mL of the selected strain described above. One gram of clay-loam soil was suspended in 10 L of nanopure water to create a turbidity of 1 NTU. This experimental water was inoculated with generic *E. coli* (TVS 356). Non-sterile reservoir water from a major agricultural region in California, collected within 24 h of use was similarly inoculated with *E. coli* O157:H7 (PTVS 81) and irrigation district system canal water was inoculated with either *E. coli* O157:H7 (PTVS 81) or *S. enterica* (PTVS 55). The inoculated bacterial concentration in the different water sources ranged from 2 to 638 CFU/10 L of water, to assess presence detection as a function of the time of enrichment. As negative controls uninoculated water samples from all three sources were utilized.

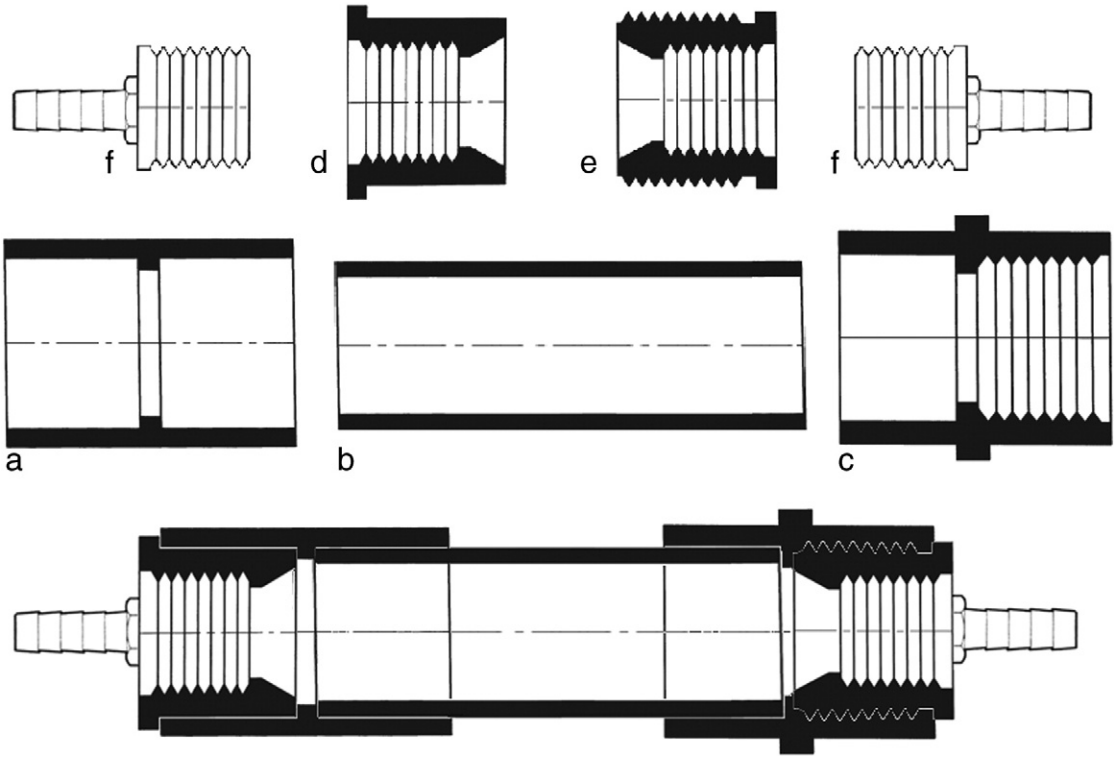

Fig. 1. Modified Moore swab cassette. a. Coupler (S×S) 3.81 cm. b. PVC pipe 10×3.81 cm. c. Female adapter (S×3FPT) 3.81×3.81 cm. d. Reducer Bushing flush style (SPG×FPT) 3.81×1.27 cm. e. Reducer Bushing (MPT×FPT) 3.81×1.27 cm. f. Hose barb to MPT fittings 1.27×0.95 cm. A cylindrical shape-swab 12×4.4 cm is tightly inserted inside MMS unit before closing with reducer bushing (e).

After filtration, the MMS cassette was separated from the tubing and sealed on both ends using European 8D silicone stoppers and placed on frozen gel-ice. Recovery was accomplished by extracting the MMS from the cassette and placing the saturated swab in a sterile bag (Nasco Whirl-Pak®, Modesto, CA). For sample enrichment, 100 mL of double strength (2×) TSB, 200 mL (2×) mEHEC (Biocontrol; Bellevue, WA) and 200 mL (2×) buffered peptone water (BPW; Difco; Sparks, MD) were utilized for recovery of generic *E. coli*, *E. coli* O157:

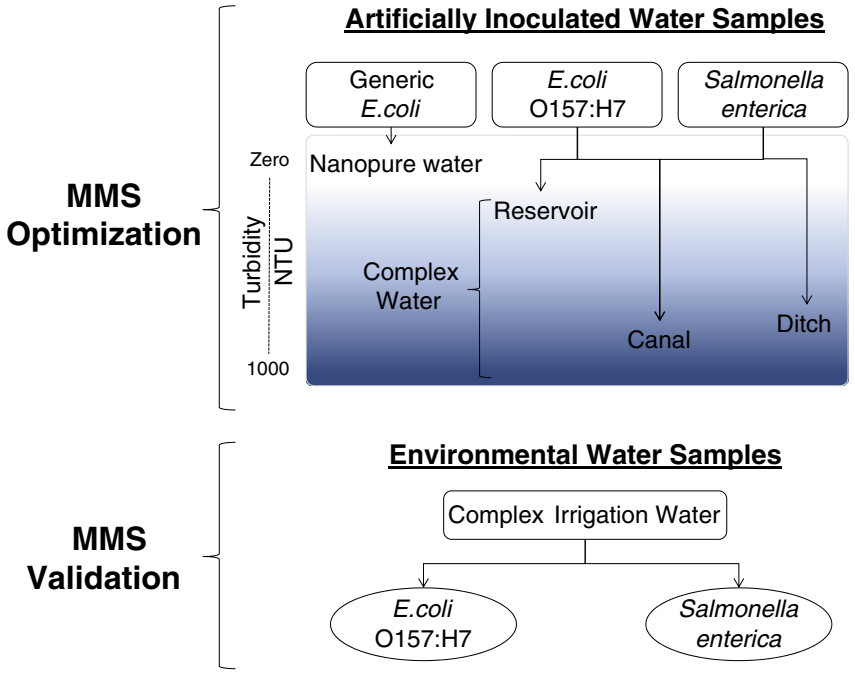

Fig. 2. MMS optimization and validation schematics. Optimization of MMS was achieved by capturing target microorganism in series of experiments in which 10 L of water, nanopure, reservoir and canal, and ditch, was artificially inoculated with generic *E. coli*, *E. coli* O157:H7 and *Salmonella enterica* respectively. Validation of MMS was accomplished by successfully capturing *E. coli* O157:H7 and *S. enterica* from complex irrigation water samples from two major agricultural regions in California.

