Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Fate of *Escherichia coli* O145 present naturally in bovine slurry applied to vegetables before harvest, after washing and simulated wholesale and retail distribution

M.L. Hutchison[1,2], D. Harrison[2], J.F. Heath[3] and J.M. Monaghan[3]

1 Hutchison Scientific Ltd, Dundee, UK
2 School of Veterinary Sciences, Food Microbiology, University of Bristol, Langford, UK
3 Department of Crop and Environment Science, Fresh Produce Research Centre, Harper Adams University, Newport, Shropshire, UK

Keywords
E. coli, fresh produce, irrigation water, livestock manure, zoonotic agent.

Correspondence
Mike Hutchison, Hutchison Scientific Ltd, Dundee, UK.
E-mail: mh@hutchisonscientific.com

2017/1629: received 17 August 2017, revised 8 September 2017 and accepted 20 September 2017

doi:10.1111/jam.13593

Abstract

Aims: To determine the fate of *Escherichia coli* on vegetables that were processed through commercial wash treatments and stored under simulated retail conditions at 4°C or wholesale at fluctuating ambient temperatures (0–25°C, dependent on season).
Methods and Results: Bovine slurry that was naturally contaminated with *E. coli* O145 was applied without dilution or diluted 1:10 using borehole water to growing potatoes, leeks or carrots. Manure was applied 1 week prior to harvest to simulate a near-harvest contamination event by manure deposition or an application of contaminated water to simulate a flooding event or irrigation from a contaminated water source. At harvest, crops were contaminated at up to 2 log cfu g^{-1}. Washing transferred *E. coli* into the water of a flotation tank used for potato washing and did not completely remove all traces of contamination from the crop. Manure-contaminated potatoes were observed to contain 0·72 cfu *E. coli* O145 g^{-1} after processing and retail storage. Manure-contaminated leeks harboured 0·73–1·55 cfu *E. coli* O145 g^{-1} after washing and storage. There was no cross-contamination when leeks were spray washed. Washing in an abrasive drum resulted in less than perfect decontamination for manure-contaminated carrots. There were five post-distribution isolations from carrots irrigated with contaminated water 24 h prior to harvest.
Conclusions: Standard commercial washing and distribution conditions may be insufficient to reliably control human pathogenic *E. coli* on fresh produce.
Significance and Impact: Previous speculation that the cause of a UK foodborne disease outbreak was soil from imperfectly cleaned vegetables is plausible.

Introduction

Consumption of fresh fruit and vegetables is associated with good nutrition in humans because they provide an important source of vitamins, minerals and biochemical cofactors (Augusto *et al.* 2015). However, in recent years, there have been a number of high-profile foodborne illness outbreaks that have been traced back to fresh produce (King *et al.* 2012; Laidler *et al.* 2013). In the United Kingdom in 2011, an outbreak of 250 infections was caused by verocytotoxigenic *E. coli* (VTEC) O157 phage type (PT) 8 (Launders *et al.* 2015). The consequent case–control-based investigation concluded that there was a significant correlation between infection and those households where there was domestic preparation of unwrapped leeks, or potatoes bought in paper sacks. Since both leeks and potatoes are cooked before consumption, a hypothesis was proposed that cross-

© 2017 The Authors. *Journal of Applied Microbiology* **123**, 1597–1606 published by John Wiley & Sons Ltd on behalf of The Society for Applied Microbiology.
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

contamination of domestic kitchens from contaminated soil on the surfaces of root vegetables was the source of the outbreak (Launders *et al.* 2015). There is a history of potatoes being implicated in foodborne illness in the United Kingdom. The first reported outbreak of haemorrhagic colitis was most likely caused by *Escherichia coli* O157:H7 associated with potatoes, which occurred in East Anglia (Morgan *et al.* 1988). Eleven patients were hospitalized and there was one fatality. Neither investigation could clearly determine the outbreak source because of a common issue with fresh produce-related outbreaks, which is a relatively short product shelf-life (Boxall *et al.* 2011). In extreme cases, contaminated food may have been consumed or spoiled and been disposed of before an outbreak is even identified. Outbreaks involving fruit and vegetables may cause reduced consumer confidence in fresh fruit and vegetables, alter eating habits and reduce the consumption of nutritious produce that is important for a healthy diet (Augusto *et al.* 2015). In particular, enforcement authorities have concerns that consumer food choices should not result in diet-related health problems (Augusto *et al.* 2015).

When assessing the food safety risks associated with particular foods, it is important to consider the survival of pathogens capable of causing human illness. Traditionally, these survival estimates have involved the use of laboratory cultured cells (Hutchison *et al.* 2004b; Islam *et al.* 2005). However, growth in nutrient-rich media (Adkins *et al.* 2006) at a defined temperature that is different from the fluctuating temperatures in natural environments (Hutchison *et al.* 2004b, 2005a; Visvalingam *et al.* 2013) can cause up- and downregulation of metabolic, virulence and stress–response genes. In combination, these control measures alter the physiological state of cultured bacterial cells prior to being placed back into a natural environment. Furthermore, in natural environments, enteric pathogens are required to compete against indigenous microflora to become established in a niche (Wanjugi and Harwood 2013). The application of a laboratory culture to a niche can result in atypically large populations of pathogen (Maks and Fu 2013), and cause artificial changes to competitive indigenous populations. An additional issue with cultured strains is the typicality of the strain cultured, although that issue can be partly addressed by culturing a selection of isolates, typically from foods previously implicated in outbreaks and infected patients (Kim *et al.* 2009). Potentially, any of the issues associated with cultured bacteria could change survival measurements and consequently invite criticism that any model that used them was an imperfect mimic for a natural system (Boysen *et al.* 2013; Van der Linden *et al.* 2014). For that reason, some of the most recent fate of pathogen studies and the current study have tended towards the use of naturally contaminated foods and other materials as a way of optimizing our estimates of the fate of human pathogens (Maks and Fu 2013).

