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The fate of surrogate verocytotoxic *E. coli* contaminating the rhizospheres of root vegetables during processing and retail and wholesale distribution.

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1 EXECUTIVE SUMMARY

In the United Kingdom in 2011, an outbreak of 250 infections, including one fatality; was caused by verotoxic *E. coli* O157. Statistics-based investigations by health-care professionals concluded that there was a significant correlation between human infections and those households where there was domestic preparation of unwrapped leeks, or potatoes bought in paper sacks. Both leeks and potatoes are typically cooked to high enough temperatures to kill bacteria and destroy verotoxins before consumption. Consequently, a hypothesis was proposed that cross-contamination of domestic kitchen surfaces from contaminated soil on the surfaces of the vegetables was the cause of the outbreak. However, the investigation could not equivocally determine the outbreak source because the relatively-short product shelf-life meant there was no material available for microbiological examination to confirm the contamination source.

The purpose of this study was to determine if soil contamination at a late stage of crop production could remain on harvested crops following standard commercial harvesting practice. The general approach was to grow crops of leek, potato and carrot using standard commercial cultivation practices and timings. The crops were contaminated with cattle slurry or irrigation water one week before harvest to simulate a worst case scenario of either gross contamination through flooding or irrigation or a livestock incursion into a crop. The slurry and irrigation water were contaminated with naturally-occurring *E. coli* O145 as a substitute for a potential pathogen such as toxigenic *E. coli* O157. Although *E. coli* O145 has caused foodborne outbreaks in the past, the strain used for the experiments lacked an ability to produce verotoxin and therefore was unable to cause human illness.

After harvest, the crops were processed according to common commercial practices. Leeks were spray washed to remove excess soil, carrots were washed by immersion and the outer surface of the root was peeled by abrasive brushing. Potatoes were washed in a flotation tank. Uncontaminated crops were washed in water that was recycled from the washing of contaminated vegetables to determine if there was any potential for cross-contamination between different batches of washed vegetables. After washing, the crops were stored under conditions that were typical for either refrigerated retail distribution, or ambient-temperature wholesale distribution. After the simulated distribution the crops were examined to determine if there was viable *E. coli* O145 still present on the surfaces of the vegetables.

Washing contaminated potatoes in a flotation tank did not remove all of the *E. coli* O145 from the crops, and transferred some *E. coli* into the wash water. Washing uncontaminated

potatoes in contaminated wash water transferred contamination to the crop. After simulated distribution, there were small numbers of *E. coli* O145 cells on potatoes. Measurable contamination was isolated only from 2/15 replicates of the slurry treatment and only from the refrigerated retail storage treatment. There was no contamination measured for the ambient temperature wholesale distribution treatment.

For the leeks, a water mist was effective at reducing the contamination, with both the slurry and irrigation water treatments showing significant reductions in numbers of *E. coli* O145 cells as a consequence of spraying. The water used for washing was collected, but it did not contain numbers of *E. coli* O145 above the detection limit of the test method, although leeks washed in the collected water did acquire low levels of *E. coli* O145 contamination. There were low level isolations from both the directly and indirectly contaminated crops after both simulated wholesale and retail distribution. As before, the highest numbers of cells was observed for the refrigerated retail distribution.

For carrots, there was significant rainfall between contamination and harvest. The soil was waterlogged at harvest and consequently contamination of the crop at harvest was low. After washing and peeling, *E. coli* O145 was detected only in one of the fifteen slurry treatment replicates and five of fifteen water treatments, when the water was applied 24h before harvest as a simulation of a soil cap softening treatment. Soil capping is the term used by commercial growers to describe a hard soil crust created by excessive sunshine and low rainfall. After storage, only the slurry-contaminated and simulated cap softening-contaminated carrots contained *E. coli* O145 cells. As before, only those carrots subjected to the simulated refrigerated retail distribution treatments remained contaminated.

In addition to crop contamination with slurry or irrigation water, experimental work was undertaken to assess the risks of field workers returning to work and hand harvesting crops whilst infected with an enteric pathogen. These studies focussed on poor hand washing practices after using portable field lavatories. Generic *E. coli* was used as a marker for an enteric pathogen to circumvent ethical concerns. The studies showed that *E. coli* from faecal material can be transferred to carrots if hands were not washed effectively after using the toilet and the worker returned to harvesting crops. In addition, some designs of field toilets had their hand-washing facilities external to the latrines. We observed a build-up of faecal indicator bacteria on the loo flush lever and internal door handles in such latrine designs.