H7 and *S. enterica*, respectively. Samples, already in enrichment culture broth, were massaged for 1 min and the full MMS incubated for a total of 24 h at 42 °C for *E. coli* O157:H7 and 37 °C for generic *E. coli* and *S. enterica*.

Duplicate aliquots of 15 mL were taken from MMS filtration of inoculated nanopure water samples at 4 and 7 h of incubation to determine the earliest point for cultural detection of generic *E. coli* isolates during enrichment. Enrichment subsamples were filtered with an IsoGrid membrane, a sterile 0.45 μm microfiltration device (Neogen Corp.; Lansing, MI), and placed on CHROMagar™-ECC (DRG International Inc.; Mountainside, NJ) supplemented with 50 mg/L of Kanamycin (ECC + Kan) and held at 22 °C for 2 h then transferred to 44.5 °C for 24–48 h. Isogrid membranes were also placed on TSA + Kan + Pyr and incubated at 37 °C for 36 h. Grids were inspected for fluorescence typical of GFP at 18 and 36 h.

Recovery of pathogenic isolates was performed by collecting, in duplicate, 200 μL aliquots of enrichment plated onto TSA + Kan + Pyr agar and held at 37 °C for up to 36 h. *E. coli* O157:H7 inoculated in irrigation reservoir water samples were recovered after 12 h and for canal samples after 18 h. *S. enterica* recovery in irrigation canal samples was accomplished after 18 and 24 h. Plates were inspected for fluorescence typical of GFP at 18 and 36 h.

2.5. Pathogen detection in inoculated complex irrigation water

2.5.1. MMS capture of E. coli O157:H7 and S. enterica in ditch, reservoir, and canal inoculated water

To measure the efficacy of the MMS in capturing pathogens and removing sediments, three different water sources (irrigation drainage ditch, two on-farm reservoirs, and three irrigation canal water samples) were inoculated with *E. coli* O157:H7 and *S. enterica* (Fig. 2). Water samples were inoculated with 100 μL of 10^0 and 10^1 CFU/mL for both pathogens. In ditch and both reservoir water samples, the final concentration of *E. coli* O157:H7 was 2.3 CFU/10 L while canal water samples had concentrations of 44 CFU/10 L. *S. enterica*, ditch and both reservoir sources were adjusted to 4.3 CFU/10 L. In tests with canal water, the resultant concentration was 32.2 CFU/10 L. Uninoculated water samples from all three water sources were used as negative controls.

To further assess the MMS efficiency, two additional tests were conducted with complex, higher environmental turbidity, irrigation water samples. Before inoculation and after water filtration, turbidity levels were determined using the most probable number (MPN) of total coliforms and *E. coli* using IDEXX Colilert reagent in the QuantiTray 2000 format (IDEXX Lab.; Westbrook, ME) in two sub-samples of 100 mL each (Eccles, Searle, Holt, & Dennis, 2004).

Water filtration was performed as described in the previous section. Filtration time varied from 20 to 30 min depending on the water turbidity and volume. Bacterial enrichment was performed as described above. Pathogen recovery was performed by plating, in duplicate, 200 μL aliquots of enrichment onto TSA + Kan + Pyr and held at 37 °C for up to 36 h. For both pathogens, ditch and reservoir samples were recovered after 18 and 24 h; canal samples, were recovered after 7, 18 and 24 h. Plates were inspected for fluorescence typical of GFP at 18 and 36 h.

2.6. Pathogen detection in environmental irrigation water

2.6.1. MMS capture of pathogens in environmental irrigation water

Water samples were collected from two major agricultural regions in California, San Joaquin and Salinas Valley. Over the course of 2 years, 2008–2009, and between the months of July and October, San Joaquin Valley water samples were collected from two irrigation sources, canal and irrigation reservoir. Sampling collection was performed at time of irrigation based on different application methods: sprinkler emitters, drip emitter, and furrow (gated-pipe and siphon tube). Canals sampled included: California Aqueduct, Delta-Mendota Canal, San Luis Drain and San Luis Canal and their associated lateral canals in Fresno, Merced and Stanislaus counties. All canal locations were selected for proximity to potential sources of fecal contamination such as concentrated animal feeding operations (CAFO's), and public access points where the surface water distribution system passed through rural communities.

Water samples from Salinas Valley were collected in 2009 during the month of September and in 2010 at the beginning of cool season vegetable production in April. Additional collections were conducted during peak production from August to September. Four reservoirs were selected for sampling due to reported wildlife intrusion and their proximity to a CAFO and a composting operation, both being a potential risk factor for contamination of the water body. In addition, an affiliated environmental water source was sampled in 2009 to test for the pathogen detection and recovery of phase of the MMS system, in the event that actual irrigation source sampling was determined to be consistently negative. This particular sampling was conducted on lettuce field irrigation run-off water obtained from a tail-water ditch passing through vegetated banks during peak flow.

2.6.2. Water collection and on-site MMS filtration

Sample collection time spanned from 0900 to 1900 h and was achieved by grab-sampling water in five 4 L sterile plastic jars per location. In addition, at some locations, MMS capture filtration was performed in the field by connecting MMS cassette on one end to a 3.5 m by 0.95 cm sterile tubing (Masterflex platinum-cured silicone tubing, L/S® 18; Cole Parmer, USA) supported with a 3.6 m extendable pole and equipped with plastic clips to secure the uptake-draw end. MMS cassette was suspended vertically over a flow-thru discharge container attached to sturdy mounts. The uptake end of the tubing was placed into the sample location, extended at least 1 m beyond the shoreline of the irrigation source. For each sample point, 10 L of water was passed through a MMS filter by means of a peristaltic pump (Masterflex L/S® Easy-Load Pump Model 7518-00, Cole Parmer, USA) powered by an external and portable power supply (EverStart ES5LBS Battery; Johnson controls, Milwaukee, WI). Additional 500 mL water samples were taken to perform further fecal indicator analysis.