This paper attempts to improve our estimates of the lengths of time that enteric pathogens can survive on potatoes, carrots and leeks following significant contamination scenarios. The crops were contaminated with undiluted and diluted bovine slurry containing nontoxigenic *E. coli* O145, as a marker for *E. coli* capable of causing human illness. The marker was a natural component of the microbiota and contamination was 1 week prior to harvest. The crops were processed by commercially relevant washing practices and held under simulated commercial distribution conditions, typical of those used in Western Europe and North America.

Materials and methods

Identification of excreta naturally contaminated with a verotoxic *E. coli* surrogate

A composite of bovine faecal deposits in a single slaughter batch was collected in the lairage from animals presented for slaughter at the University of Bristol teaching slaughterhouse. After the pens were cleared of livestock, three masses (1–2 g) of excreta were taken from not less than five different faecal depositions on the floor of the pen and combined into a single sample. Selective enrichment of the excreta was undertaken to ensure a high degree of confidence that the material did not contain *E. coli*-harbouring *stx* genes. Excreta (5 g) was enriched in an equal volume of modified tryptone soya broth (mTSB; Oxoid, Basingstoke, UK) supplemented with 20 mg l^{-1} novobiocin (Sigma, Poole, UK), 1·5 g l^{-1} bile salts (Oxoid) and 1·5 g l^{-1} K_2HPO_4 (Sigma) with incubation at 41·5°C for 12 h. Cells from 1 ml of enriched broth were pelleted (10 400 *g* for 5 min at 16–18°C; Eppendorf centrifuge model 5415C, Stevenage, UK) and resuspended in sterile distilled water (1 ml). The resuspended pellet was placed in a boiling water bath (2 min) to generate a crude DNA template.

Confirmation and characterization of *E. coli* isolated from enriched manure

Samples were initially screened by PCR for the presence of verotoxin genes stx_1 and stx_2 and virulence factors eae, ehxA and saa using previously described primers with minor changes to the reaction conditions (Paton and Paton 2002).

In brief, DNA lysates (2 μl) were added to a 48-μl reaction mix containing 200 mM concentration each of adenine, cytosine, guanine and thymine triphosphates,

 © 2017 The Authors.

250 nM concentration of each primer, and 1 U of Taq polymerase (New England Biolabs, Hitchin, Herts). The manufacturer-supplied buffer contained 10 mM Tris-HCl (pH 8·3), 50 mM KCl, 2 mM $MgCl_2$, 0·1% gelatin, 0·1% Tween 20. Samples were subjected to 35 amplification cycles as described previously (Paton and Paton 2002). Amplified DNA was visualized on 1·5% (w/v) agarose gels (Bio Rad, Hemel Hempstead, Herts) stained with ethidium bromide and under ultraviolet light of 220 nm wavelength.

Samples that did not contain verotoxin DNA were characterized further by plating using a filter resuscitation protocol described below that allowed for the recovery of sublethally stressed cells. Confirmation of *E. coli* was by biochemical profiling (API 20E biochemical profiling strip; bioMérieux, Baskingstoke, Hampshire), according to the manufacturer's instructions.

PCR determination of serotype and the presence of loci encoding H antigens was undertaken using a previously described methodology (Perelle *et al.* 2004). Visualization of the amplicons was undertaken as described above.

On farm slurry collections

Slurry samples (1 kg) from the herds of interest identified by a slaughterhouse sample were collected for confirmatory microbiological examination using a rope tied to the handle of a brick-weighted bucket. A sample comprised six combined subsamples collected from different depths and areas of storage lagoons or tanks. Slurry was refrigerated at 4°C during shipping to the laboratory and testing commenced within 24 h.

After confirmation of the presence of a human pathogenic-relevant *E. coli* serotype in sufficient numbers to be of use in field studies, slurry for the inoculation of crops was pumped directly from the storage lagoon into 1 000-l intermediate bulk containers (IBC) the same day that crops were contaminated. To generate simulated contaminated irrigation water, one part slurry was mixed into nine parts borehole water prior to transport. Transport to the field site was *c.* 1 h and without refrigeration.

Determination of *E. coli* O145 numbers in slurry, water and on vegetables

Numbers of *E. coli* O145 were determined using a previously described filter resuscitation method designed to recover sublethally stressed cells (Hutchison *et al.* 2004a). In brief, vegetables were chopped using sterile knives into rough blocks. The length of each block side was *c.* 1 cm, although blocks that included sides from the vegetable's outer surfaces could be irregularly shaped. All samples were diluted decimally in modified tryptone soya broth (mTSB) supplemented with 40 μg ml^{-1} novobiocin and stomached (Colworth 400, Seward, Thetford, UK) for 1 min in mesh bags (6041/STR; Seward). A 10-ml volume for each liquid homogenate was filtered for all samples, except carrots; where, as a precaution against excessive dilution from recent high rainfall, a 20-ml volume was used. The original homogenate, a 1:10 dilution and a 1:100 dilution were filtered. Each dilution was plated once. A recovery for 5 h at 37°C was allowed by placing the filters onto a sterile felt pad soaked in mTSB supplemented with 40 μg ml^{-1} novobiocin (Hutchison *et al.* 2004a). After recovery, the filters were placed onto a previously described chromogenic agar developed for the selective isolation of non-O157 STEC (Possé *et al.* 2008). The media was manufactured in-house. Plates were incubated for 16–20 h at 41°C before counting. The theoretical limit of detection of the test method was 1 cfu g^{-1} for potatoes and leek, and 0·5 cfu g^{-1} for carrots; based on the detection of a single cell in the most concentrated dilution volume that was plated.

Motility

Cultures to be tested were stabbed into motility test agar, (Becton Dickson, Franklyn Lake, NJ, USA) and the agar and checked after a 24-h incubation period (37°C) for the diffuse growth indicative of swarming.