The results of this study have shown crops grown under commercial conditions and contaminated close to harvest with slurry and irrigation water can remain contaminated

through simulated distribution. It is likely that this contamination could be spread around a domestic kitchen environment if the crops were not segregated from cooked foods. Refrigerated retail distribution was consistently more likely to preserve the *E. coli* O145 used in these studies when compared with wholesale distribution at ambient temperature. The statistically most-likely explanation for the 2011 UK outbreak associated with leeks and potatoes was cross-contamination to domestic kitchen environments. The results from these studies have shown that the hypothesis was plausible if a relatively large crop contamination event occurred close to harvest.

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2 PROJECT BACKGROUND

This study was commissioned primarily to determine whether it was plausible for soil contaminating the surfaces of potatoes or leeks to have caused on outbreak of foodborne illness that affected 250 people in the UK in late 2010 and early 2011. Given there have been considerable improvements over the last 15-20 years to the hygienic application of treated excreta as crop fertilisers, it was considered unlikely that routine modern farming activities involving faecal material would cause crop contamination. Thus, there were three accidental contamination scenarios investigated. These scenarios were:

1. A field worker colonised with an enteric pathogen returning to work too early during a crop harvest and the potential for unhygienic use of a field latrine as a cause of crop contamination.
2. The application of irrigation water contaminated with an enteric pathogen causing crop contamination. This scenario was designed to provide information about pathogen fate during flood events, and also during cap softening (water application to hardened soil crusts [called caps], to allow root crops such as carrots to be harvested).
3. Stray domesticated livestock such as cattle or wildlife defecating directly onto crops around 7 days before harvest.

The crops that were used for these studies were carrot, leek and potato, chosen primarily because leeks and potatoes were implicated in the 2010-2011 outbreak, and raw, grated carrots had been implicated in a number of outbreaks overseas (section 3). Crops were cultivated after soliciting advice from professional growers and according to routine commercial practices found in the UK. Harvest was at an appropriate time of year. The

chosen crops also had the advantage of being harvested at different seasons facilitating harvest, washing and laboratory examinations with a relatively small number of staff.

This study was undertaken as a series of deliverables that are listed in Table 1. The various sections of this report correspond to these deliverables.

Table 1 Project deliverable descriptions and corresponding sections of the report

Deliverable number	Deliverable description	Report section for deliverable
1	Outbreaks and causes review of the literature updated, industry discussions completed.	Section 3 - Section 6
2	A. Crops drilled and growing B. Report of final non-VTEC molecular methods submitted.	A: Section 8.3.4 B: Section 8.3.1
3	Hand wash cross contamination experiments completed.	Section 7
4	Potatoes and leeks harvested and the fate of non-VTEC serotypes down the chain completed and reported.	Section 8
5	Crop washing cross-contamination experiments completed for leeks and potatoes.	Section 8
6	Carrots harvested and the fate of non-VTEC serotypes down the chain completed and reported. Crop washing cross-contamination experiments completed and reported for carrots.	Section 8
7	Submission of draft final report.	This document

Sections 7 and 8 were prepared so as to be straightforwardly converted into manuscripts targeted to different journals. Primarily, that is the reason that references are listed at the end of each report section, with different sections using different citation and bibliographical formats.

3 A SYSTEMATIC REVIEW OF LITERATURE RELATING TO OUTBREAKS OF VEROCYTOTOXIC *E. COLI* (VTEC) AND RELATED ENTERIC BACTERIA LINKED TO THE SUPPLY OF ROOT VEGETABLES AND A SUMMARY OF INDUSTRY ADVICE AND DISCUSSIONS.

3.1 INTRODUCTION

Fresh produce is associated with 3% of the total cases of foodborne illness in the UK (Adak et al. 2002). This percentage equates to roughly 50,000 cases of illness per year and consequently this class of food is of particular concern to regulatory authorities. A key factor is that some fruits and vegetables are likely to be consumed after minimal processing and without cooking; and so any human pathogens present on produce have an increased likelihood of causing foodborne illness compared with foods that are cooked. Consequently, the farming of fresh produce requires exceptional care. The potential consequences of inadequate care were demonstrated by the first recognised outbreak of haemorrhagic colitis due to *Escherichia coli* O157:H7 in the United Kingdom. The outbreak was associated with the handling of leeks and potatoes in particular (HPA, 2011), showing that care is still needed in the production and handling of crops that are generally heated before consumption.

