Prevalence and Characterization of *Escherichia coli* and *Salmonella* Strains Isolated from Stray Dog and Coyote Feces in a Major Leafy Greens Production Region at the United States-Mexico Border

Michele T. Jay-Russell*, Alexis F. Hake, Yingjia Bengson, Anyarat Thiptara, Tran Nguyen

Western Center for Food Safety, University of California Davis, Davis, California, United States of America

Abstract

In 2010, Romaine lettuce grown in southern Arizona was implicated in a multi-state outbreak of *Escherichia coli* O145:H28 infections. This was the first known Shiga toxin-producing *E. coli* (STEC) outbreak traced to the southwest desert leafy green vegetable production region along the United States-Mexico border. Limited information exists on sources of STEC and other enteric zoonotic pathogens in domestic and wild animals in this region. According to local vegetable growers, unleashed or stray domestic dogs and free-roaming coyotes are a significant problem due to intrusions into their crop fields. During the 2010–2011 leafy greens growing season, we conducted a prevalence survey of STEC and *Salmonella* presence in stray dog and coyote feces. Fresh fecal samples from impounded dogs and coyotes from lands near produce fields were collected and cultured using extended enrichment and serogroup-specific immunomagnetic separation (IMS) followed by serotyping, pulsed-field gel electrophoresis (PFGE), and antimicrobial susceptibility testing. A total of 461 fecal samples were analyzed including 358 domestic dog and 103 coyote fecals. STEC was not detected, but atypical enteropathogenic *E. coli* (aEPEC) strains comprising 14 different serotypes were isolated from 13 (3.6%) dog and 5 (4.9%) coyote samples. *Salmonella* was cultured from 33 (9.2%) dog and 33 (32%) coyote samples comprising 29 serovars with 58% from dogs belonging to Senftenberg or Typhimurium. PFGE analysis revealed 17 aEPEC and 27 *Salmonella* distinct pulsotypes. Four (22.2%) of 18 aEPEC and 4 (6.1%) of 66 *Salmonella* isolates were resistant to two or more antibiotic classes. Our findings suggest that stray dogs and coyotes in the desert southwest may not be significant sources of STEC, but are potential reservoirs of other pathogenic *E. coli* and *Salmonella*. These results underscore the importance of good agriculture practices relating to mitigation of microbial risks from animal fecal deposits in the produce production area.

Citation: Jay-Russell MT, Hake AF, Bengson Y, Thiptara A, Nguyen T (2014) Prevalence and Characterization of *Escherichia coli* and *Salmonella* Strains Isolated from Stray Dog and Coyote Feces in a Major Leafy Greens Production Region at the United States-Mexico Border. PLoS ONE 9(11): e113433. doi:10.1371/journal.pone.0113433

Editor: Dongsheng Zhou, Beijing Institute of Microbiology and Epidemiology, China

Received July 10, 2014; Accepted October 28, 2014; Published November 20, 2014

Copyright: © 2014 Jay-Russell et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Funding for this project was provided by the Center for Produce Safety (http://cps.ucdavis.edu) and University of California Agriculture and Natural Resources (UCANR) United States Department of Agriculture/National Institute of Food and Agriculture (http://www.csrees.usda.gov/) grant #2010-34608-20788 (SA7670). Additionally, JV Farms is a Center for Produce Safety contributor (https://cps.ucdavis.edu/campaign_contributors.php). Except JV Farms, the funders had no role in study design or data collection. JV Farms food safety staff assisted in enrolling local animal shelters and collecting fecal samples for shipment to the authors. All funders had no role in the analysis, decision to publish, or preparation of the manuscript.

Competing Interests: This study was funded in part by JV Farms (Yuma, Arizona) through a contribution to the Center for Produce Safety Campaign for Research. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

* Email: mjay@ucdavis.edu

Introduction

Foodborne disease illnesses caused by pathogen contamination of fresh produce are being recognized in greater numbers in the United States (U.S.) and abroad [1], [2]. An analysis of Centers for Disease Control and Prevention (CDC) data on reported foodborne illnesses from 1973 to 1997 indicated that outbreaks associated with fresh produce accounted for 6% of all reported foodborne disease outbreaks in the 1990s compared with just 0.7% in the 1970s [3], [4]. A more recent survey of CDC outbreak data from 1998–2008 showed these numbers are still rising, with 46% of foodborne illnesses being attributed to produce and 22% specifically attributed to leafy greens [5]. While norovirus infections transmitted downstream during post-harvest handling are likely the major driver of these statistics, reports of fresh produce-associated outbreaks from zoonotic agents potentially spread by domestic and wild animal reservoirs in the pre-harvest environment are clearly contributing to this disease burden [6], [7].

Approximately 90% of commercial lettuce reservoirs for the U.S. market is grown in two major produce production regions that rotate seasonally [8]: the Salinas Valley in the central California coast (April through October) and the desert southwest at the U.S.-Mexico border (November through March). The desert southwest growing region includes Yuma, Arizona, California’s Imperial Valley, and northern Mexico. The role of domestic animals and wildlife as potential sources and transmitters of zoonotic bacterial pathogens to lettuce and other leafy greens and agriculture water has been studied at length in the central California coast [9], [10], [11], [12], [13]. There is limited information, however, on the importance of animal reservoirs in the pre-harvest bacterial contamination of fresh produce in other
parts of the country [14]. The desert presents unique pre-harvest food safety challenges including urban encroachment where produce fields and irrigation canals may be adjacent to housing developments and recreation vehicle (RV) parks. In addition to concerns about human sources of foodborne pathogens near leafy green production areas of the desert, growers report problems with unleashed, free-roaming domestic dogs (Canis familiaris) entering their fields. Off-leash or stray dog intrusions into produce fields and the surrounding production area may result in damage to crops and destruction of potentially contaminated plants (Figure 1). Growers also report frequent coyote (Canis latrans) sightings and signs (tracks, scat, feces) on roads adjacent to produce fields where tractors and other equipment are used.