Crop cultivation

Crops were field-grown to comply with the Red Tractor assurance scheme grower guidance (Red Tractor Assurance 2014a, 2014b, 2014c) on a secure field site at Harper Adams University, Shropshire in the west of England (geo: 52·777404, −2·429197). Prior to planting each crop, the field soil was destoned and 1·8 m width beds formed from raised soil following standard commercial practice. The potatoes (*Solanum tuberosum* cv. Harmony) were planted in mid-April 2014 in double rows across the bed at a spacing of 30 cm between seed potatoes. The leeks (*Allium ampeloprasum* cv. Krypton) were transplanted in early May 2014 as young plants in four rows per bed with 10 cm spacing along the row. The carrots (*Daucus carota* cv. Nairobi) were drilled as seed in late-May 2014 in three rows per bed with 5–10 cm spacing along the row. All crops were irrigated and maintained free from weeds, pests and diseases following standard commercial practices. The commercially relevant planting densities produced *c.* 400–600 potatoes, leeks and carrots over a 5 m length of bed at harvest.

The potato and carrot experimental plots were planted in adjacent rows of 100 m length. Experimental plots were 5-m long with a 15-m untreated buffer strip

between experimental plots. The experimental plots in the adjacent bed were staggered such that there was a 5-m shared buffer strip across both beds. Leeks were planted in a 4 × 3 block. Each plot was 5-m long with a grass buffer of 5 m between plots along the beds. Experimental plots were randomly assigned for potatoes and carrots but in leeks the three high treatments were kept at the edge of each row of four plots with an untreated plot of leeks separating them from the next treated plot to minimize the risk of cross-contamination through run-off.

Application of slurry to crops

There were three independent plots for each treatment. In addition to uncontaminated controls, there were treatments to mimic a single bovine depositing 60 l of slurry or a 60-l contaminated irrigation or flood event in a section of field containing produce. The contamination was applied 1 week before harvest using watering cans with the rose removed and each application of material was uneven, sporadic and random as a mimic for direct excreta deposition by livestock.

Crop harvest and washing

Potatoes were harvested mechanically using a tractor-pulled potato harvester (Del Morino s.r.l., Arezzo, Italy; model DM 50), that removed a proportion of the soil and laid the tubers on the ground's surface. Leeks were harvested by randomly selecting plants and manually trimming the roots and leaves using a knife followed by stripping back the flag leaves to expose the shank. One month prior to harvest, the carrots were insulated with fleece and covered in polythene as protection from frost. The insulation was not replaced after contamination. Carrots were harvested using a hand fork to lift the roots to the surface followed by manual lifting. Five samples were collected from each of the three independent treatments and control plots at harvest ($n = 15$). Each sample was composed of five vegetables, which were collectively chopped at the testing laboratory. The test sample was 25 g of randomly selected, chopped sample.

Potatoes were washed in unchlorinated rainwater by immersion in a 200-l flotation tank with a 10 l min^{-1} water overflow and 10 l min^{-1} air sparge applied from the bottom of the tank. The bottoms of the leek shank were spray washed (McGeary Spray System Solutions, Dungannon, Ireland; 6× Nozzle DNN114) with rainwater. A pilot-scale brush washer (Niagri Engineering, Norfolk, UK; model Cleanwash 25) was used to remove dirt from the carrots and the outer surface; a process known as 'polishing'. Carrots were cleaned with unchlorinated borehole water. The vegetables were washed in increasing order of contamination. Wash water was collected for each replicated treatment ($n = 15$). Three batches of previously unwashed, uncontaminated vegetables, each with five replicate samples, were washed immediately in the contaminated water generated by washing contaminated crops to determine if there was a degree of cross-contamination between consecutive batches. All wash treatments were completed within 48 h of harvest.

Simulated crop distribution

For simulated wholesale distribution, crops were stored at ambient temperature. For retail distribution, storage was at 4°C. For carrots and potatoes, the storage duration was 2 weeks. For leeks, it was 1 week. The timings for all crops were based on typical shelf lives. Carrots and potatoes were stored in paper sacks inside unlined crates. Leeks were stored in polythene-lined, opaque transport crates, with an empty crate stacked on top. The storage materials and environmental conditions were typical for wholesale and retail distribution in the United Kingdom. For potatoes, only the postwash, directly contaminated produce was stored under simulated distribution conditions. For carrots and leeks, the washed, directly contaminated produce and the indirectly contaminated vegetables generated by washing uncontaminated produce in contaminated water were stored.

Chemical characterization of slurry

Dry matter was determined by weighing the manure before and after drying in an oven (Unitherm, forced draught drying oven, Meerssen, the Netherlands) with air circulation set to 80% at a temperature of 80°C until no further weight loss was observed (*c.* 40 h). Ammonia concentration was estimated by chemical titration with 0·05 M sulphuric acid using methyl red and bromocresol green as indicators (Hutchison *et al.* 2004a). The pH of the slurry was determined directly using a pH meter (model Jenway M3540, Cole-Palmer, Stone, UK) and conductivity was measured by, dilution of the slurry in deionised water if required and, a conductivity meter (Model CD 4303, RS components, Corby, UK).

Recording of climatic conditions

Weather conditions for the field plots were recorded at a Meteorological Station located 200 m east of the trial field. Air temperature was recorded at 20 cm above the soil. Storage temperatures for the simulated retail and wholesale distribution chains were recorded using Tinytag plus 2 temperature loggers (Gemini Data Systems,

Chitchester, UK), set to record air temperature every minute. Relative humidity was recorded using model RC-4HC meters (Elitech, Berkhamstead, UK), again with records made each minute.

Statistical analyses

Paired *t*-tests, analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) *post hoc* analyses were undertaken using statistical software (Statplus professional, build 5·9·9·1, AnalystSoft, Walnut, CA, USA). Statistical testing was used to compare numbers of *E. coli* or the physicochemical properties of manure between treatments. For all tests, a $P < 0{\cdot}05$ was used to determine if any differences were significant.