Commercial production of vegetables in the UK tends to look to quality assurance (QA) schemes for advice on the avoidance of contamination in produce. The advice and guidance contained within QA schemes is set by technical committees that are a mix of experienced growers (who advise on what's practically attainable) and academics (with a theoretical knowledge of the hazards and the likelihood that these will occur). The largest QA scheme in the UK is the Red Tractor Fresh Produce scheme (part of Assured Food Standards), which takes a pragmatic approach of ranking crops by risk (Table 2). The lowest risks are assigned to those crops that are always cooked before consumption. Red Tractor requires that growers manage the potential for contamination of soil, water and the production environment on a stringency basis that is linked with crop risk.

Table 2 Red Tractor Farm Assurance Fresh Produce Crop Classification

Category 1 - crops you can eat raw and which do not have a protective skin that is removed before eating. Category 1 crops may also have a significant risk or history of pathogen contamination.
e.g. Wholehead Lettuce, Leafy Salads (including any vegetable leaf you can eat raw), Celery, Salad Onions, Radish, Fresh and Frozen Herbs are examples of Category 1 crops.
Category 2 - crops you can eat raw and which either have a protective skin, or grow clear of the ground, or have no significant history of pathogen contamination.
*e.g. Apple, Beetroot, Blackcurrant, Blueberry, Broad Bean, Broccoli, Cabbage, **Carrot**, Capsicum, Cauliflower, Celeriac, Cherry, Courgette, Cucumber ,Garlic, Green Beans (other than runner beans), Melon, Mushroom, Onion (red and white), Pea, Pear, Peach, Plum, Peanut, Raspberry, Strawberry, Sugar Snap Peas, Sweet Corn, Tomato and Tree Nuts are examples of Category 2 crops.*
Category 3 - crops that the customer always cooks.
*e.g. Artichoke, Aubergine, Runner Bean, **Leek**, Marrow, Parsnip, **Potato**, Pumpkin, Squash, Swede, Sweet Potato and Turnip are examples of Category 3 crops.*

Source: Red Tractor Farm Assurance (2011)

3.1.1 WITHIN THE EUROPEAN UNION

Fresh vegetables, as a consequence of high water activity, nutrient content and neutral pH are generally capable of supporting the growth of bacteria (Doan and Davidson, 2000). Consequently, there have been infrequent reports of foodborne outbreaks between 2007-11 in other European Union (EU) countries (including Norway and Switzerland) that were linked with fresh produce (EFSA, 2013). The low numbers of outbreak reports throughout the EU highlight that the safety of fresh produce is an issue of concern not confined to the UK. One of the findings of this study was there were very few outbreaks associated with root vegetables contaminated with enteric human pathogenic bacteria. Furthermore, root vegetables were not routinely contaminated with viruses or protozoa. Between 2007 and 2011, there was one outbreak each linked with onion, leek and potato, with two outbreaks associated with carrots. In contrast, leafy greens that are eaten raw were linked to eight outbreaks and sprouted seeds with 15 (EFSA, 2013). Although the number of outbreaks was low, outbreaks linked to the supply of root vegetables have the potential to affect large numbers of consumers. There were 250 cases of human illness linked with the handling of raw leeks and potatoes in the 2011 VTEC outbreak in the UK (HPA, 2011).

This review has identified outbreaks of foodborne illness linked to the supply of root vegetables in North America and Europe and discusses the identified routes of contamination, where established. The second part of this project deliverable summarises discussions with the UK industry on standard production and supply chain conditions that may influence persistence of enteric bacterial contamination on root vegetables through the supply chain.

3.2 METHODOLOGY

A systematic review of the literature was undertaken to identify outbreaks of human illness associated with root vegetables. Specifically, the primary question was to critically review and evaluate the literature relating to outbreaks of verocytotoxic *E. coli* (VTEC) and related enteric bacteria linked with the consumption or handling of root vegetables.