In spring 2010, an outbreak of *Escherichia coli* O145:H28 infections involving 27 confirmed and 4 probable case-patients from 5 states was linked to Romaine lettuce grown in southern Arizona [15], [16]. This was the first known leafy green-related infections involving 27 confirmed and 4 probable case-patients

**Methods**

**Ethics Statement**

Permission to access privately owned lands was obtained from the produce companies enrolled in the study. Animal shelter administrative directors in the U.S. and Mexico approved participation in the study. Dog fecal samples from the shelter in Mexico were transported by vehicle across the Mexico-US border by one of our industry collaborators. A permit for importation of dog feces was not required per the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) “Animal and Animal Product” import guidelines (#1102 Feline and Canine Material). Wildlife scientific collection permits and university animal care and use approval were not necessary in this study because fecal samples were collected from the ground and no animals were handled.

**Sampling**

Three animal shelters were enrolled in the study, one each in Yuma, Arizona, Imperial Valley, California, and northern Mexico. These facilities were chosen because animal control officers had worked historically with leafy green growers in the region to remove stray dogs from agriculture fields. We aimed to sample once monthly during the desert southwest leafy greens growing season (November to March) with a goal to collect ~300 samples based on sample size calculations. Due to limits in the number of impounded dogs available each month and logistics with the shelter personnel, each facility was sampled six times spread variably from November 3, 2010 to May 5, 2011 (Table 1).

**Laboratory**

Our overall goal was to culture Shiga toxin-producing *E. coli* belonging to serogroups STEC O103, O145, O157, O26, and non-typhoidal *Salmonella enterica* using a combined pre-enrichment step followed by immunomagnetic separation (IMS), selective plating, latex agglutination, and PCR or biochemical confirmation as described below. Isolates were then characterized by presence of virulence factors, genetic relatedness, and antibiotic resistance.

---

**Figure 1. Examples of animal intrusions into produce production areas of the desert southwest: a stray dog traveling next to an irrigation canal in northern Mexico (A); coyote feces adjacent to a lettuce field in southern California (B); dog feces on a lettuce plant in southern Arizona (C); areas of intentionally destroyed lettuce crop (arrow) following evidence of animal intrusion.**

doi:10.1371/journal.pone.0113433.g001
Table 1. Monthly prevalence of atypical enteropathogenic *Escherichia coli* (aEPEC) and *Salmonella enterica* isolated from coyote and dog fecal samples, southwestern desert, November 3, 2010 through May 5, 2011.

| Source | Animal | Location | Nov | Dec | Jan | Feb | Mar | May | Total |
|--------|--------|----------|-----|-----|-----|-----|-----|-----|-------|
|        |        |          | No. positive/No. tested (%) |     |     |     |     |     |      |
| aEPEC  | COYOTES| Arizona  | 0/10 | 0/11 | 1/11 (9.1) | 0/7 | 0/0 | 0/0 | 1/39 (2.6) |
|        |        | California | 0/10 | 3/16 (18.8) | 0/13 | 0/11 | 1/14 (7.1) | 0/0 | 4/64 (6.3) |
|        |        | Subtotal  | 0/20 | 3/27 (11.1) | 1/24 (4.2) | 0/18 | 1/14 (7.1) | 0/0 | 5/103 (4.9) |
|        | DOGS   | Arizona  | 0/16 | 1/21 (4.8) | 0/0 | 0/17 | 3/45 (6.7) | 0/25 | 4/124 (3.2) |
|        |        | California | 1/24 (4.2) | 3/18 (16.7) | 0/23 | 1/18 (5.6) | 1/17 (5.9) | 0/0 | 6/100 (6.0) |
|        |        | Mexico   | 0/30 | 0/28 | 1/27 (3.7) | 2/49 (4.1) | 0/0 | 0/0 | 3/134 (2.2) |
|        | Subtotal | 1/70 (1.4) | 4/67 (6.0) | 1/50 (2.0) | 3/84 (3.6) | 1/62 (1.6) | 0/25 | 13/358 (3.6) |
|        | Total   | 1/90 (1.1) | 7/94 (7.4) | 2/74 (2.7) | 3/102 (2.9) | 2/76 (2.6) | 0/25 | 18/461 (3.9) |
| Salmonella enterica | COYOTES | Arizona | 6/10 (60.0) | 2/11 (18.2) | 5/11 (45.5) | 0/7 | 0/0 | 0/0 | 13/39 (33.3) |
|        |        | California | 5/10 (50.0) | 7/16 (43.6) | 3/13 (23.1) | 3/11 (27.3) | 2/14 (14.3) | 0/0 | 20/64 (31.3) |
|        | Subtotal | 11/20 (55.0) | 9/27 (33.3) | 8/24 (33.3) | 3/18 (16.7) | 2/14 (14.3) | 0/0 | 33/103 (32.0) |
|        | DOGS   | Arizona  | 0/16 | 2/21 (9.5) | 0/0 | 0/17 | 2/45 (4.4) | 0/25 | 4/124 (3.2) |
|        |        | California | 4/24 (16.7) | 1/18 (5.6) | 1/23 (4.3) | 0/18 | 3/17 (17.6) | 0/0 | 9/100 (9.0) |
|        |        | Mexico   | 13/30 (43.3) | 1/28 (3.6) | 3/27 (11.1) | 3/49 (6.1) | 0/0 | 0/0 | 20/134 (14.9) |
|        | Subtotal | 17/70 (24.3) | 4/67 (6.0) | 4/50 (8.0) | 3/84 (3.6) | 5/62 (8.1) | 0/25 | 33/358 (9.2) |
|        | Total   | 28/90 (31.1) | 13/94 (13.8) | 12/74 (16.2) | 6/102 (5.9) | 7/76 (9.2) | 0/25 | 66/461 (14.3) |