Results

Sixty-two slaughter batches of animals from 36 different farms were examined for *stx* genes over a period of 4 months from April to July. With the exception of a single batch of animals, the enriched cultures all contained a stx_2 amplicon. For the batch of animals that did not harbour any toxin genes, plating onto the chromogenic media gave rise to two blue-green coloured colony morphologies that were identified by biochemical testing to be *E. coli* and by PCR to be serotype O145. The strain was characterized as lacking stx_1, stx_2, *eae*, the H7 antigen and *hylA*. Samples of manure were re-examined immediately before the excreta was used to contaminate each crop. *Escherichia coli* O145 was exclusively determined as the serotype of the blue colonies for all three vegetables on each occasion immediately prior to crop contamination.

The numbers of *E. coli* O145 in each batch of slurry used to contaminate crops was variable and decreased over time (Table 1). The changes in numbers were likely due to several factors including seasonal changes in diet for the livestock, stirring of the store contents to promote reductions in enteric micro-organism numbers and rainfall dilution of slurry store contents. The physicochemical properties of the excreta, however, remained similar throughout the course of the study (Table 1), although the excreta used for the carrots had a significantly lower dry matter content (ANOVA; Tukey HSD), which may have been a consequence of dilution in the slurry store from elevated rainfall in the months prior to harvest (Fig. 1).

For the slurry-contaminated potatoes, *E. coli* O145 was present at around 2 log cfu per g vegetable at harvest (Fig. 2). For the irrigation water treatment, the contamination was lower at around 0·35 log cfu per g. Two of the 15 replicates for the uncontaminated control each contained a single *E. coli* O145 cell. However, no further isolations were made from the uncontaminated controls during subsequent washing and storage. Washing the slurry-contaminated potatoes did not significantly change the numbers of *E. coli* O145 contaminating the vegetable, whereas a near-significant reduction was observed for the irrigation water treatment (paired t-test, $P = 0{\cdot}051$). Both the irrigation water and slurry treatments released 0·5 log cfu per g and 1 log cfu per g into the wash water respectively (Fig. 2). No generic *E. coli* was isolated from any of the water sources used for any of the crop washing treatments prior to use. Washing uncontaminated crops in the contaminated water resulted in crops acquiring *E. coli* O145 at a concentration of 0·75 log cfu per g for the slurry. A single colony was isolated from a single replicate when potatoes were washed in the contaminated wash water from the irrigation water treatment. The relative humidities during storage are shown in Table 2. After simulated distribution at ambient (diurnal cycling, 16 to 25°C, Table 2) for potatoes, *E. coli* O145 was isolated only from 2 of 15 replicates (11 colonies in total) of the slurry treatment and only from the refrigerated (4°C constant) retail storage (Fig. 2).

For the leeks, there were no isolations of *E. coli* O145 from any of the uncontaminated controls. Despite visible faecal material on the surface of the leeks at harvest, contamination was lower than for the potatoes. For the slurry there were 1·4 log cfu per g contaminating the crop, with 0·4 cfu per g for the irrigation water treatment. A water rinse was effective at reducing the contamination on leeks, with both the slurry and irrigation water treatments showing significant reductions (paired *t*-test $P < 0{\cdot}05$) as a consequence of spraying (Fig. 3). As before, the water used for washing was collected and tested, but did not contain numbers of *E. coli* O145

Table 1 Concentration of *Escherichia coli* O145 and physicochemical properties of the slurry used to contaminate crops. Results are the mean of five (chemical analyses) or 10 (*E. coli* counts) replicates ± SD

Crop	Geometric mean count *E. coli* O145 (log cfu per g)	Dry matter content (% w/w)	pH	Conductivity (mSi cm^{-1})	Ammonium N (mg NH_4-N kg^{-1})
Potato	4·00 ± 0·08	7·34 ± 0·09	6·88 ± 0·08	3·96 ± 0·12	988 ± 17·89
Leek	3·78 ± 0·11	7·22 ± 0·04	6·90 ± 0·07	3·96 ± 0·11	1090 ± 65·19
Carrot	3·30 ± 0·13	6·98 ± 0·00	6·78 ± 0·04	4·27 ± 0·13	1006 ± 66·56

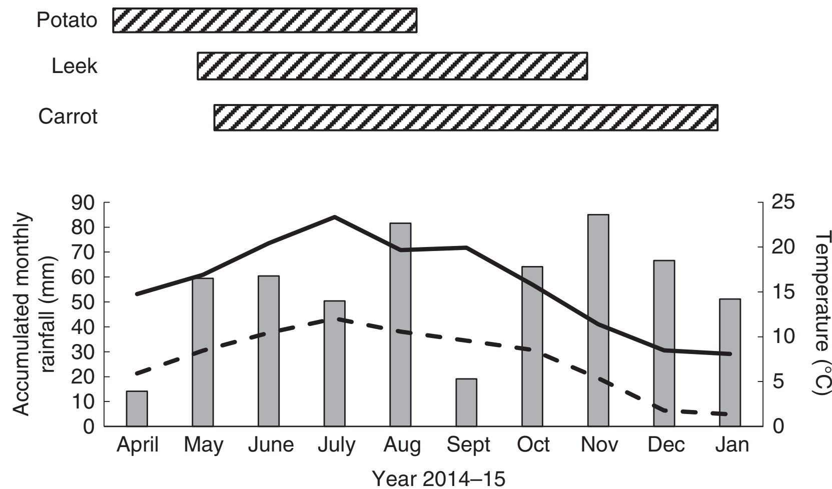

Figure 1 A summary of climactic conditions across the cultivation periods (horizontal diagonal-hatched bars) for potato, leek and carrot. Accumulated monthly rainfall is shown as grey bars and the monthly average of daily maximum and minimum temperatures are shown as solid and dashed lines respectively.