In addition to the primary question, evidence regarding two sub-questions was also evaluated. These sub-questions were:

1. The identification of human outbreaks caused by VTEC and caused or associated with root vegetables.
2. The identification of human outbreaks caused by other enteric bacteria and caused or associated with root vegetables.

Searching was undertaken online using the ISI Web of Knowledge, PubMed, Medline (within Web of Knowledge), European Food Safety Authority (EFSA), DEFRA, Health Protection Agency (HPA), the United States Food and Drug Administration (FDA), and the United States Centres for Disease Control (CDC) resources to find relevant literature. In addition, the bibliographies of relevant articles were examined to identify missed relevant papers and outbreak reports. The search terms used in the web searches within the field topic are shown as Table 3.

Table 3 Search phrases used to identify outbreak reports and publications relating to root vegetables

	Keywords	AND Qualifier	AND Qualifier	AND Qualifier	NOT Qualifier
Q. 1	"O157:H7" OR "*O157*" OR "haemolytic-uremic syndrome" OR "HUS" OR "verocytotoxin-producing *E. coli*" OR "verotoxin-producing *E. coli*" OR "VTEC" OR "enterohemorrhagic *E. coli*" OR "EHEC" OR "shiga-like toxin-producing *E. coli*" OR "STEC" OR "SLTEC" OR "haemolytic uremic syndrome-associated enterohemorrhagic *E. coli*" OR "HUSEC"	"human" OR "consum*" OR "ingest*"	"outbreak*" OR "disease*" OR "infect*" OR "illness*"	"root vegetable*" OR "carrot*" OR "potato*" OR "tuber*" OR "leek*" OR "onion*" OR "rhizosphere*" OR "parsnip*" OR "turnip*"	"tuberculosis"
Q. 2	"enteric" OR "*Salmonella*" OR "*Campylobacter*" OR "*Listeria*" OR "*E. coli*" OR "*Escherichia coli*"	"human" OR "consum*" OR "ingest*"	"outbreak*" OR "disease*" OR "infect*" OR "illness*"	"root vegetable*" OR "carrot*" OR "potato*" OR "tuber*" OR "leek*" OR "onion*" OR "rhizosphere*" OR "parsnip*" OR "turnip*"	"tuberculosis"

*An asterisk is used to denote a database-specific wildcard character as a search term

A record of the search results was made using Microsoft Excel (version 2010, Microsoft Corporation, Redmond, WA, USA) and is included in Appendix 4.

3.3 IDENTIFICATION, ASSESSMENT AND RELEVANCE SCREENING OF NEW LITERATURE

There were very few recent peer reviewed publications that contained the information required to answer the primary or sub questions. As such, Health Protection Agency (HPA; subsequently renamed as Public Health England) reports, which tended not to be peer-reviewed were also included in the searches. We noted that enteric human pathogens were, by definition, associated with faecal matter and that between 10-15 years ago there were widespread changes made to routine farming practices in Britain. In particular, the use of raw livestock excrement as a fertiliser declined and the composting and stirring of solid and

liquid wastes respectively to oxygenate the material and reduce microbiological loads became widespread (Appendix 2).

The titles and abstracts were screened for relevance using the following inclusion criteria:

(1) any reference not relating to root vegetables

(2) any reference not relating to enteric human pathogens

(3) any reference dealing specifically with lab based identification, diagnostics or treatment papers

(4) any reference referring to cooked products (i.e. cooked vegetable salad)

(5) any reference referring to an outbreak outside of Europe and N. America

(6) any reference not written in English, was not included.

The literature search between Jan 1990 – March 2014 provided:

Primary question – 71 results from Web of Knowledge

Sub questions – 361 results from Web of Knowledge

Most of the literature identified for primary and sub questions made reference to enteric pathogens being linked to foodborne illness but did not identify specific outbreaks of foodborne illness linked to contamination of root vegetables during primary production or the supply chain. Only a few reports detailed the potential routes of contamination and some papers gave only brief details of an investigation. After sifting for relevance, 22 publications were identified that referred to a specific outbreak of foodborne illness associated with enteric pathogens linked to root vegetables. These included duplicate reporting of the same outbreak and only 10 recent outbreaks of relevance were identified for the literature review.