2.6.3. Physicochemical and microbiological analysis of environmental water samples

Readings for source water pH and temperature were executed on site, immediately after collection and only for San Joaquin samples. For transportation, either jars holding water samples or MMS cassette were placed in a cooler and covered with ice-packs for 3 h until arrival to UC Davis. After samples were brought to the lab, turbidity levels were measured as described above. Water samples were processed to quantify total coliforms and *E. coli* with Quanti-tray 2000. For generic *E. coli* confirmation, periodic subsets of samples were extracted from the broth contents of individual UV-positive cells (indicating presumptive *E. coli*) from the Quanti-Tray® grid. The paper side of the template was wiped twice with a KimWipe saturated with 70% ethanol and pierced with a fine hypodermic needle. Approximately 0.25 mL of the broth was extracted and 10 μL placed on Chromagar™ ECC (DRG International Inc. Mountainside, NJ) to observe for typical blue colonies after 24–32 h at 44.5 °C. For the purpose of this study, this was accepted as adequate verification of generic *E. coli* presence and accuracy in MPN enumeration.

2.6.4. Pathogen detection and culture confirmation in environmental water samples

Samples were filtered with the MMS system as described above and screened for pathogenic *E. coli* O157:H7 and *S. enterica*, accomplished by extracting the rolled swab and placing it in a sterile Whirlpak® bag. The MMS was enriched as described above with

mEHEC and BPW for *E. coli* O157 and *Salmonella*, respectively, and incubated for up to 18 h at 37 °C. Detection of *E. coli* O157:H7 in MMS mEHEC enrichment was achieved using the Biocontrol GDS®-O157 (Biocontrol Systems, Bellevue, WA) following manufacturer instructions. Detection of *S. enterica* in MMS BPW enrichment was done utilizing the GDS®-Salmonella (Biocontrol Systems, Bellevue, WA) and BAX®-Salmonella (Dupont Qualicon) commercial kits according to manufacturer instructions. GDS® and BAX® were performed with 1 mL and 5 μL of enrichment broth respectively.

In addition, MMS mEHEC enrichments of samples obtained from the Salinas water sources were screened for the presence of *stx*1, *stx*2 and *rfb*E genes. Detection of shiga-like toxin genes was done utilizing GDS® for shiga toxins and multiplex PCR (mPCR) which also included the detection of the *rfbE* gene as described by Bertrand and Roig (2007).

Additionally, MMS enrichments were screened to detect pathogenicity markers of *Salmonella* and pathogenic *E. coli* using quantitative real-time PCR with Taqman® probes. An aliquot of 200 μL of each MMS enriched mEHEC and BPW was collected in tubes and placed in a heating block at 95 °C for 10 min to obtain a culture lysate. For *Salmonella*, 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). Amplification of *invA* gene was conducted using forward primer *inv*A(Tq)-F (5′-TGGGCGACAAGACCATCA-3′), reverse primer *inv*A(Tq)-R (5′-TTGTCCTCCGCCCTGTCTAC-3′) and *inv*A(Tq) probe (6FAMCAATGGT CAGCATGGTATA-MGBNFQ). For detection of a broader group of pathogenic *E. coli* (TPEC) forward primer TPEC-F (TGATCACTGGCGGCGATA), reverse primer TPEC-R (TATGATGTCCTCATCTTCAGAGAGAAC) and TPEC probe (6FAM-TGCTTCTGTGTATCAGGG-MGBNFQ) were utilized (Lio & Syu, 2004). 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. Each 20 μL reaction to amplify either *invA* or TPEC contained 10 μL of a 2× Taqman® Gene expression master mix (Applied Biosystems Inc., Foster City, CA, USA), 0.5 μM of each forward and reverse primers, 2.5 pmol of probe targeting genes aforementioned (Applied Biosystems Inc., Foster City, CA, USA) and 2 μL of the lysate. Amplification was conducted in a thermocycler (7300 Real Time PCR System, Applied Biosystems Inc., Foster City, CA, USA) with a protocol consisting in 1 cycle of 50 °C for 5 min, 1 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. Amplification lower or equal to the Ct value of a standard containing 5 copies of target gene sequence was classified as positive. All set of primers used in this study were previously tested for inclusivity and exclusivity of the target species, utilizing a culture collection of other related *Enterobacteriaceae* as well as non related microorganisms including Gram positive microorganisms. Primers were further tested in a range of 10^1 to 10^7 copies to determine reaction efficiency and sensitivity (data not shown).

2.6.5. Pathogen isolation and culture confirmation

E. coli O157:H7 culture isolation was obtained by plating GDS®-O157 immunomagnetic beads onto CHROMagar™-O157 (DRG International Inc. Mountainside, NJ) followed by 24 h incubation at 37 °C. *Salmonella* culture isolation was obtained by plating GDS®-Salmonella immunomagnetic beads onto XLT4 (Difco, Sparks, MD) followed by 24 h incubation at 37 °C. Confirmation of *E. coli* O157:H7 isolates was performed by mPCR to detect four genes: *eae*A, *stx*1, *stx*2, *hlyA* and *rfbE* as described by Haack et al. (2009). For *S. enterica*, isolate confirmation was done with PCR 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.

2.7. Statistical evaluation of MMS capture efficiency

Statistical analysis was performed with Statistical Analysis Software V. 9.2 (SAS Institute Cary NC). Differences in *E. coli* and total coliform populations among different environmental water samples were achieved after ANOVA analysis using the GLM procedure and Tukey pair wise comparison was applied for mean separation. To establish significant difference among treatments a p-value lower than 0.05 was utilized. Pearson correlation analysis was determined using the CORR procedure.

3. Results

3.1. MMS capture filtration from zero turbidity water

Application of the MMS bacterial capture system was tested with various water sources. Initial evaluations demonstrated the capacity and functional recovery of generic *E. coli* with concentrations that varied approximately from 10^0 to 10^2 CFU suspended in 10 L of nanopure water. Culture-based detection, from water containing 100 CFU/10 L was possible after only 4 h of MMS enrichment, while for samples containing 2 CFU/10 L, incubation time required 7 h before colony detection of generic *E. coli* on solid media was observed using standard plating techniques (Table 1).