*The Arizona shelter was sampled twice in March and in May. The California shelter was sampled twice in November. The shelter in Mexico was sampled twice in December and twice in February.*

doi:10.1371/journal.pone.0113433.t001
Pre-enrichment. Initially, non-selective pre-enrichment for the simultaneous culture of STEC and Salmonella was performed by adding 10 grams of feces to 100 mL of universal pre-enrichment broth (UPB; Difco, Becton Dickinson, Sparks, MD) and incubating for 20 hours at 35°C using a protocol modified in our laboratory for dog fecal material [10]. One milliliter of enriched UPB was then transferred to 9 mL tryptic soy broth (TSB; Becton Dickinson, Sparks, MD) for STEC detection and incubated for 2 hours at 25°C with shaking at 100 rpm (Innova 44, Eppendorf North America, Hauppauge, NY), followed by 8 hours at 42°C with shaking at 100 rpm, then at 6°C without shaking until processing the following day [10], [19]. One milliliter of enriched UPB was also transferred to 9 mL of buffered peptone water (BPW; Hardy Diagnostics, Santa Maria, CA) for Salmonella detection and incubated for 24 hours at 37°C with shaking at 50 rpm [11]. In 2011, we discontinued the use of the UPB pre-enrichment step to streamline the protocol. Instead, pre-enrichment was performed by adding 10 grams of feces directly into a WhirlPak bag containing 100 mL TSB followed by the same incubation parameters as just described [10], [19]. Spiking experiments comparing the UPB and TSB pre-enrichment methods revealed no statistical difference (p = 0.32) in recovery of STEC or Salmonella (data not shown). As such, we completed this study using the streamlined protocol without the UPB step. Aliquots of the primary enrichment broths were mixed with sterile glycerol to a final concentration of 14.3%, and stored at −20°C [11].

Escherichia coli. IMS using Dynal anti-E. coli O157, O26, O103, and O145 beads (Invitrogen, Grand Island, NY) was performed on TSB enrichment broths with the automated Dynal BeadRetriever (Invitrogen) per the manufacturer’s instructions. Following incubation and washing, 50 µL of the resuspended beads were plated onto Rainbow agar (Biolog, Hayward, CA) with novobiocin (20 µg/mL) and tellurite (0.8 µg/mL) (MP Biomedicals, Solon, OH) and streaked for isolation [10], [19]. Another 50 µL were plated onto Sorbitol MacConkey Agar (BD Becton, Sparks, MD) with ceftaxime (0.05 mg/L) and tellurite (2.5 mg/L), streaked for isolation, and incubated at 37°C overnight. E. coli O157:H7 RM1484 and three non-O157 STEC strains (O103, O145, O26) were used as positive controls to detect EPEC and STEC) E. coli O157, O26, O103, and O145 beads (Invitrogen, Grand Island, NY) was performed on BPW broths as described previously using the Dynal BeadRetriever (Invitrogen) [11]. Following incubation and washing, 100 µL of separated broth was further enriched in 3 mL Rappaport-Vassiliadis Soya Peptone (RVS; Difco, Becton Dickinson, Franklin Lakes, NJ) broth for 48 hours at 42°C [23]. Ten microliters of RVS broth were then plated on xylose lysine deoxycholate (XLD; Difco, Becton Dickinson, Franklin Lakes, NJ) agar and incubated at 37°C overnight. Samples with growth of hydrogen sulfide positive colonies were confirmed for Salmonella by performing biochemical profiles (triple sugar iron, urea, citrate, and lysine decarboxylase) on up to six individual colonies from each XLD agar. Salmonella Enteritidis ATCC BAA1045 was used as a positive control to observe the expected phenotype. The same colonies used for biochemical profiling were also streaked onto LB agar and incubated at 37°C overnight. Two bacterial colonies from each positive plate were banked onto Cryobeads (ProLab Diagnostics, Round Rock, TX) and stored at −80°C. Serotyping using the Kauffmann-White scheme was conducted by the United States Department of Agriculture (USDA) National Veterinary Services Laboratories in Ames, Iowa [24].

Pulse field Gel Electrophoresis. E. coli and Salmonella isolates were retrieved from frozen storage at −80°C and clonal relationships were assessed by pulsed-field gel electrophoresis (PFGE) according to the CDC’s PulseNet standard procedure using Salmonella Braenderup ATCC BAA664 as the molecular size standard [25]. Briefly, bacterial isolates were suspended in buffer containing 10 mM Tris pH 8 and 10 mM EDTA for DNA isolation. DNA was digested in enzyme buffer with restriction enzyme XbaI. Images were analyzed, and the similarity among different strains was characterized using BioNumerics version 7.1 software (Applied Maths, Austin, TX). Pattern comparisons were made using the software cluster analysis tool and confirmed by visual examination to assign pulsotypes [26], [27].

Antimicrobial Susceptibility testing. Frozen E. coli and Salmonella isolates were thawed and streaked onto trypticase soy agar (TSA; Difco, Becton Dickinson, Franklin Lakes, NJ) with 5% sheep blood agar, then incubated at 37°C for 24 hours. The broth microdilution method for antimicrobial susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [28], [29]. Isolates were evaluated for susceptibility to 12 antimicrobial drugs (ampicillin, amoxicillin/clavulanic acid, ceftriaxone, azithromycin, chloramphenicol, sulfisoxazole, cefoxitin, kanamycin, streptomycin, trimethoprim/sulfamethoxazole, tetracycline, cefurox) using the National Antimicrobial Resistance Monitoring System (NARMS) Gram negative tray (Trek Diagnostic Systems, Westlake, OH). E. coli ATCC 25922, E. coli ATCC 35218, and Pseudomonas aeruginosa ATCC 27853 were used as quality control organisms.
for MIC determination in accordance with CLSI guidelines. Breakpoints guidelines were adopted from the NARMS report, with the exception of azithromycin, for which no breakpoints have been published [30]. Based on a recent publication proposing an epidemiologic cut-off for wild-type *Salmonella* of ≤16 µg/ml, isolates with MIC values >16 µg/ml were considered resistant to azithromycin for the purpose of this study [31].

**Statistical Analysis**

WinEpi online software (http://www.winepi.net/uk/index.htm) was used to calculate sample size with a confidence level set at 95% and a population size of 1,000–10,000. Prevalence estimates were based on data from a longitudinal study of coyote populations in the central California coast in 2008-2010 [11, 19], with 1% for *E. coli* O157, 5% for non-O157 STEC, and 10% for *Salmonella enterica*; using these estimates, the required number of samples would be 258–294 for *E. coli* O157, 57–59 for non-O157 STEC, and 29 for *Salmonella* detection. Based on the expected low prevalence of *E. coli* O157, our goal was to collect at least 300 fecal samples.