3.4 POTATOES

A number of outbreaks have been linked to consumption of cooked potato salad (comprehensively reviewed by Doan and Davidson, 2000; recent EU data is presented by EFSA, 2013). In general, these outbreaks were unlikely to have been caused by the potato

ingredient of the salad because they were initially prepared by boiling. It was more likely that contaminated mayonnaise or herbs or an infected food handler was the source for these outbreaks (Doan and Davidson, 2000). Although the main focus of this study is the transmission of enteric bacteria through the retail supply chains, for thoroughness, a summary of the comprehensive review of Doan and Davidson (2000) has been included.

Doan and Davidson (2000) summarise that there were more than 60 foodborne illness outbreaks globally linked to potatoes and potato products between 1967 and 1993. In total they estimate around 6,500 people were infected. Potato salad, containing more ingredients than just potatoes, was associated with more than 90% of the outbreaks. The pathogens involved in the outbreaks and the known number of cases associated with each were: *Salmonella* (3,997 cases, 58.0%), *Shigella* (1,447 cases, 21.0%), *S. aureus* (590 cases, 8.6%), *B. cereus* (502 cases, 7.3%); *Streptococcus* (251 cases, 3.6%), *C. botulinum* (79 cases, 1.1%) and *E. coli* O157:H7 (24 cases, 0.3%; Doan and Davidson 2000). In general, the causes of these outbreaks tended to be linked with either contaminated food handlers or the unhygienic handling and storage of cooked potatoes, rather than contaminated raw potatoes. The largest outbreak associated with potatoes occurred in 1989. There was an outbreak of shigellosis aboard a cruise ship that involved 85 people. Potato salad was tested and found to contain *Shigella flexneri*. The implicated *S. flexneri* was also isolated from members of the kitchen staff that had prepared the food (Lew et al., 1991).

In the UK, the first reported outbreak of haemorrhagic colitis associated with potatoes contaminated with *Escherichia coli* O157:H7 occurred in East Anglia (Morgan et al., 1988). The outbreak lasted two weeks and affected 24 people. Eleven patients were hospitalised and there was one fatality. Most of the patients had not eaten out of their home in the two weeks preceding the outbreak. The source of the outbreak was never unequivocally determined or confirmed by laboratory testing. However, a case-control study determined that there was not a significant association with buying or eating fresh meat, beef burgers, salad vegetables, other vegetable or fruit but identified handling vegetables, particularly potatoes and lettuce, as significant risk factors. The regional distribution of the cases suggested that the produce was locally grown and industry information suggested that potatoes best fitted the pattern of distribution. Although it was highly speculative, Morgan et al (1988) hypothesised that poorly composted livestock excreta used to fertilise the potatoes was the likely source of the VTEC. The route of contamination was through the handling of

contaminated potatoes followed by hand-to mouth transmission, rather than foodborne infection.

A summary of the key points from the East Anglia outbreak is:

- Food handling and not consumption of product was the likely route of transmission
- Livestock waste was the likely source of the contamination
- The retail chain used to distribute the contaminated potatoes was likely to have been wholesale

Potatoes were also involved in a large VTEC outbreak in the UK from December 2010 to July 2011 with 252 cases reported (HPA, 2011). Of these cases, 74 patients went to hospital and the infection was fatal for one patient with an underlying health condition. A case-control investigation involving 30 of the people infected indicated that cases were forty times more likely to have been in a household where people handled leeks sold loose (i.e. not pre-packed) compared with the control group. In addition, there was a twelve times increased likelihood to have infection in a household where people handled potatoes bought in or sold from sacks. There was no evidence to link a particular retail source or variety of produce to the outbreak. It was suggested, speculatively, that the outbreak was caused by traces of soil containing verocytotoxic *E. coli* O157 was present on leeks and potatoes and that there was subsequent cross-contamination during storage or kitchen handling was the likely route of transmission.

A summary of the outbreak is:

- Food handling rather than consumption was assessed as the vehicle of contamination
- The source of contamination was suggested to be contaminated soil
- The retail chain used to distribute the contaminated vegetables was not determined

3.5 LEEKS

There were no outbreaks identified that implicated leeks only. However, a single outbreak has been associated jointly with leeks and potatoes (HPA, 2011). The details have been previously summarised above in Section 3.4.