MMS bacterial capture system was also functional in recovering low numbers of pathogenic bacteria *E. coli* O157:H7 from reservoir and canal inoculated water samples and *S. enterica* from inoculated canal water. In these samples, concentrations of both pathogens ranged from 10^0 to 10^1 CFU in 10 L. Pathogen detection was achieved after 12 and 18 h in *E. coli* O157:H7 inoculated reservoir and canal water, respectively. Confirmation of *S. enterica* capture in canal water was achieved after 18 h of MMS enrichment (Table 1). Uninoculated water samples tested negative for target organisms at all time points.

3.2. MMS indicator and pathogen detection in inoculated complex water samples and efficiency in removing sediments

MMS effectiveness was tested with *E. coli* O157:H7 and *S. enterica* in more complex irrigation water samples (drainage ditch, reservoir and canal). Pathogen detection from initial concentrations that ranged between 10^0 and 10^1 CFU/10 L was feasible after MMS enrichment of 7 h for canal and 18 h for ditch and reservoir (Table 2). Assessment of changes in turbidity and the indigenous

Table 1
Detection of generic *E. coli*, *E. coli* O157:H7 and *S. enterica* after filtration of inoculated water through the MMS capture system.

Water source	Target microorganism	Initial bacterial concentration CFU/10 L	Recovery time (h)[a]				
			4	7	12	18	24
Nanopure[b]	Generic *E. coli*	638	+	+	+	+	+
		21	+	+	+	+	+
		2	−	+	+	+	+
Irrigation1	*E. coli* O157:H7	43	ND	ND	+	+	+
(reservoir)		6	ND	ND	+	+	+
Irrigation2[c]	*E. coli* O157:H7	16	ND	ND	ND	+	+
(canal)	*S. enterica*	22	ND	ND	ND	+	+

(ND) not determined for this time point.
[a] (+) and (−) indicate positive and negative recoveries of the targeted bacteria, respectively.
[b] Three set of experiments were conducted with Generic *E. coli* and nanopure water.
[c] Two sets of experiments per water sample were conducted with *E. coli* O157:H7 and *S. enterica*.

Table 2
Modified Moore swab detection of *E. coli* O157:H7 and *S. enterica* in artificially inoculated complex irrigation water in relation to sediment removal and background bacteria reduction.

		Water source					
		Ditch water[a]	Reservoir[a]		Canal[a]		
			1	2	1	2	3
Initial pathogen concentration (CFU/10 L)	*E. coli* O157:H7	2.3			44		
	S. enterica	4.3			32.2		
MMS enrichment recovery time (h)[b]	7 h	ND	ND	ND	+	+	+
	18 h	+	+	+	+	+	+
	24 h	+	+	+	+	+	+
Total coliforms[c]	Before filtration	4.38	3.94	2.51	5.15	3.57	5.24
	After filtration	3.54 ± 0.28	3.59 ± 0.21	1.84 ± 0.15	4.00 ± 0.11	2.62 ± 0.26	5.05 ± 0.03
	% Capture[d]	85.6%	55.3%	78.6%	92.9%	88.8%	35.4%
E. coli[c]	Before filtration	1.75	2.03	1.24	1.59	0.49	1.89
	After filtration	1.51 ± 0.10	1.60 ± 0.11	0.89 ± 0.20	0.36 ± 0.36	0.22 ± 0.27	2.02 ± 0.09
	% Capture[d]	42.5%	62.9%	55.3%	94.1%	46.3%	34.9%
Turbidity (NTU)	Before filtration	225.50	21	3	900	27	11
	After filtration	17.50	8.50	2	15	7	0
	% Reduction	92.2%	59.5%	33.3%	98.3%	74.1%	100%

(ND) not determined for this time point.
[a] Two sets of experiments per water sample were conducted with *E. coli* O157:H7 and *S. enterica*.
[b] (+) and (−) indicate positive and negative recovery of both targeted pathogens, *E. coli* O157:H7 and *S. enterica*, respectively.
[c] Mean log MPN/100 mL ± standard deviation.
[d] Percentage capture represents the percentage of the population trapped in the MMS.

population densities of *E. coli* and total coliforms were tested from influent and effluent following MMS filtration. In the three water types, reduction in populations of total coliforms and *E. coli* was less than 1-log regardless of the initial water turbidity. The singular exception was in one canal water sample with an initial turbidity level of 900 NTU, in which a 1.15 and 1.23 log-reductions were observed for total coliforms and *E. coli*, respectively, however pathogen detection was achieved. MMS ability to reduce total coliforms and *E. coli* showed an average percentage reduction of 72.8% and 56.0% respectively. Results indicate that greater values of turbidity were associated with greater reduction of total coliforms and *E. coli* after filtration.

MMS sediment removal varied among samples depending on initial water turbidity. Values before filtration ranged from 3 to 900 NTU; however, MMS filtration clearly results in substantial water clarification with values decreasing to between 0 and 17.50 NTU. Water samples with initial turbidity greater than 225.5 NTU showed not only a greater decrease in turbidity (at least 92.2% clarification), but also in quantitative influent:effluent differential of indigenous total coliforms (85.6 to 92.9% capture) and *E. coli* (42.5 to 94.1% capture). In contrast, water samples with turbidity lower than 20 NTU appeared to limit the efficacy of capturing/removing total coliforms and *E. coli* (Table 2).

3.3. Pathogen detection in environmental water samples

3.3.1. San Joaquin Valley

Pathogen presence assessments of surface water samples were performed from various San Joaquin Valley sources. Detection of *E. coli* O157:H7 with GDS®-O157 was achieved after 18 h of MMS enrichment in 4 out of 114 samples, all associated with sampling of irrigation canal water. Isolation of colonies was achieved by plating GDS®-O157 immunomagnetic beads onto CHROMagar™-O157 colony. Confirmation by mPCR, showed that isolated colonies contained *eaeA*, *stx2* and *rfbE* genes. *S. enterica* was not present in any of the 114 samples filtered with MMS (Table 3).

Analysis of variance showed no significant difference ($p > 0.05$) in the population of total coliforms among the different sources tested. Moreover, the population of *E. coli* was not significantly different, with a p-value of $p = 0.07$, among the sources, particularly for water collected from furrows and irrigation runoff water, often returned to an environmental water body or district irrigation canal system where greater populations of *E. coli* were recovered (Table 3).