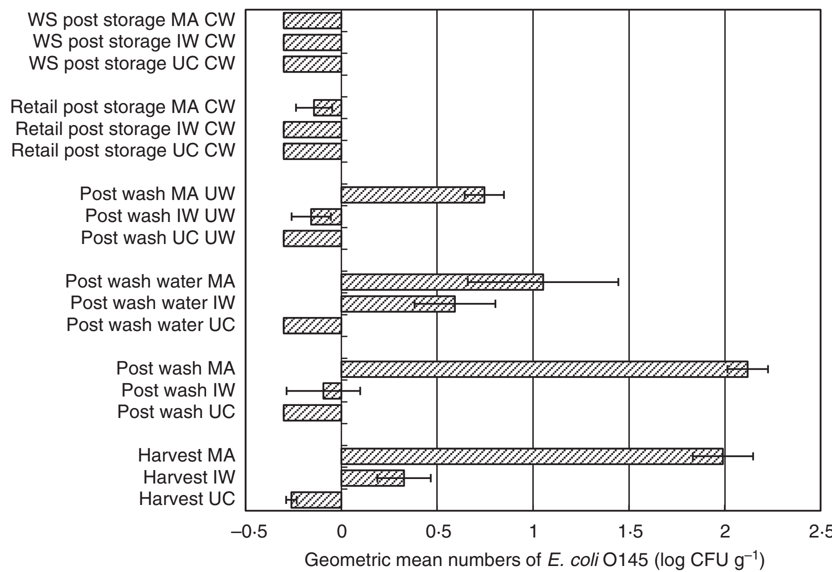

Figure 2 Numbers of *Escherichia coli* O145 on potatoes contaminated 1 week before harvest with slurry (MA) or irrigation water (IW) and uncontaminated controls (UC). Contaminated potatoes were washed in uncontaminated water (CW) and previously uncontaminated potatoes subsequently washed in the same wash water (UW). The contaminated potatoes were stored under conditions to simulate retail and wholesale (WS) distribution. Error bars are the SE of the mean log of 15 replicates per treatment.

above the detection limit of the test method. However, leeks washed in the collected water did acquire low levels of *E. coli* O145 contamination (Fig. 3). For the leeks, both the directly contaminated washed produce was followed through simulated distribution as well as the leeks contaminated by the recycled contaminated wash water. There were low level isolations from both the directly and indirectly contaminated crops for both wholesale ambient (6–16°C, Table 2) and retail (4°C) distributions. As before, the highest numbers of cells were observed for the retail distribution, although for the indirectly contaminated slurry treatment.

Table 2 Average relative humidities during simulated crop distribution through a retail and wholesale supply chain. The ambient temperature fluctuated between values shown in parentheses

Crop	Average humidity (%) during simulated distribution for	
	Retail at 4°C	Wholesale at (ambient temperature)
Potato	91·07	62·23 (16–25°C)
Leek	90·52	59·93 (6–16°C)
Carrot	90·87	60·51 (0–15°C)

For carrots, there was exceptionally atypical elevated rainfall (Fig. 1) for the 3 months prior to harvest. The soil was too waterlogged to support the weight of a tractor and consequently a manual harvest rather than the planned mechanical one was undertaken. The high rainfall also meant that contamination of the crop at harvest was lower than expected. After washing and polishing, *E. coli* O145 was detected only in 1 of the 15 slurry treatment replicates and 5 of 15 water treatments, when the water was applied 24 h before harvest as a simulation of flooding or a soil cap softening treatment. (Soil capping is the term used by commercial growers to describe a