3.6 CARROTS

Carrots have been implicated in six outbreaks of foodborne illness caused by enteric bacteria in the EU and North America. Three similar outbreaks of *Yersinia pseudotuberculosis* were reported as being linked to the consumption of grated raw carrots in Finland in 2003 (Yalava et al., 2006), 2004 (Kangas et al., 2008) and 2006 (Rimhanen-Finne et al., 2009). All three outbreaks were linked to schools and caused illness in 111, 53 and 400 children respectively. All three outbreaks showed an epidemiological linkage with the consumption of raw carrots. The carrots were traced back to both the farm and processing units in all three outbreaks. For the 2003 outbreak, environmental samples from the carrot washing and peeling equipment, and the carrot-peeling line in the processing plant were collected. For the 2003 and 2004 outbreaks, samples from spoiled carrots and fluid draining from spoiled carrots were taken. For the 2004 outbreak, a pooled sample of common shrew (*Sorex araneus*) intestines from one of the two suspected source farms were collected. During the 2006 outbreak, a carrot distributor's storage facility was sampled. All of the samples listed above tested positive for *Y. pseudotuberculosis*. Of note, was that the strains had indistinguishable or closely-related serotypes and genotypes across all three outbreaks.

The exact mechanism of the contamination of carrots in the farm in all three outbreaks was not conclusively established. It was suggested that probably routes of contamination were direct contact with wildlife faeces during storage (Yalava et al., 2006); or contamination from shrews picked up with the carrots by harvesting machinery and held in storage bins along with the carrots (Kangas et al., 2008). For the storage bin contamination scenario, there was speculation that further spread may have been achieved by cross-contamination of equipment during subsequent washing and peeling (Yalava et al., 2006).

A summary of all three carrot associated *Yersinia* outbreaks is:

- The outbreaks were all caused by the consumption of raw produce
- The source of contamination for at least one outbreak was wildlife faeces
- The retail chain used to distribute the contaminated carrots was wholesale followed by food service

Carrots have also been linked to illness outbreaks caused by *Shigella* in Canada and Sweden. In August 2007, the Canadian Food Inspection Agency recalled baby carrots grown by a US-based producer (Kozak et al., 2013). The recall was in response to four cases of Shigellosis. No further details were reported on this outbreak.

An outbreak of Shigellosis also occurred in Sweden in 2008 with 140 cases linked to *S. sonnei* (EFSA, 2013). All the cases had consumed food prepared in a single restaurant. Epidemiological evidence suggested that consumption of grated raw carrot was the cause of the outbreak, however there was no sample available for laboratory-confirmation.

A summary of the Swedish outbreak is:

- Illness was caused by the consumption of raw produce
- The source of the contamination was not identified
- The retail chain used to distribute the contaminated carrots was wholesale followed by food service

In 1993 an outbreak in Rhode Island, USA caused by enterotoxigenic *E. coli* was linked to the possible contamination of raw grated carrots used in salads served on an internal flight amongst other ingredients (MMWR, 1994). There were 47 cases. Another outbreak occurred at the same time in New Hampshire, USA linked to consumption of a salad containing raw carrots, amongst other ingredients, with 121 cases. The carrots in each outbreak were supplied from the same US state and the strains involved in each outbreak had identical genotypes. A trace-back did not identify a single source and the route of contamination was not established (MMWR, 1994).

In summary,

- The outbreaks were associated with the consumption of raw produce (including carrot)
- The source of contamination was not identified
- The retail chain used to distribute the contaminated carrots was wholesale followed by food service

3.7 ONION

This review refers to dried bulb onions (also known as Spanish onions). These are different to green onions (also known as salad onions, scallions or spring onions). Green onions have been associated with a number of food borne illness outbreaks (particularly viruses), are classed as a high risk crop and treated as a ready to eat (RTE) product. Green onions are considered more risky than Spanish onions by a number of QA schemes including Red Tractor because they are manually harvested green and retailed immediately, have a shorter growth duration and so are closer to any contamination event in the field. Green onions are commonly irrigated close to harvest and frequently consumed raw. In contrast, bulb onions are mechanically harvested and dried over a number of weeks. Furthermore, bulb onions may be stored for several months and are frequently (but not exclusively) cooked before consumption.