The effect of water temperature, turbidity, pH, month and time of sample collection were evaluated using a parametric bivariate and Pearson's correlation analysis between the populations of total coliforms and *E. coli* and the corresponding water variables. For total coliforms, a significant correlation was described among water turbidity, temperature, and month of collection, which might indicate an effect of seasonality in the deposition or growth of this sub-population of potential quality indicators. In contrast, pH values and the time of sample collection showed no significant linear correlation with total coliform population densities. For the population of *E. coli*, when a quadratic polynomial model was utilized, it was determined that there was a significant correlation with water temperature and pH ($p < 0.05$) (data not shown). *E. coli* levels had no

Table 3
Comparison of total coliforms and *E. coli* population by source in relation to pathogen presence in water from San Joaquin Valley.

Water source	N	Total coliforms[a]	*E. coli*[a]	Log diff	MMS enrichment positives after 18 h incubation		
		log MPN/100 mL				GDS®-O157[b]	GDS®-Sal BAX®-Sal[b]
Canal	83	$3.90^{A} \pm 0.61$	$0.75^{B} \pm 0.75$	3.15 ± 0.76		4/83	0/83
Drip	4	$4.16^{A} \pm 1.47$	$0.87^{A,B} \pm 0.66$	3.29 ± 1.25		0/4	0/4
Furrow	4	$4.54^{A} \pm 0.99$	$1.11^{A,B} \pm 1.06$	3.43 ± 0.46		0/4	0/4
Reservoir	6	$4.24^{A} \pm 0.91$	$0.60^{B} \pm 1.11$	3.64 ± 0.74		0/6	0/6
Siphon	12	$4.13^{A} \pm 0.92$	$0.88^{A,B} \pm 0.77$	3.25 ± 0.65		0/12	0/12
Tailwater	4	$4.67^{A} \pm 1.09$	$2.06^{A} \pm 0.83$	2.61 ± 0.41		0/4	0/4
Sprinkler	1	3.65^{A}	$0.80^{A,B}$	2.85		0/1	0/1
Mean		4.00 ± 0.74	0.83 ± 0.80	3.18 ± 0.76	Total	4/114	0/114

[a] Results represent the mean ± standard deviation (N = sample size). Different letters within the same column denote significant difference ($p < 0.05$) among different sources.
[b] Results represent positive detection/total number of analyzed samples with Biocontrol GDS®-O157, GDS®-Salmonella and Dupont Qualicon BAX®-Salmonella respectively.

Table 4
Pearson's correlation values for total coliforms and *E. coli* among the environmental and temporal water constituent traits during sample collection.

Variables	N	Pearson correlation coefficient	
		Total coliforms	*E. coli*
Day collected	114	−0.24*	−0.17
Month	113	−0.23*	−0.11
Temperature	108	0.19*	0.17
Turbidity	108	0.35**	0.10
E. coli	114	0.53***	1.00
Total coliforms	114	1.00	0.53***
E. coli O157:H7 presence	114	−0.18[a]	−0.05

(*, **, ***) asterisks denote significance of the Pearson's correlation value (*$p<0.05$, **$p<0.001$, and ***$p<0.0001$).
N = sample size.
[a] p-value = 0.059.

significant correlation with any of the other collection assessment parameters. In addition, for these water sources, total coliform populations were significantly correlated with numbers of *E. coli* in the same processed sample (Table 3). Pearson's correlation analysis showed a significant correlation between turbidity and total coliforms, but none with *E. coli* (Table 4).

3.3.2. Salinas Valley

Pathogen testing and detection of *E. coli* O157:H7 with GDS®-O157 were achieved after 18 h of MMS enrichments in 3 out of 16 samples, in which culture confirmation was achieved in 2 samples. Genotyping of isolated colonies by mPCR showed the presence of *eae*A, *stx*1, *stx*2, *hyl*A and *rfb*E genes. Screening for TPEC resulted in positive detection in 3 of 7 samples, while *S. enterica* was present in 2 of 4 reservoir samples and also in an affiliated environmental water source, lettuce field irrigation run-off water. Colony isolation and confirmation were achieved from both sources. Taxonomic determination resulted in *S. enterica* subsp. *arizonae* and *S. diarizonae*. Molecular testing of *rfb*E gene was only performed in 2010 samples and detection outcomes were negative in all cases. Population of total coliforms and *E. coli* remained similar among the samples with a mean of log 3.56 MPN/100 mL and 2.28 MNP/100 mL, respectively. Due to the limited number of samples obtained in this region, no statistical analysis was performed (Table 5).

4. Discussion

Moore swabs and other modified versions have been previously utilized as low-cost, coarse capture filters to evaluate microbiological water quality. The use of conventional and advanced molecular techniques such as PCR, mPCR, quantitative PCR (qPCR), real time PCR (qrt-PCR), reverse transcriptase PCR (RT-PCR), fluorescent in situ hybridization (FISH), and nucleic acid sensor have been variously applied for pathogen qualitative or quantitative detection in diverse water bodies or sources (Csordas, Delwiche, & Barak, 2008; Gilbride, Lee, & Beaudette, 2006; Girones et al., 2010; Noble & Weisberg, 2005). Regardless of the methodological sensitivity and efficiency to quantify pathogens, these analytical tools remain limited by the scope of the sample collection strategy and volume being analyzed, typically limited to 100 mL (Noble & Weisberg, 2005). As most irrigation sources are expected to be non-homogeneous, especially flowing surface water, the potential for failing to identify risk potential at practical levels of pathogen presence in a water body with small volume samples is probable (Pachepsky et al., 2011; Suslow, 2010). As a simple and low-cost approach to irrigation or environmental water sampling, the cassette-MMS method of capturing pathogens allowed the filtration of larger than standard water volumes, 10 L in this study, including high clarity and complex turbid water sources with higher suspended solids. Moreover, traditional grab sampling techniques for environmental water require keeping water refrigerated for transportation, thus often limiting the practical amount of analyzed material transferred to a contract lab. The MMS method, in our experience, has proven to be a simple process enabling the opportunity for daily on-site capture filtration of greater than 10 L, with the aid of a power supply and portable pump, and compact transport or shipping of MMS cassettes or extracted swabs under chilled conditions.

In addition to sampling larger volumes, MMS is able to capture sediments, suspended particles and algal fragments (e.g. *Cladophora* spp.) present in the water typical of canals and lakes that are known to harbor pathogens and likely to be a source of chronic contamination (Badgley, Nayak, & Harwood, 2010; Badgley, Thomas, & Harwood, 2010; Byappanahalli et al., 2009; Ishii et al., 2006). There is a particular concern for algae presence in shoreline stabilizing rocks, soil banks of unlined irrigation conveyance systems, concrete-lined canal sidewalls and in irrigation booster-pump basins. Environmental assessment for water quality, in related studies, has shown that sampling non-disturbed bulk water most often results in non-detection of target pathogens, whereas sampling of algal filaments from the same site resulted in positive detection of EHEC or *Salmonella* (unpublished data).