Data were entered in Microsoft Excel 2007 spreadsheets and exported for analysis in STATA (Stata 11.0, College Station, TX). McNemar’s chi-square test was used to compare the sensitivities of *Salmonella* isolation from coyote scat due to any specific serovar. All 278 isolates were negative by PCR for genes encoding stx1 and stx2. Additionally, 461 frozen fecal TSB-enrichment broths were negative using a multiplex qPCR assay to detect stx1 and stx2 genes [19]. Isolates (n = 187) lacking any virulence factors and not belonging to one of the eight serogroups identified by the reference laboratory’s multiplex PCR were not further characterized. Among the remaining 91 isolates, a total of 29 different *E. coli* serotypes were identified (Table 4). Excluding clones from the same samples, there were 18 isolates comprising 14 serotypes with genes encoding *eaeA*, including one (O26:H11) with both *eaeA* and *hlyA* genes; these isolates were classified as aEPEC (Table 4) [32, 33]. There was more diversity among serotypes from coyotes compared with dogs. Specifically, 11 of 15 coyote fecal isolates were different serotypes with none being dominant. In contrast, almost half of the dog samples contained two dominant serotypes that were negative for virulence markers, O103:H11 and O103:H49 (Table 4).

A total of 17 pulsotypes (PT) were found among 18 aEPEC strains from dogs and coyote feces (Figure 2). Two non-pathogenic *E. coli* O145:H11 isolates were included in the dendrogram for comparison with the *E. coli* O145:H28 human clinical strain (PT-8) associated with the 2010 outbreak linked to Romaine lettuce. Of note, *E. coli* O145 strains isolated from dog feces during the study had different H types (O145:H11 and O145:H34) and were genetically unrelated to the 2010 outbreak strain based on PFGE analysis (Table 4, Figure 2).

All aEPEC isolates were tested for antibiotic resistance, and 6 (33.3%) were found to be pan-susceptible to the antimicrobial drugs we used in the NARMS panel (Table 5). One isolate from coyote and 3 isolates from dog feces were resistant to two or more antibiotics. Two aEPEC isolates, serotypes O167:H9 and O114:H8, from dog feces collected in Arizona and Mexico, respectively, were resistant to four antibiotics including ampicillin, ceftriaxone, chloramphenicol, tetracycline (O167:H9), and chloramphenicol, sulfisoxazole, streptomycin, and trimethoprim/sulfamethoxazole (O114:H9).

**Phenotypic and molecular characterization of *Salmonella* isolates**

Overall, 29 different *Salmonella enterica* serovars were identified with 46 (70%) of 66 isolates belonging to subspecies Group I (Table 6). Two dominant serovars, Senftenberg and Typhimurium comprised 38% of the isolates from dog samples. In contrast, no predominant *Salmonella* serovars were identified among strains isolated from coyotes. A significant association was observed between serovar Senftenberg and the date of sample collection (P = 0.03), with 10 of the 14 S. Senftenberg isolations occurring in the month of November, primarily from the shelter in Mexico.
As shown in Figure 3, PFGE analysis of Salmonella isolates revealed 27 distinct pulsotypes. S. Senftenberg isolates (n = 8) from dog feces collected on two dates at the shelter in Mexico belonged to four different but closely related pulsotypes (PT-9, 10, 11, 12). In contrast, S. Senftenberg isolates (n = 6) from California shelter dogs collected on three sampling dates belonged to a single pulsotype (PT-13). S. Typhimurium PT-18 and PT-21 were the only shared pulsotypes among samples from different locations and species including 2 California coyote and an Arizona dog isolate (PT-18), and two dogs from Mexico and a California coyote (PT-21).

Antibiotic resistance testing revealed that 58 (87.9%) of the 66 Salmonella isolates, evenly distributed between dogs and coyotes, were susceptible to the antibiotics tested (Table 5). Of the 8 Salmonella isolates with resistance to at least one antibiotic, 4 were resistant to 2 or more drugs. An S. Newport isolate from coyote feces collected in California showed the most antibiotic resistant phenotype in this study including resistance to ampicillin, amoxicillin/clavulanic acid, ceftriaxone, chloramphenicol, and trimethoprim/sulfamethoxazole.

### Discussion

In this study, we show that stray dogs and free-roaming coyotes in the southwest desert leafy greens production region at the U.S.-Mexico border do not appear to be significant reservoirs of E. coli O157 and other STEC, but aEPEC and Salmonella were prevalent in fecal samples using the methods described herein.

### E. coli detection and characterization

Previous studies of STEC occurrence in domestic dogs in the U.S. have focused on detection of Shiga toxin genes among animals with and without gastroenteritis. In one survey, a higher prevalence of stx1 (3% and 15%) and stx2 (36% and 23%) was found in diarrheic and non-diarrheic greyhounds, respectively [34]. In another study, there was no occurrence of stx1 or stx2 in 52 healthy Midwestern research colony dogs [35]. For logistical reasons, we were not able to sample unhealthy dogs with diarrhea in isolation wards at the U.S. shelters, and health status information was not available at the shelter in Mexico; thus, we collected only fresh, normally formed fecal samples from dogs.

Outside the U.S., a survey in Japan revealed an extremely low prevalence of E. coli O157:H7 in dogs and cats, with only 1 of 614 (0.2%) fecal samples testing positive [36]. If we over-estimated E. coli O157:H7 prevalence at 1% in our sample size calculations, it is possible we would have needed to collect more than 300 fecal samples to detect E. coli O157:H7 in the shelter dog population. In another Japanese survey, a positive association between the presence of dogs or cats on beef cattle farms and prevalence of O157 in cattle was found [37]. This association, however, could be attributed to the hygiene practices of the farm (e.g., farms that allow dogs to run loose may have poorer hygiene and biosecurity practices than farms that do not), rather than evidence of colonization. In Argentina, stx1 and stx2 were detected in 3.7% and 4.2% of dog samples, respectively, and STEC was culture confirmed in 4% of the samples [38]. While our results suggest that STEC is rare in southwest dog populations, there are caveats to consider when comparing our results with prevalence surveys in

### Table 2. Summary of population characteristics from domestic dogs sampled in a southwest United States and northern Mexico produce production region, November 3, 2010 through May 5, 2011 (N = 358).