An outbreak of *E. coli* O157:H7 occurred in Canada in 2008 with 235 cases, of which 26 were admitted to hospital (North Bay Perry Sound District Health Unit, 2009). The outbreak was centred on a restaurant and epidemiological and case control studies linked the outbreak to onions contaminated on-farm. The onions were peeled and manually sliced before being diced by machine and eaten raw as garnish on a beef burger. Environmental samples from the Canadian source farm did not find evidence of contamination of soil or onions. No other outbreaks were associated with onions supplied to other outlets at the same time from this farm. There was also an alternative hypothesis that the contamination may have come from a food handler and only half of the employees identified as having contact with onions were successfully contacted and interviewed. However, the authors concluded that there was good evidence that kitchen staff were exposed simultaneously with customers rather than the source of the infection. The authors concluded that it was most likely a small contaminated batch of onions had contaminated an onion dicer and improper cleaning of the onion dicer may have prolonged the outbreak.

In summary, the Canadian outbreak was:

- Associated with the consumption of raw onions
- The source of contamination was not determined
- The retail chain used to distribute the contaminated carrots was wholesale followed by food service

Thirty cases of *Salmonella haifa* were identified in Sweden in 2011. Imported red onion was suspected as the vehicle of transmission, but no link was established (EFSA, 2013). No further information on this outbreak was available.

3.8 SWEET POTATO

There was an outbreak of campylobacteriosis in a retirement home in the USA in 1997 (Winquist et al., 2001). A case-control study involving 16 cases established that the most implicated food was sweet potatoes. The authors suggested that the most likely explanation was cross-contamination from raw meats, although there was no direct evidence to support the speculation. However, a review of food preparation procedures in the kitchens identified multiple opportunities for general cross-contamination.

A summary of the retirement home outbreak is:

- The illness was likely caused by the consumption of cooked sweet potato
- There was speculation that the source of contamination was after the potatoes were cooked, from raw meat in the kitchen
- The retail chain used to distribute the sweet potatoes was wholesale followed by food service

3.9 VEGETABLE PASTA SALAD

In May 2010, there was an outbreak of *Salmonella* Enteritidis PT8/7 linked to a private barbecue (Mertins et al., 2013). The number of people affected was 11, three people were hospitalised and two developed acute pancreatitis. An investigation of the source was undertaken by the German authorities and a cohort study revealed that vegetable pasta salad was the most likely source of the outbreak. Part of the evidence supporting that

conclusion was that a single infected patient that did not attend the barbeque, but had consumed some of the vegetable pasta salad after the event and no other barbeque food. The symptoms of infection in the person that prepared the vegetable salad began around two hours after preparing the food (which is exceptionally short incubation for an infection by *Salmonella*). Symptoms in other patients commenced 6-24h after the barbeque (Mertins et al., 2013). In the opinion of the report's authors, the short incubation supports a supposition that person that prepared the food was infected from an unknown source prior to the barbeque. The report authors considered that contamination of the pasta vegetable salad was likely to be from the infected food handler, rather than a contaminated ingredient. However, the source was not unequivocally determined and the outbreak is included in this review so that a complete record of outbreaks with potential vegetable sources is reported. Evidence to support an infected food handler as the source included there were no pathogens found in samples of pasta purchased after the outbreak. For the outbreak, poor traceability information prevented identification of the vegetable sources. The report's authors considered that a contributory factor to the outbreak might have been that the vegetable salad had been stored without refrigeration for almost 24h after preparation, which had allowed bacterial multiplication to >200 cfu/g food. Further evidence, which generally supported a theory of a high degree of contamination was that the mean age of the infected people was 27 years, all were healthy and none were particularly vulnerable to infection.

3.10 OVERALL SUMMARY AND CONCLUSIONS

Although there have been historical outbreaks of foodborne illness associated with potatoes in particular, root vegetables have been associated with very few outbreaks since 1990. For the recent outbreaks, root vegetables consumed both raw and cooked have been implicated. A number of outbreaks can be explained by contamination during production or storage. It is also common however for vegetables to become contaminated as a consequence of poor hygiene during further processing or other handling and from infected kitchen staff. A number of reports have concluded for some outbreaks that where crops were cooked before consumption, the illness may have been caused by kitchens contaminated with soil from the unwashed vegetables.