When using MMS with environmental samples, a common challenge resides in analyzing capture-swabs that co-concentrate large amounts of background bacteria that may interfere with pathogen enrichment and detection. While direct PCR amplification of pathogens from water remains a difficult task considering their low concentrations, water bacterial heterogeneity and presence of inhibitors; the use of filtration followed by an enrichment step prior to molecular

Table 5
Pathogen presence in water from reservoirs in Salinas Valley.

		Background bacteria		Molecular detection[a]					BAX®-Sal
		Total coliforms[b]	*E. coli*[b]	GDS®			Taqman® assays		
		log MPN/100 mL		O157	stx1	stx2	TPEC	*invA*	
2009	September	3.74 ± 0.28	2.96 ± 0.09	3/8[c]	3/8[c]	5/8[c]	ND	1/1[d]	ND
2010	April	nd	nd	0/3	0/3	0/3	1/3	1/3[e]	1/3[e]
	August	4.08	2.20	0/1	0/1	0/1	1/1	1/1	1/1
	September	3.47 ± 1.93	1.95 ± 1.58	0/4	0/4	0/4	1/3	nd	nd
Mean		3.56 ± 1.51	2.28 ± 1.33	3/16	3/16	5/16	3/7	3/5	2/4

(ND) not determined for this time point.
[a] Results represent positive detection/total number of analyzed samples with Biocontrol GDS®-O157, stx1, stx2, and Dupont Qualicon BAX®-Salmonella and qrt-PCR Taqman® assays for pathogenic *E. coli* and *invA* genes which target a negative regulator of the LEE pathogenicity island in pathogenic *E. coli* and the invasion gene *invA* in *Salmonella*.
[b] Mean log MPN/100 mL ± standard deviation.
[c] Colony confirmation was obtained from 2 of 3 GDS®0157 positives. Colonies were further screened for genotyping which evidenced presence of *rfbE*, *hylA*, *eaeA*, *stx1* and *stx2* genes.
[d] Colony confirmation was obtained from qrt-PCR Taqman® assays positive, serotype *Salmonella arizonae* and *S. diarizonae*.
[e] Colony confirmation was obtained from one BAX®-Salmonella positive. Colony was further screened for genotyping which evidenced presence of *invA* gene.

techniques seems to be the most reasonable approach (Noble & Weisberg, 2005). In this study, environmental sample analysis after MMS enrichment was performed by means of immunomagnetic separation system (IMS) using the GDS detection system for *E. coli* O157:H7 and *S. enterica*. Similar detection systems, without an IMS process step, developed by Qualicon BAX were also utilized. These applications of molecular detection platforms, though originally intended to be used for rapid testing of pathogens without an enrichment step (Fu, Rogelj, & Kieft, 2005), provide the opportunity to give sensitive results for quality assessments of larger water volumes within a 24 h timeframe. Since the MMS capture filtration process described was followed by enrichment, and a limited incubation time, typically 7 h, this allowed for a relatively rapid time to a decision point on qualifying a given irrigation water source within routine or risk-related sampling programs. Enrichment analyses of artificially inoculated samples were determined to be positive after 4 and 18 h for zero-measurably turbidity and complex water samples, respectively. For environmental samples enrichment analysis was performed after 18 h, but as short as 7 h, of incubation with an additional 3 h for qPCR results; still within the desired 24 h decision point timing and considering the volume of water being analyzed.

When filtering samples with a native turbidity at a collection of more than 200 NTU, MMS was able to significantly lower turbidity by capturing sediments and target indicator bacteria, up to 92.9% and 94.1% of coliforms and *E. coli* respectively. Most likely bacteria are attached to the silt-clay fraction or organic micro-aggregates of the suspended solids (Pandey, Soupir, & Rehmann, 2012). Impediment of solids on the cheesecloth may further aid to capture planktonic cells within the filter. Although the target bacterial retention in the MMS filter was limited to about 1-log, results showed that this level of capture was sufficient for non-quantitative pathogen retention and subsequent detection (Table 2). When filtering near zero-turbidity water samples, it is advisable to add small amounts of sterile clay texture soil or commercial clay (e.g. Bentonite; Sigma Chemical) to the water with pre-filtration mixing to improve MMS capturing capabilities. However, selected microorganisms used to inoculate water were successfully detected, qualitatively, regardless of the initial water turbidity level and indigenous coliform and *E. coli* concentrations.

The application of the MMS system in environmental water samples in San Joaquin and Salinas Valley evidences its applicability for pathogen monitoring. In both regions pathogen detection after MMS enrichment and molecular detection was achieved. In Salinas' environmental samples, 1 out of 3 *E. coli* O157:H7 positive water sources and both Salinas region positives for *S. enterica*, indicator *E. coli* populations, at the time of collection, were slightly above the 126 MPN/100 mL (log 2.1 MPN/100 mL) standards. In contrast, in all four samples from San Joaquin in which *E. coli* O157:H7 was detected, indicator *E. coli* populations, at the time of collection, were below the 126 MPN/100 mL standards for overhead and drip/furrow irrigation adopted for the CA leafy green industry and several other produce commodities, such as fresh market tomatoes (United Fresh, 2011). These results suggest that indicator *E. coli* may inadequately reflect the presence of pathogens in the water, an issue extensively reviewed in a recent critical review by (Pachepsky et al., 2011). Separate from the main objective of this study, our assessment provides additional evidence that neither total coliform nor *E. coli* numbers in a water source, determined from a 'snapshot' sample, provide predictive insights to the presence of pathogenic forms. Environmental results showed that total coliform populations showed no significant difference among the different sample types; conversely, *E. coli* concentrations in tailwater (irrigation runoff) showed higher counts when compared to the other sources. These two particular sample types tend to carry more sediments and soil particles hence increasing the turbidity and background bacterial counts. Environmental data shows a link between total coliforms population and seasonality, especially at the end of summer and beginning of fall. These results are also supported by the temperature relationship with total coliform concentrations in the hot summer days that coincide with algal bloom season observed in San Joaquin valley canals in early August to early September and in the CA Central Coast surface water reservoirs in late August to early October. As typical for the region, irrigation district sources were the most numerous of the samples collected and not previously identified as harboring viable pathogens in assessment with the typical 100 mL sampling protocols (US EPA, 2001). This outcome highlights the importance of sample procedure and sample size if the objective is to functionally improve the assessment of pathogen presence in a water source intended for application to fresh produce. The expanding experiences with programmatic monitoring of fresh produce irrigation sources suggest the need for alternative indicator organisms and critical limits to better assess the risk of water fecal contamination.