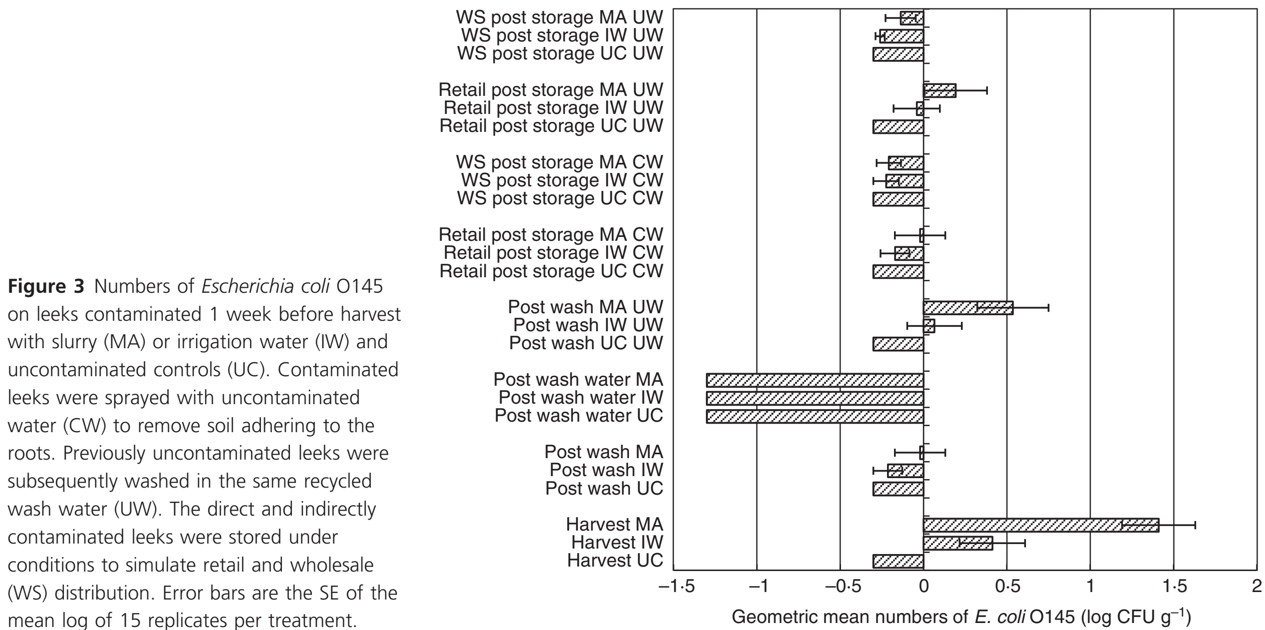

Figure 3 Numbers of *Escherichia coli* O145 on leeks contaminated 1 week before harvest with slurry (MA) or irrigation water (IW) and uncontaminated controls (UC). Contaminated leeks were sprayed with uncontaminated water (CW) to remove soil adhering to the roots. Previously uncontaminated leeks were subsequently washed in the same recycled wash water (UW). The direct and indirectly contaminated leeks were stored under conditions to simulate retail and wholesale (WS) distribution. Error bars are the SE of the mean log of 15 replicates per treatment.

hard soil crust created as a consequence of excessive rainfall followed by rapid drying from intense sunlight or wind prior to harvest.)

Discussion

The slurry used for this study contained *E. coli* O145 that lacked some key virulence genes required to cause human illness. However, enterohaemorrhagic strains of the same serotype have been previously implicated as causes of foodborne disease associated with fresh produce (Sonntag *et al.* 2004; Taylor *et al.* 2013). A history of human illness caused by the serotype makes it useful as a non-pathogenic surrogate for *E. coli* capable of causing human illness. The reasons why the O145 used for this work did not contain toxin genes were not extensively investigated as part of the current study, however, we noted that some O145 serotypes have been reported to lack motility and lack the H (Haunch) antigen that is commonly a receptor for stx-harbouring phages. Although the O145 strain did not contain H7, PCR analyses of the genome revealed the presence of genes encoding a type H28 flagella. H7 has been shown to be important for bacterial attachment to the surface of vegetables (Rossez *et al.* 2014), although it is unclear if this is a general trait of all flagellar types or a property peculiar to H7.

The volume of slurry spread on each plot was selected as typical for the quantity produced by a single bovine in a day (Phillips 2010). Although the numbers of O145 in the slurry changed over the course of the study, a constant volume of excreta, rather than a constant number of O145 was applied to each crop. We justify the approach by consideration that animals shed different numbers of bacteria into their wastes (Hutchison *et al.* 2004a) as a consequence of their age, stage of infection and other factors such as diet (Hutchison *et al.* 2005b).

We were unable to find significant information describing the fate of enteric human pathogens on leeks and potatoes during distribution. However, there is previous work that discusses related findings for other vegetables, albeit with a focus more on the postdistribution storage of produce processed by shredding. A Korean study inoculated a range of lettuce and sprouted seeds with four different lab-cultured pathogens including *E. coli* O157:H7 and *Salmonella* Typhimurium (Tian *et al.* 2012). Storage and changes in bacterial populations were followed over time at either 4°C or 15°C. *Escherichia coli* O157 did not survive on uncut sprouts at either temperature. However, an important finding of the study was that bacterial growth was observed when pathogens were inoculated onto cut vegetable leaf surfaces such as lettuce. There was no significant influence on bacterial populations between the different storage temperatures. For some treatments, bacterial growth could exceed an increase of three logs. A possible role for nutrient release from cut-damaged plant cells supporting the observed bacterial growth was not investigated, although a general conclusion from the work was that refrigeration of cut vegetables during storage is important as it impedes bacterial growth (Tian *et al.* 2012).

More recent work undertaken in Ireland has investigated the impact of slicing and peeling and storage temperature on carrots contaminated with lab-cultured *E. coli* O157:H7 (O'Beirne *et al.* 2014). A summary of the

study is that blunt cutting blades used to slice carrots distributed *E. coli* deeper into the carrot tissue and enhanced survival compared with sharp blades. There were no significant differences when hand and machine peeling of carrots were compared. An important observation made by the Irish study was that bacterial growth occurred at 10°C compared with decline at 4°C, and also that survival on cut surfaces was better than on peeled surfaces (O'Beirne *et al.* 2014). The authors noted that in contrast to peeled carrots, transverse cutting damaged vascular tissues, including phloem, thereby releasing salt and sugar to support bacterial multiplication. Although some historical studies (Finn and Upton 1997) have reported the decline of *Salmonella* inoculated onto shredded carrot in naturally modified atmospheres, the majority of workers report observations (Sant'Ana *et al.* 2012; Likotrafiti *et al.* 2013; O'Beirne *et al.* 2014) that are at odds with refrigeration preserving potential human pathogens. The apparent conflict highlights the importance of having an accurate mimic for commercial food production and processing practices, and the dangers of extrapolating from one set of conditions to another. Modern commercial processing and washing of carrots and potatoes is designed to protect against crop damage and although carrots were surface abraded the nutrients released following cellular injury of vascular tissue that were an integral part of some previous models were not present in our mimic. Consequently, we did not observe bacterial growth during simulated distribution, although the temperature and duration of storage for our study were similar to those used by previous workers (Sant'Ana *et al.* 2012; Likotrafiti *et al.* 2013; O'Beirne *et al.* 2014).

A common commercial process in Western countries is for the tops of leek leaves to be trimmed with the outer leaves being removed. Thus, for leeks used in this study, there was damage to the vascular tissue and nutrient release, with the potential to support bacterial multiplication. There are few publications in the literature that report the survival of enteric pathogens during storage of leeks. However, one study assessed whether there was an impact for the presence of Mycorrhizae on the survival of *Salmonella* and *E. coli* O157:H7 in young leek plants (Gurtler *et al.* 2013). The no-fungus controls from the study agree broadly with our results that there is survival of *E. coli* for at least a week after contamination. We were unable to find any information describing the effect of damaged leaves, although we note that leeks are members of the allium family, which generate a class of natural antimicrobials called allicins (De Wet *et al.* 1999). Any role for allicins in the fate of enteric pathogens in leeks has not been investigated and is likely to be complex because allicin concentration changes between batches of crops (Burt 2004).