| Demographic            | Number Sampled (%) |
|------------------------|--------------------|
| **Shelter**            |                    |
| Arizona                | 124 (34.6)         |
| California             | 100 (27.9)         |
| Mexico                 | 134 (37.4)         |
| **Reason Impounded**   |                    |
| Stray                  | 297 (83.0)         |
| Othera                 | 44 (12.3)          |
| Unknown                | 17 (4.7)           |
| **Age**                |                    |
| Puppy                  | 58 (16.2)          |
| Adult                  | 279 (77.9)         |
| Unknown                | 21 (5.9)           |
| **Sex**                |                    |
| Male                   | 165 (46.1)         |
| Female                 | 186 (52.0)         |
| Unknown                | 7 (2.0)            |
| **Breed**              |                    |
| Chihuahua/Mix          | 41 (11.5)          |
| Labrador/Shepherd Mix  | 56 (15.6)          |
| Pit Bull Terrier/Mix   | 60 (16.8)          |
| Other                  | 37 (10.3)          |
| Unknown                | 140 (39.1)         |

*aIncludes all dogs born in shelter, relinquished by owner, confiscated from owner, or dogs being kept for quarantine or treatment purposes.

doi:10.1371/journal.pone.0113433.t002
Table 3. Comparison of results from Shiga toxin-producing E. coli (STEC) O-group-specific (O103, O145, O157, O26) commercial latex agglutination screening tests and a multiplex PCR confirmatory test to detect 8 major STEC O-groups (O103, O111, O113, O121, O145, O157, O26, O45) used to serotype isolates cultured from fecal samples by selective enrichment and serogroup-specific (O103, O145, O157, O26) immunomagnetic separation (IMS).

| Multiplex STEC PCR | O103 | O111 | O113 | O121 | O145 | O157 | O26 | Total |
|--------------------|------|------|------|------|------|------|-----|-------|
| No. Isolates<sup>a</sup> | 199  | 62   | 59   | 1    | 2    | 1    | 1   | 278   |
| O103               |      |      |      |      |      |      |     |       |
| O111               |      |      |      |      |      |      |     |       |
| O113               |      |      |      |      |      |      |     |       |
| O121               |      |      |      |      |      |      |     |       |
| O145               |      |      |      |      |      |      |     |       |
| O157               |      |      |      |      |      |      |     |       |
| O26                |      |      |      |      |      |      |     |       |

<sup>a</sup>All isolates were stx<sub>1</sub> and stx<sub>2</sub> positive.

<sup>b</sup>Two isolates classified as O103 by latex agglutination screening and negative by multiplex STEC PCR belonged to serotype O103 (Table 4). We also found discordant results between O-group specific latex agglutination and confirmatory tests, which has been described previously [48]. The utility of using a commercial latex agglutination screen to identify presumptive STEC from environ-
mental samples such as feces needs further assessment and comparison with other methods including the serology gold standard. Nevertheless, we concluded that screening for Shiga toxin genes by qPCR would be a more efficient approach to detect STEC colonies in the future, rather than screening for STEC O-groups.

Salmonella detection and characterization

Reported Salmonella prevalence in dogs varies greatly between studies, from 5% to over 70% [49], [50], [51], [52]. The predominance of two Salmonella serovars in the shelter dog population, but not in the sampled coyote population, is interesting. According to a USDA NVSL 2011 annual report, Typhimurium and Senftenberg, followed by Muuenchen, Newport, and Javiana, were the most common serovars isolated nationally from dogs and cats during 2011 [53]. We speculate that the dominant serovars among shelter dog samples could indicate a common source of exposure to Salmonella in the environment. For example, contaminated dog food is increasingly recognized as a risk factor for Salmonella infections in dogs [54], [55], [56]. The findings could also be indicative of poor sanitation practices and overcrowding. Staffing at the facility in Mexico was noticeably limited and animals were typically kenneled in large groups of ten or more dogs, making the transmission of Salmonella between

Table 4. Serotypes and virulence factors of Escherichia coli strains isolated from dog and coyote fecal samples, southwestern desert, November 3, 2010 through May 5, 2011.

| Source | Serotype* | Coyote | Dog | Total | Virulence Factor |
|--------|-----------|--------|-----|-------|-----------------|
|        |           | stx1/stx2 | eaeA | hlyA |
| aEPEC  | O::H2     | 1      | 0   | 1     | -               |
|        | O::H8     | 0      | 1   | 1     | -               |
|        | O::H25    | 0      | 1   | 1     | -               |
|        | O114: H8a | 0      | 1   | 1     | -               |
|        | O123: H+  | 0      | 2   | 2     | -               |
|        | O126: H9  | 0      | 1   | 1     | -               |
|        | O128: H2  | 1      | 0   | 1     | -               |
|        | O145: H34 | 0      | 3   | 3     | -               |
|        | O153: H21/36 | 0      | 1   | 1     | -               |
|        | O157: H+  | 2      | 0   | 2     | -               |
|        | O167: H9  | 0      | 1   | 1     | -               |
|        | O26: H11  | 0      | 1   | 1     | -               |
|        | O26: H8   | 1      | 0   | 1     | -               |
|        | O64: H19  | 0      | 1   | 1     | -               |
| Subtotal|          | 51     | 3   | 1     | 8               |
| OTHER  | O103: H+  | 1      | 0   | 1     | -               |
|        | O103: H2  | 1      | 4   | 5     | -               |
|        | O103: H7  | 0      | 7   | 7     | -               |
|        | O103: H9  | 0      | 2   | 2     | -               |
|        | O103: H16b| 0      | 11  | 11    | -               |
|        | O103: H19 | 1      | 0   | 1     | -               |
|        | O103: H21 | 1      | 0   | 1     | -               |
|        | O103: H21/36 | 2      | 1   | 3     | -               |
|        | O103: H40/44 | 2      | 1   | 3     | -               |
|        | O103: H43 | 0      | 1   | 1     | -               |
|        | O103: H49b| 2      | 26  | 28    | -               |
|        | O113: H4b | 0      | 3   | 3     | -               |
|        | O145: H11b| 0      | 2   | 2     | -               |
|        | O26: H2   | 0      | 1   | 1     | -               |
|        | O26: H32b | 0      | 3   | 3     | -               |
| Subtotal|          | 10     | 62  | 72    |                 |
| TOTAL  |          | 15     | 76  | 91    |                 |

*aO-, O antigen non-determinant.