5. Conclusions

The results reported in this study provide data that support validation of an optimized MMS system for laboratory and in-field/on-farm capture-filtration of low concentrations of human pathogens from low turbidity water and high turbidity complex water and environmental water samples. As a practical and readily accessible system for applied research or Good Agricultural Practices preventive controls and compliance monitoring, it is an economical and sufficiently sensitive approach to agricultural water testing. Understanding the source of contamination, presence and prevalence of pathogens in irrigation water is crucial in fresh produce safety management. The application of this strategy to increase the sample size analyzed through filtration and bacterial trapping in water will provide a more informative technique for monitoring water quality intended for fresh produce irrigation and foliar contact applications as well as a better tool for risk assessment of various water sources, where access to more sophisticated, precise, and expensive equipment is not available.

Acknowledgments

This study was partially funded by the California Leafy Greens Research Board and the California Melon Research Board. The cooperation of regional growers to allow access to private on-farm access points for irrigation water sampling is gratefully acknowledged.

References

Al-Qadiri, H. M., Lu, X., Al-Alami, N. I., & Rasco, B. A. (2011). Survival of *Escherichia coli* O157:H7 and *Campylobacter jejuni* in bottled purified drinking water under different storage conditions. *Journal of Food Protection*, *74*(2), 254–260. http://dx.doi.org/10.4315/0362-028X.JFP-10-368.

Badgley, B. D., Nayak, B. S., & Harwood, V. J. (2010). The importance of sediment and submerged aquatic vegetation as potential habitats for persistent strains of enterococci in a subtropical watershed. *Water Research*, *44*(20), 5857–5866. http://dx.doi.org/10.1016/j.watres.2010.07.005.

Badgley, B. D., Thomas, F. I. M., & Harwood, V. J. (2010). The effects of submerged aquatic vegetation on the persistence of environmental populations of *Enterococcus* spp. *Environmental Microbiology*, *12*(5), 1271–1281. http://dx.doi.org/10.1111/j.1462-2920.2010.02169.x.

Bertrand, R., & Roig, B. (2007). Evaluation of enrichment-free PCR-based detection on the *rfbE* gene of *Escherichia coli* O157—application to municipal wastewater. *Water Research*, *41*(6), 1280–1286. http://dx.doi.org/10.1016/j.watres.2006.11.027.

Bisha, B., Perez-Mendez, A., Danyluk, M. D., & Goodridge, L. D. (2011). Evaluation of modified Moore Swabs and continuous flow centrifugation for concentration of *Salmonella* and *Escherichia coli* O157:H7 from large volumes of water. *Journal of Food Protection*, *74*(11), 1934–1937. http://dx.doi.org/10.4315/0362-028X.JFP-11-133.

Byappanahalli, M. N., Sawdey, R., Ishii, S., Shively, D. A., Ferguson, J. A., Whitman, R. L., et al. (2009). Seasonal stability of Cladophora-associated *Salmonella* in Lake Michigan watersheds. *Water Research*, *43*(3), 806–814. http://dx.doi.org/10.1016/j.watres.2008.11.012.

Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M. T., Myers, C., Rose, C., et al. (2007). Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production

region in California. In D.Fox (Ed.), *PLoS One*, *2*(11), e1159. http://dx.doi.org/10.1371/journal.pone.0001159.

Craun, G. F., Brunkard, J. M., Yoder, J. S., Roberts, V. A., Carpenter, J., Wade, T., et al. (2010). Causes of outbreaks associated with drinking water in the United States from 1971 to 2006. *Clinical Microbiology Reviews*, *23*(3), 507–528. http://dx.doi.org/10.1128/CMR.00077-09.

Csordas, A., Delwiche, M., & Barak, J. (2008). Automatic detection of *Salmonella enterica* in sprout irrigation water using a nucleic acid sensor. *Sensors and Actuators B: Chemical*, *134*(1), 9–17. http://dx.doi.org/10.1016/j.snb.2008.05.020.

D'Lima, C. B., & Suslow, T. V. (2009). Comparative evaluation of Practical functionality of rapid test format kits for detection of *Escherichia coli* O157:H7 on lettuce and leafy greens. *Journal of Food Protection*, *72*(12), 2461–2470.

Eccles, J. P., Searle, R., Holt, D., & Dennis, P. J. (2004). A comparison of methods used to enumerate *Escherichia coli* in conventionally treated sewage sludge. *Journal of Applied Microbiology*, *96*(2), 375–383.

Fu, Z., Rogelj, S., & Kieft, T. L. (2005). Rapid detection of *Escherichia coli* O157:H7 by immunomagnetic separation and real-time PCR. *International Journal of Food Microbiology*, *99*(1), 47–57. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.07.013.

Galan, J. E., Ginocchio, C., & Costeas, P. (1992). Molecular and functional characterization of the *Salmonella* invasion gene *invA*: Homology of *InvA* to members of a new protein family. *Journal of Bacteriology*, *174*(13), 4338–4349.

Gelting, R. J., Baloch, M. A., Zarate-Bermudez, M. A., & Selman, C. (2011). Irrigation water issues potentially related to the 2006 multistate *E. coli* O157:H7 outbreak associated with spinach. *Agricultural Water Management*, *98*(9), 1395–1402. http://dx.doi.org/10.1016/j.agwat.2011.04.004.

Gilbride, K. A., Lee, D. -Y., & Beaudette, L. A. (2006). Molecular techniques in wastewater: Understanding microbial communities, detecting pathogens, and real-time process control. *Journal of Microbiological Methods*, *66*(1), 1–20. http://dx.doi.org/10.1016/j.mimet.2006.02.016.

Girones, R., Ferrus, M. A., Alonso, J. L., Rodriguez-Manzano, J., Calgua, B., Correa Ade, A., et al. (2010). Molecular detection of pathogens in water—The pros and cons of molecular techniques. *Water Research*, *44*(15), 4325–4339. http://dx.doi.org/10.1016/j.watres.2010.06.030.