One important finding from this study was that it was more likely to isolate *E. coli* O145 from vegetables stored at a constant refrigerated temperature compared with crops stored at ambient temperature. Ambient temperature fluctuates diurnally, and it has been previously reported that the decline of enteric pathogens such as *E. coli* O157 in excreta is more rapid under conditions of temperature fluctuation (Semenov *et al.* 2007).

Fresh vegetables are becoming increasingly implicated as sources of foodborne illness (Likotrafiti *et al.* 2013). This study was undertaken primarily to assess whether it was a plausible hypothesis that contaminated soil on the surfaces of leeks or root vegetables could have contaminated a domestic kitchen to a degree that cross-contamination occurred (Launders *et al.* 2015). Our observations were that washing reduces, but seldom completely removes, all of the soil on crops, and many individual vegetables still had visible soil deposits on their surfaces. Replicating standard commercial processes reduced contamination in all three crops and most markedly with the brushing and washing in carrots. However, based on the results of this study, it is possible that pathogenic *E. coli* could survive washing and cool distribution prior to retailing. It has been reported several times that some VTEC require exposure only to small numbers of cells to establish a human infection. Furthermore, the maximum numbers of cells observed in the slurry were 4 logs for this study and there are reports in the literature of 'super-shedding' animals that can excrete more than 8 log cfu per g pathogenic *E. coli* (Hutchison *et al.* 2004a). There are challenges with the identification of such highly and naturally contaminated wastes for use in studies of this type. However, it seems likely that higher numbers of pathogenic cells applied to crops near harvest would result in higher numbers of pathogens on crops at retail.

Acknowledgements

This study was funded by Food Standards Scotland as project FSS00014 and the UK Food Standards Agency as project FS101052. The authors thank Dr Monika Tchòrzewska and Dr Fran Whittington for microbiological examinations and laboratory support.

Conflict of Interest

None of the authors declare a conflict of interest.

References

Adkins, J.N., Mottaz, H.M., Norbeck, A.D., Gustin, J.K., Rue, J., Clauss, T.R.W., Purvine, S.O., Rodland, K.D. *et al.*

(2006) Analysis of the *Salmonella typhimurium* proteome through environmental response toward infectious conditions. *Mol Cell Proteom* **5**, 1450–1461.

Augusto, R.A., Cobayashi, F. and Cardoso, M.A. (2015) Associations between low consumption of fruits and vegetables and nutritional deficiencies in Brazilian schoolchildren. *Pub Health Nutrit* **18**, 927–935.

Boxall, N.S., Adak, G.K., de Pinna, E. and Gillespie, I.A. (2011) A *Salmonella* Typhimurium phage type (PT) U320 outbreak in England, 2008: continuation of a trend involving ready-to-eat products. *Epidemiol Infect* **139**, 1936–1944.

Boysen, L., Wechter, N.S. and Rosenquist, H. (2013) Effects of decontamination at varying contamination levels of *Campylobacter jejuni* on broiler meat. *Poult Sci* **92**, 1425–1429.

Burt, S. (2004) Essential oils: their antibacterial properties and potential applications in foods – A review. *Int J Food Microbiol* **94**, 223–253.

De Wet, P.M., Rode, H., Sidler, D. and Lastovica, A.J. (1999) Allicin: a possible answer to antibiotic resistant campylobacter diarrhoeal infection? *Arch Dis Childhood* **81**, 278.

Finn, M.J. and Upton, M.E. (1997) Survival of pathogens on modified-atmosphere-packaged shredded carrot and cabbage. *J Food Prot* **60**, 1347–1350.

Gurtler, J.B., Douds, D.D., Dirks, B.P., Quinlan, J.J., Nicholson, A.M., Phillips, J.G. and Niemira, B.A. (2013) *Salmonella* and *Escherichia coli* O157:H7 survival in soil and translocation into leeks (*Allium porrum*) as influenced by an arbuscular mycorrhizal fungus (*Glomus intraradices*). *Appl Env Microbiol* **79**, 1813–1820.

Hutchison, M.L., Walters, L., Avery, S.M., Synge, B.A. and Moore, A. (2004a) Levels of zoonotic agents in British livestock manures. *Let Appl Microbiol* **39**, 207–214.

Hutchison, M.L., Walters, L., Moore, A., Crookes, K.M. and Avery, S.M. (2004b) Effect of length of time before incorporation on the survival of pathogenic bacteria present in livestock wastes applied to agricultural soil. *Appl Env Microbiol* **70**, 5111–5118.

Hutchison, M.L., Walters, L.D., Avery, S.M. and Moore, A. (2005a) Decline of zoonotic agents in livestock waste and bedding heaps. *J Appl Microbiol* **99**, 354–362.

Hutchison, M.L., Walters, L.W., Avery, S.M., Munro, F. and Moore, A. (2005b) Analyses of livestock production, waste storage and pathogen levels and prevalences in farm manures. *Appl Env Microbiol* **71**, 1231–1236.

Islam, M., Doyle, M.P., Phatak, S.C., Millner, P. and Jiang, X.P. (2005) Survival of *Escherichia coli* O157:H7 in soil and on carrots and onions grown in fields treated with contaminated manure composts or irrigation water. *Food Microbiol* **22**, 63–70.

Kim, J., Shepherd, M.W. and Jiang, X.P. (2009) Evaluating the effect of environmental factors on pathogen regrowth in compost extract. *Microbial Ecol* **58**, 498–508.