Twelve dog fecal samples contained two different serotypes including O103:H16/O113:H4 (n = 6); O26:H32/O103:H49 (n = 5); and O114:H8/O145:H11 (n = 1).

doi:10.1371/journal.pone.0113433.t004
individuals more likely to occur. We controlled for this variable by limiting fecal collections to fresh feces from individual animals impounded within the last 24 hours. Often, however, it was noted that most of the individual animals sampled on a given day from the Mexican shelter had been collected from the same location and were likely living in groups together. Thus, dogs shedding genetically related strains may have shared common exposures prior to or during impound.

The fact that nearly one in three (32%) coyote fecal samples collected near leafy green fields were positive for *Salmonella* was surprising. In contrast, a survey from the central California coast found that the organism was recovered from only 3 (7.5%) of 40 coyote colonic fecal sample enrichment broths [11]. Variations in sampling and laboratory culture methods between the two studies could, in part, explain these differences. It is also possible that individual coyotes were re-sampled over the course of this study. However, given the wide geographic and temporal distribution of sampling locations in combination with the high diversity of serovars and PFGE subtypes (Figure 3), we do not believe that repeat sampling of individual coyotes contributed significantly to our overall prevalence. Even in the event of re-sampling, the apparent high prevalence of *Salmonella* in coyote fecal material found in or near the production area is of importance to growers given the potential to contaminate the plants directly, or indirectly via agriculture water sources and farm equipment. Additional studies using trapping or hunting techniques are needed to determine actual prevalence of foodborne pathogens in the southwest desert coyote population.

There was some seasonality observed in *Salmonella* recovery from both dog and coyote samples including a significantly higher prevalence in November compared with samples collected in late winter and spring months. More long-term studies, however, are indicated to reveal any true seasonality or temporal patterns of *Salmonella* occurrence. The relatively high prevalence of *Salmonella* shedding in dogs and coyotes may be due in part to the hunting/scavenging behaviors of canids in the region. In desert regions where prey and water resources are limited, both coyotes and stray dogs opportunistically forage on fresh and rotten nutritional sources, including garbage and other refuse, vegetable and fruit matter, and the meat of dead animals. Such scavenging behaviors may put dogs and coyotes at a higher risk of exposure to *Salmonella*. High prevalence of *Salmonella* has previously been found in other scavenging species of the southwest, such as turkey vultures (*Cathartes aura*) [57]. Additionally, these animals may be obtaining water from anthropogenic sources, such as irrigation ditches, sediment basins, and camp-sites in the absence of natural water sources. Water samples, from both static sources and flowing streams, rivers, and creeks, often yield high percentages of *Salmonella* positive samples [10], [58], [59].

We found more antibiotic resistance among aEPEC isolates compared with *Salmonella* isolates (Table 5). Four (22.2%) of 18 aEPEC and 4 (6.1%) of 66 *Salmonella* isolates were resistant to two or more antibiotic classes; two dog aEPEC isolates (O114:H8 and O167:H9), a dog S. Senftenberg, and a coyote S. Newport displayed resistance to 3 or more antibiotic classes. Of note, Newport and Senftenberg have been identified as emerging multi-drug-resistant serovars worldwide [60], [61]. In a wildlife study conducted during the same time period in the central California coast, a majority of *Salmonella enterica* subspecies Group IIIa and IIIb isolates from wild-caught amphibians and reptiles captured near produce fields were resistant to at least one antibiotic [12]. It appears that antibiotic resistance among *Salmonella* isolates is less

Figure 2. *Escherichia coli* (XbaI restriction) pulsotypes of 18 aEPEC isolates and 2 non-pathogenicirulent *E. coli* O145:H11 isolates from dog and coyote fecal samples in the southwest desert produce growing areas of Arizona, California, and northern Mexico, November 3, 2010 through May 5, 2011. A human clinical *E. coli* O145:H28 outbreak strain associated with a Romaine lettuce-related outbreak traced to Arizona in May 2010 is also shown. doi:10.1371/journal.pone.0113433.g002
Table 5. Antimicrobial resistance patterns among 18 atypical enteropathogenic *Escherichia coli* (aEPEC) and 66 *Salmonella enterica* isolates from coyote and dog fecal samples, southwestern desert, November 3, 2010 through May 5, 2011.

| Drug resistance patterna | Source (No. of isolates) | Serotype or antigenic formula b |
|--------------------------|--------------------------|--------------------------------|
|                          | aEPEC Coyote (n = 5) Dog (n = 13) Total (% of all isolates) |                                |
| Pansusceptible           | 2 4 6 (33.3)             | O-H2                           |
|                          |                         | O-H25                          |
|                          |                         | O126:H9                        |
|                          |                         | O145:H34                       |
|                          |                         | O26:H8                         |
| FIS                      | 2 6 8 (44.4)            | O-H8                           |
|                          |                         | O123:H+                        |
|                          |                         | O128:H2                        |
|                          |                         | O145:H34                       |
|                          |                         | O153:H21/36                    |
|                          |                         | O157:H+                        |
|                          |                         | O26:H11                        |
|                          |                         | O64:H19                        |
| FIS-STR                  | 1 0 1 (5.6)             | O157:H+                        |
| FIS-TET                  | 0 1 1 (5.6)             | O123:H+                        |
| AMP-AXO-CHL-TET          | 0 1 1 (5.6)             | O167:H9                        |
| CHL-FIS-STR-SXT          | 0 1 1 (5.6)             | O114:H8                        |
| Salmonella               | Coyote (n = 33) Dog (n = 33) Total (% of all isolates) |                                |
| Pansusceptible           | 29 29 58 (87.9)         | Aqua                           |
|                          |                         | Barranquilla                   |
|                          |                         | Drac                           |
|                          |                         | Duisburg Enteritidis           |
|                          |                         | Javiana                        |
|                          |                         | Livingstone; Montevideo        |
|                          |                         | Muenchen                       |
|                          |                         | Newport                        |
|                          |                         | Oranienburg Sandiego           |
|                          |                         | Senftenberg Typhimurium        |
|                          |                         | Typhimurium var S-             |
|                          |                         | II 47:bi:1,5                   |
|                          |                         | III 17:229:-                  |
|                          |                         | III 62a:36:-; III 40:24; 32:- |
|                          |                         | III 48:z51; III 48:z4; IV      |
|                          |                         | 44z36:-                        |
|                          |                         | IV 47;i,x,e,u,x                |
| AMP                      | 0 1 1 (1.5)             | Enteritidis                    |
| STR                      | 1 1 2 (3.0)             | Typhimurium                    |
|                          |                         | IV Rough O: autoagglutinate    |
| XNL                      | 1 0 1 (1.5)             | Sandiego                       |
| AXO-TET                  | 1 1 2 (3.0)             | Mbandaka IV 44z36:-           |
| AMP-STR-SXT              | 0 1 1 (1.5)             | Senftenberg                    |
| AMP-AUG2-AXO-CHL-SXT     | 1 0 1 (1.5)             | Newport                        |