Greene, S. K., Daly, E. R., Talbot, E. A., Demma, L. J., Holzbauer, S., Patel, N. J., et al. (2008). Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. *Epidemiology and Infection*, *136*(02). http://dx.doi.org/10.1017/S095026880700859X.

Haack, S. K., Duris, J. W., Fogarty, L. R., Kolpin, D. W., Focazio, M. J., Furlong, E. T., et al. (2009). Comparing wastewater chemicals, indicator bacteria concentrations, and bacterial pathogen genes as fecal pollution indicators. *Journal of Environment Quality*, *38*(1), 248. http://dx.doi.org/10.2134/jeq2008.0173.

Ishii, S., Yan, T., Shively, D. A., Byappanahalli, M. N., Whitman, R. L., & Sadowsky, M. J. (2006). *Cladophora* (Chlorophyta) spp. harbor human bacterial pathogens in Nearshore Water of Lake Michigan. *Applied and Environmental Microbiology*, *72*(7), 4545–4553. http://dx.doi.org/10.1128/AEM.00131-06.

Koster, W., Egli, T., Ashbolt, N., Botzenhart, K., Burlion, N., Endo, T., et al. (2003). Analytical methods for microbiological water quality testing. *Assessing microbial safety of drinking water* (pp. 237). London: Published on behalf of the World Health Organization and the Organisation for Economic Co-operation and Development by IWA.

Lio, J. C. W., & Syu, W. J. (2004). Identification of a negative regulator for the pathogenicity island of enterohemorrhagic *Escherichia coli* O157:H7. *Journal of Biomedical Science*, *11*(6), 855–863. http://dx.doi.org/10.1159/000081833.

Loge, F. J., Thompson, D. E., & Call, D. R. (2002). PCR detection of specific pathogens in water: a risk-based analysis. *Environmental Science & Technology*, *36*(12), 2754–2759. http://dx.doi.org/10.1021/es015777m.

Macler, B. A., & Merkle, J. C. (2000). Current knowledge on groundwater microbial pathogens and their control. *Hydrogeology Journal*, *8*(1), 29–40. http://dx.doi.org/10.1007/PL00010972.

Meays, C. L., Broersma, K., Nordin, R., & Mazumder, A. (2004). Source tracking fecal bacteria in water: a critical review of current methods. *Journal of Environmental Management*, *73*(1), 71–79. http://dx.doi.org/10.1016/j.jenvman.2004.06.001.

Miller, W. G., Leveau, J. H., & Lindow, S. E. (2000). Improved *gfp* and *inaZ* broad-host-range promoter-probe vectors. *Molecular Plant–Microbe Interactions*, *13*(11), 1243–1250.

Noble, R., & Weisberg, R. (2005). A review of technologies for rapid detection of bacteria in recreational waters. *Journal of Water and Health*, *3*, 381–392.

Olsen, S. J., Miller, G., Breuer, T., Kennedy, M., Higgins, C., Walford, J., et al. (2002). A waterborne outbreak of *Escherichia coli* O157:H7 infections and hemolytic uremic syndrome: implications for rural water systems. *Emerging Infectious Diseases*, *8*(4), 370–375. http://dx.doi.org/10.3201/eid0804.000218.

Pachepsky, Y., Shelton, D. R., McLain, J. E. T., Patel, J., & Mandrell, R. E. (2011). Irrigation waters as a source of pathogenic microorganisms in produce: a review. *Advances in Agronomy*, *vol. 113*. (pp. 75–141): Elsevier Retrieved from http://linkinghub.elsevier.com/retrieve/pii/B9780123864734000026

Pandey, P. K., Soupir, M. L., & Rehmann, C. R. (2012). A model for predicting resuspension of *Escherichia coli* from streambed sediments. *Water Research*, *46*(1), 115–126. http://dx.doi.org/10.1016/j.watres.2011.10.019.

Soderstrom, A., Osterberg, P., Lindqvist, A., Jonsson, B., Lindberg, A., Blide Ulander, S., et al. (2008). A large *Escherichia coli* O157 outbreak in Sweden associated with locally produced lettuce. *Foodborne Pathogens and Disease*, *5*(3), 339–349. http://dx.doi.org/10.1089/fpd.2007.0065.

Solomon, E. B., Yaron, S., & Matthews, K. R. (2002). Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied and Environmental Microbiology*, *68*(1), 397–400. http://dx.doi.org/10.1128/AEM.68.1.397-400.2002.

Solomon, E. B., Potenski, C. J., & Matthews, K. R. (2002). Effect of irrigation method on transmission to and persistence of *Escherichia coli* O157:H7 on lettuce. *Journal of Food Protection*, *65*(4), 673–676.

Steele, M., & Odumeru, J. (2004). Irrigation water as source of foodborne pathogens on fruit and vegetables. *Journal of Food Protection*, *67*(12), 2839–2849.

Suslow, T. V. (2010). *Standards for irrigation and foliar contact water: produce safety project issue brief.* (Retrieved from http://www.pewhealth.org/uploadedFiles/PHG/Content_Level_Pages/Issue_Briefs/PSP_Water-Suslow-1.pdf)

Thurston-Enriquez, J. A., Watt, P., Dowd, S. E., Enriquez, R., Pepper, I. L., & Gerba, C. P. (2002). Detection of protozoan parasites and microsporidia in irrigation waters used for crop production. *Journal of Food Protection*, *65*(2), 378–382.

United Fresh (2011). *Food safety programs and auditing protocol for the fresh tomato supply chain.* (Retrieved from http://www.unitedfresh.org/newsviews/food_safety_resource_center/fresh_tomato_supply_chain)

US EPA (). *Protocol for developing pathogen TMDLs*. Washington, DC: US Environmental Protection Agency (EPA-841-R-00-002).

US FDA (). *United States Food and Drug Administration.* Guide to minimize microbial food safety hazards for fresh fruits and vegetables (Retrieved from http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/UCM169112.pdf)

Wang, G., & Doyle, M. P. (1998). Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *Journal of Food Protection*, *61*(6), 662–667.

Ziemer, C. J., & Steadham, S. R. (2003). Evaluation of the specificity of *Salmonella* PCR primers using various intestinal bacterial species. *Letters in Applied Microbiology*, *37*(6), 463–469. http://dx.doi.org/10.1046/j.1472-765X.2003.01430.x.