King, L.A., Nogareda, F., Weill, F.X., Mariani-Kurkdjian, P., Loukiadis, E., Gault, G., Jourdan-DaSilva, N., Bingen, E. *et al.* (2012) Outbreak of Shiga toxin-producing *Escherichia coli* O104:H4 associated with organic fenugreek sprouts, France, June 2011. *Clin Infect Dis* **54**, 1588–1594.

Laidler, M.R., Tourdjman, M., Buser, G.L., Hostetler, T., Repp, K.K., Leman, R., Samadpour, M. and Keene, W.E. (2013) *Escherichia coli* O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer. *Clin Infect Dis* **57**, 1129–1134.

Launders, N., Locking, M.E., Hanson, M., Willshaw, G., Charlett, A., Salmon, R., Cowden, J., Harker, K.S. *et al.* (2015) A large Great Britain-wide outbreak of STEC O157 phage type 8 linked to handling of raw leeks and potatoes. *Epidemiol Infect* **144**, 171–181.

Likotrafiti, E., Smirniotis, P., Nastou, A. and Rhoades, J. (2013) Effect of relative humidity and storage temperature on the behavior of *Listeria monocytogenes* on fresh vegetables. *J Food Safety* **33**, 545–551.

Maks, N. and Fu, T.J. (2013) Evaluation of PCR Detection of *Salmonella* in alfalfa sprouts and spent irrigation water collected during sprouting of naturally contaminated eed. *J Food Prot* **76**, 314–317.

Morgan, G.M., Newman, C., Palmer, S.R., Allen, J.B., Shepherd, W., Rampling, A.M., Warren, R.E., Gross, R.J. *et al.* (1988) 1st recognised community outbreak of hemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157:H7 in the UK. *Epidemiol Infect* **101**, 83–91.

O'Beirne, D., Gleeson, E., Auty, M. and Jordan, K. (2014) Effects of processing and storage variables on penetration and survival of *Escherichia coli* O157:H7 in fresh-cut packaged carrots. *Food Cont* **40**, 71–77.

Paton, A.W. and Paton, J.C. (2002) Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for stx(1), stx(2), eae, ehxA, and saa. *J Clin Microbiol* **40**, 271–274.

Perelle, S., Dilasser, F., Grout, J.L. and Fach, P. (2004) Detection by 5 ‘-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. *Molec Cell Probe* **18**, 185–192.

Phillips, C.J.C. (2010) *Principles of Cattle Production*. Oxford: CABI Publishing.

Possé, B., De Zutter, L., Heyndrickx, M. and Herman, L. (2008) Novel differential and confirmation plating media for Shiga toxin-producing *Escherichia coli* serotypes O26, O103, O111, O145 and sorbitol-positive and -negative O157. *FEMS Microbiol Lett* **282**, 124–131.

Red Tractor Assurance. (2014a) *Crop-specific protocol – Carrots*. Available from: http://assurance.redtractor.org.uk/contentfiles/Farmers-5300.pdf. Accessed 8 September 2017.

Red Tractor Assurance. (2014b) *Crop-specific Protocol – Leeks*. Available from: http://assurance.redtractor.org.uk/contentfiles/Farmers-5284.pdf. Accessed 8 September 2017.

Red Tractor Assurance. (2014c) Crop-specific protocol – Potatoes. Available from: http://assurance.redtractor.org.

uk/contentfiles/Farmers-5312.pdf. Accessed 8 September 2017.

Rossez, Y., Holmes, A., Wolfson, E.B., Gally, D.L., Mahajan, A., Pedersen, H.L., Willats, W.G.T., Toth, I.K. *et al.* (2014) Flagella interact with ionic plant lipids to mediate adherence of pathogenic *Escherichia coli* to fresh produce plants. *Env Microbiol* **16**, 2181–2195.

Sant'Ana, A.S., Barbosa, M.S., Destro, M.T., Landgraf, M. and Franco, B.D.G.M. (2012) Growth potential of *Salmonella* spp. and *Listeria monocytogenes* in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelf-life. *Int J Food Microbiol* **157**, 52–58.

Semenov, A.V., van Bruggen, A.H.C., van Overbeek, L., Termorshuizen, A.J. and Semenov, A.M. (2007) Influence of temperature fluctuations on *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in cow manure. *FEMS Microbiol Ecol* **60**, 419–428.

Sonntag, A.K., Prager, R., Bielaszewska, M., Zhang, W.L., Fruth, A., Tschape, H. and Karch, H. (2004) Phenotypic and genotypic analyses of enterohemorrhagic *Escherichia coli* O145 strains from patients in Germany. *J Clin Microbiol* **42**, 954–962.

Taylor, E.V., Nguyen, T.A., Machesky, K.D., Koch, E., Sotir, M.J., Bohm, S.R., Folster, J.P., Bokanyi, R. *et al.* (2013) Multistate outbreak of *Escherichia coli* O145 infections associated with romaine lettuce consumption, 2010. *J Food Prot* **76**, 939–944.

Tian, J.Q., Bae, Y.M., Choi, N.Y., Kang, D.H., Heu, S. and Lee, S.Y. (2012) Survival and growth of foodborne pathogens in minimally processed vegetables at 4 and 15 degrees C. *J Food Sci* **77**, M48–M50.

Van der Linden, I., Cottyn, B., Uyttendaele, M., Vlaemynck, G., Maes, M. and Heyndrickx, M. (2014) Evaluation of an attachment assay on lettuce leaves with temperature- and starvation-stressed *Escherichia coli* O157:H7 MB3885. *J Food Prot* **77**, 549–557.

Visvalingam, J., Gill, C.O. and Holley, R.A. (2013) Morphological and viability changes in *Escherichia coli* and *E. coli* O157:H7 cells upon rapid shift from 6 degrees C to 37 degrees C. *Food Microbiol* **34**, 95–99.

Wanjugi, P. and Harwood, V.J. (2013) The influence of predation and competition on the survival of commensal and pathogenic fecal bacteria in aquatic habitats. *Env Microbiol* **15**, 517–526.