aAMP, ampicillin; AUG2, amoxicillin/clavulanic acid; AXO, ceftriaxone; AZI, azithromycin; CHL, chloramphenicol; FIS, sulfisoxazole; FOX, cefoxitin; KAN, kanamycin; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; XNL, ceftiofur.
bO-, O antigen non-determinant.
doi:10.1371/journal.pone.0113433.t005
prominent in canid populations tested in the southwest desert compared with the cold-blooded vertebrates surveyed in coastal California.

Prevention and Control Recommendations

The produce industry currently addresses foodborne pathogen hazards from domestic and wild animal sources through adherence to best practices established by the Arizona and California Leafy Green Marketing Agreements [62], [63]. For example, fecal material in the production area is removed, and a minimal 5-foot radius no-harvest buffer zone (Figure 1D) is used to prevent contaminated plants from entering the food supply. Repeated intrusions into produce fields by dogs and coyotes can be managed by use of fencing, control of strays, and depredation (coyotes). In our study region, animal control officers on both sides of the border work closely with the produce growers to assist with stray animal problems in fields. In the Imperial-Yuma region, loose dogs can be particularly problematic in the fall-winter season—which is also the leafy greens growing season—when the area becomes a popular tourist (“snowbird”) destination for recreational vehicle enthusiasts who often travel with their dogs. In Mexico, stray dog control is more challenging because of limited resources, large numbers of un-owned dogs, and cultural barriers to dog population control.

It is worth noting that although intrusions by stray dogs may represent a food safety risk for fresh produce, trained working dogs if used properly can actually be an asset and should not be discouraged. For example, dogs have been used to help disperse and deter large flocks of nuisance birds, and scent detection dogs have been used experimentally to identify in-field fecal contamination of raw produce [64].

Conclusions

In summary, results from this study will assist the produce industry by providing baseline information on the occurrence of zoonotic enteric pathogens found in fecal material from common

| Table 6. Subspecies and serovars of *Salmonella* isolated from dog and coyote fecal samples, southwestern desert, November 3, 2010 through May 5, 2011. |
|---|
| **Source (No. isolates)** |
| **Subspecies (Group)** | **Serovar or antigenic formula** | **Coyote** | **Dog** | **Total** |
| Enterica (I) | Aqua | 1 | 0 | 1 |
| | Barranquilla | 1 | 0 | 1 |
| | Derby | 0 | 1 | 1 |
| | Drac | 1 | 0 | 1 |
| | Duisburg | 1 | 0 | 1 |
| | Ealing | 0 | 1 | 1 |
| | Enteritidis | 0 | 2 | 2 |
| | Javiana | 2 | 0 | 2 |
| | Livingstone | 0 | 1 | 1 |
| | Mbandaka | 0 | 1 | 1 |
| | Montevideo | 1 | 0 | 1 |
| | Muenchen | 2 | 0 | 2 |
| | Newport | 3 | 0 | 3 |
| | Oranienburg | 0 | 1 | 1 |
| | Sandiego | 2 | 0 | 2 |
| | Senftenberg | 0 | 14 | 14 |
| | Typhimurium | 5 | 5 | 10 |
| | Typhimurium var. 5- | 0 | 1 | 1 |
| Salmae (II) | 47:b:1,5 | 1 | 0 | 1 |
| Arizona (Illa) | 17:z29- | 3 | 0 | 3 |
| | 35:z29- | 0 | 1 | 1 |
| | 62:z36- | 1 | 0 | 1 |
| | 40:z4, z32:- | 3 | 0 | 3 |
| | 48:g, z51:- | 0 | 3 | 3 |
| Dianzizone (IIib) | 48:z2 | 2 | 0 | 2 |
| | 50:xz | 1 | 0 | 1 |
| Houtenae (IV) | 44:z36- | 1 | 1 | 2 |
| | 47, v,e,n,x | 2 | 0 | 2 |
| Unknown | Rough O:autoagglutinate | 0 | 1 | 1 |
| Total | 33 | 33 | 66 |

doi:10.1371/journal.pone.0113433.t006
domestic and wild canid populations in the desert southwest produce production region. The findings underscore the importance of good agriculture practices for leafy greens and other produce, especially those relating to animal intrusions and pre- and post-harvest environmental assessments. Follow-up surveys are warranted to determine pathogenic \textit{E. coli} and \textit{Salmonella} prevalence in other potential domestic and wildlife reservoirs in this region, and comparison with other possible environmental sources of microbial contamination (e.g., canals, irrigation water, and soil amendments).

Acknowledgments

We are deeply grateful to the growers for their assistance with sample collections and access to study locations. We thank Fatima Corona and Ed Morales for providing information about the leafy greens industry in the southwest desert and on-site coordination. We are also indebted to the local governments and animal shelters that provided us with access to their facilities and data on impounded animals. For technical assistance, we thank Diana Carychoao from the USDA ARS Produce Safety and Microbiology Research Unit, Beth Roberts from the Pennsylvania State University \textit{E. coli} Reference Center, and Peiman Aminabadi at UC Davis. We also thank Robert E. Mandrell for providing the \textit{E. coli} O145:H28 clinical outbreak strain, and Maria D. Pereira for providing the \textit{E. coli} O103, O145, and O26 isolates used as positive controls.

Author Contributions

Conceived and designed the experiments: MTJ AFH YB AT TN. Performed the experiments: MTJ AFH YB AT TN. Analyzed the data: MTJ AFH. Wrote the paper: MTJ AFH.

References

1. Lynch MF, Tauxe RV, Hedberg CW (2009) The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol Infect 137: 307–315.
2. Berger CN, Sodha SV, Shaw RK, Griffin PM, Pink D, et al. (2010) Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ Microbiol 12: 2385-2397.
3. Lynch M, Painter J, Woodruff R, Braden C (2006) Surveillance for foodborne-disease outbreaks—United States, 1998–2002. MMWR Surveill Summ 55: 1–34.
4. Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV (2004) Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J Food Prot 67: 2342–2353.
5. Painter JA, Hoskstra RM, Ayers T, Tauxe RV, Braden CR, et al. (2013) Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg Infect Dis 19: 407–415.
6. Mandrell RE (2011) Tracing pathogens in fruit and vegetable production chains. In: Brul S, Fratamico PM, McMeekin TA, editors. Tracing pathogens in the food chain. Woodhead Publishing Ltd, pp. 540–595.
7. Jay-Russell MT (2013) What is the risk from wild animals in food-borne pathogen contamination of plants? CAB Reviews 8: 040.
8. Borrs H, Brunke H. (2012) Produce Profile. Agricultural Issues Center, University of California. Available: http://www.agmrc.org/commodities__products/vegetables/lettuce-profile/. Accessed 18 October 2014.

Figure 3. \textit{Salmonella} Pulsotypes (\textit{XbaI} restriction) isolated from dog and coyote fecal samples collected in the southwest desert produce growing areas of Arizona, California, and northern Mexico, November 3, 2010 through May 5, 2011. doi:10.1371/journal.pone.0113433.g003
E. coli and Salmonella in Dog and Coyote Feces

strains: a basis for molecular risk assessment of typical and atypical EPEC strains. BMC Microbiol 11: 142.

34. Staat JJ, Chengappa MM, DeBey MC, Ficklhorn B, Oehser RD (2003) Detection of Escherichia coli Shiga toxin (stx) and enterotoxin (eae and elt) genes in foods from non-diarrheic and diarrheal greyhounds. Vet Microbiol 94: 303–312.

35. Holland RE, Walker RD, Sraranganathan N, Wilson RA, Ruhl DC (1999) Characterization of Escherichia coli isolated from healthy dogs. Vet Microbiol 63: 133–162.

36. Kazakova Y, Irie Y, Sawada T, Nakazawa M (2010) A 5-year epidemiological surveillance of Escherichia coli O157:H7 in dogs and cats in Japan. J Vet Med Sci 72: 791–794.

37. Sasaki Y, Yamada Y, Konokawa M, Murakami M, Katayama S, et al. (2011) Prevalence and characterization of Shiga toxin-producing Escherichia coli O157 and O26 in beef farms. Vet Microbiol 150: 140–145.

38. Bentancor A, Runi MV, Gentilini MV, Sardy C, Irino K, et al. (2007) Shiga toxin-producing and attaching and effacing Escherichia coli in cats and dogs in a high-income urban environment. Scand J Clin Lab Invest 67: 243–262.

39. Taylor EV, Nguyen TA, Machesky KD, Koch E, Sotir MJ, et al. (2013) Prevalence and distribution of Salmonella enterica in a major produce production region in California. PloS ONE 8: e65716.

40. Anonymous (2008) Performance standards for antimicrobial susceptibility testing; Twentieth Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute.

41. Anonymous (2012) National Antimicrobial Resistance Monitoring System– Enteric Bacteria (NARMS): 2010 Executive Report. U.S. Food and Drug Administration (FDA). Available: http://www.fda.gov/downloads/Food/RecallsOutbreaksEmergencies/UCM312356.htm. Accessed 18 October 2014.

42. Anonymous (2008) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard – Third Edition. Wayne, PA: Clinical and Laboratory Standards Institute CLSI document M31-A3.

43. Anonymous (2000) Performance standards for antimicrobial susceptibility testing; Twentieth Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute CLSI document M2-A13.

44. Anonymous (2013) Detection of diarrheagenic Escherichia coli strains isolated from dogs and cats in Brazil. Vet Microbiol 166: 676–680.

45. Anonymous (2008) Multistate outbreak of human Salmonella enterica serotype Typhimurium from shrimp products. U.S. Food and Drug Administration (FDA). Available: http://www.fda.gov/downloads/RecallsOutbreaksEmergencies/UCM235923.pdf. Accessed: 18 October 2014.

46. Anonymous (2008) Performance standards for antimicrobial susceptibility testing; Twentieth Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute CLSI document M100–S20.

47. Bielaszewska M, Prager R, Kock PH, Nelson S, Cohen JG, et al. (2010) Characterization of non-Shiga-toxin-producing and attaching and effacing Escherichia coli O157 strains isolated from dogs. Food Microbiol 25: 1195–1208.

48. Bielaszewska M, Prager R, Kock PH, Nelson S, Cohen JG, et al. (2010) Characterization of non-Shiga-toxin-producing and attaching and effacing Escherichia coli O157 strains isolated from dogs. Food Microbiol 27: 102–113.

49. Bielaszewska M, Prager R, Kock PH, Nelson S, Cohen JG, et al. (2010) Characterization of non-Shiga-toxin-producing and attaching and effacing Escherichia coli O157 strains isolated from dogs. Food Microbiol 27: 102–113.
59. Wilkes G, Edge TA, Gannon VP, Jokinen C, Lyautey E, et al. (2011) Associations among pathogenic bacteria, parasites, and environmental and land use factors in multiple mixed-use watersheds. Water Res 45: 5807–5825.
60. Whichard JM, Gay K, Stevenson JE, Joyce KJ, Cooper KL, et al. (2007) Human Salmonella and concurrent decreased susceptibility to quinolones and extended-spectrum cephalosporins. Emerg Infect Dis 13: 1681–1688.
61. Anonymous (2005) Drug-resistant Salmonella. World Health Organization. Available: http://www.who.int/mediacentre/factsheets/fs139/en/ Accessed 18 October 2014.
62. Anonymous (2013) Arizona Leafy Green Marketing Agreement. Available: http://www.arizonaleafygreens.org/. Accessed 18 October 2014.
63. Anonymous (2013) California Leafy Green Marketing Agreement. Available: http://www.caleafygreens.ca.gov/. Accessed 18 October 2014.
64. Partyka ML, Bond RF, Farrar J, Falco A, Cassens B, et al. (2014) Quantifying the sensitivity of scent detection dogs to identify fecal contamination on raw produce. J Food Prot 77: 6–14.