3.11 DISCUSSIONS WITH COMMERCIAL GROWERS OF CARROTS, LEEKS AND POTATOES

The overall purpose of this study was to determine the fate of zoonotic agents (with a particular focus on VTEC) contaminating root vegetables as they travelled down a typical distribution chain. The majority of growers in the UK subscribe to the Red Tractor Assurance Scheme (RTAS) and therefore have controls in operation. Over the last 20 years, the opinion of the project team is that there have been many changes to UK horticulture. Once common practices, such as the use of processed human excreta as crop fertilisers have disappeared and those growers that do not respect the safe timings advice in guidance such as the FSA livestock manure guidelines are in a tiny minority. Consequently, the majority of root crop contaminations are not likely to be a consequence of poor growing practices but a consequence of an accident. This programme of work will mimic experimentally six accidental scenarios that could result in inadvertent contamination or spread of contamination.

These scenarios are:

Pre-harvest

1. The deposition of contaminated excreta onto root crops close (around one week) to harvest
2. The application of contaminated irrigation water onto a root crop close (around one week) to harvest. This scenario would also provide the Agency with information on crop contact with contaminated runoff during a heavy rainfall or flood event.
3. Water application the night before harvest, which is common for some crops during periods of low rainfall (because crops such as baby carrots can be damaged by capped soil [a crust of dry surface soil]).

Post-harvest

4. The use of contaminated wash water for root vegetable washing and polishing
5. The impact of previously washing a contaminated batch of crops on an uncontaminated batch of crops without changing the wash water

6. Crop handling by a gloved or ungloved simulated shedding harvest worker with poor hygienic practices after visiting a bathroom

This section of the report summarises the standard UK production of carrots, leeks and potatoes as determined by a targeted survey of growers to establish key facts regarding the pre-harvest use of livestock excreta and irrigation water and the postharvest processing steps and handling of crops in the distribution chain. This section of the study was undertaken to ensure that a commercially-relevant model of crop production was used for the experimental field work involving the contamination of pre-harvest crops.

3.12 TARGETED SURVEY OF GROWERS

Information on the industry standard growing and handling conditions for the three crops was collected from a targeted phone survey of five UK businesses for each crop. Farming businesses and relevant contacts were suggested by the grower advisors to the project: Martin Brittain (FreshGro - Carrots), James Lee (Greenvale - Potatoes) and Philip Lilley (Hammond Produce - Leeks), as being businesses representative of standard UK production methods. The survey questions asked are included in Appendix 3.

3.13 THE GENERAL USE OF FAECAL WASTE AS FERTILISER FOR CARROTS, LEEKS AND POTATOES

No raw or composted or treated excreta was applied to cropping areas used for carrots, leeks or potato crops by any the businesses interviewed. Use of FYM is very restricted and largely driven by compliance to the requirements of customers (e.g. the Tesco Nurture and M&S field to fork supply protocols) as well compliance with the Red Tractor Assurance Scheme. It is possible there are growers that are not part of an assurance scheme that have a potential to use untreated nitrogenous wastes in an irresponsible manner. However, the use of untreated faecal waste as fertiliser for some crops is also discouraged for non-assured growers because the practice can lead to non-microbiological problems such as ‘fanging’ (carrots with multiple conical outgrowths) and a loss of quality.

3.14 CARROT PRODUCTION

The responses from the carrot growers were that carrot production for human consumption in the UK is now a large and specialised enterprise for most growers. Carrots are grown for many different markets–fresh, frozen and processing – and the quality requirements of some buyers can be exacting. Uniformity of plant size and mass and freedom from damage and disease are very important buyer considerations.

3.14.1 SOILS AND CLIMATE

The climate in most arable areas of the UK is suitable for carrots; the main limiting factor is soil type, which should not restrict root growth. Usually the soil depth is required to be 50-75mm deeper than the required length of carrot. Soils with a loose structure such as sandy loams, loamy sands and fen peats are ideal for carrots. Soils should be well drained but moisture retentive, stone free (to avoid 'fanging' where carrot shape is affected by stones preventing uniform growth) and non-capping (a cap is a crust that forms on a soil's surface in periods of low rainfall. Hard caps can damage crops during harvest). Carrot crops are grown around the UK from north east of Scotland to the south west of England with regionally-staggered growing seasons providing a continuity of supply.

3.14.2 CULTIVATIONS

To produce a crop of carrots with more than 70% complying with a specified size is very difficult. A number of the growers that were asked mentioned the need to prepare a uniform seedbed. In brief, stones are removed from the soil which is typically formed into raised beds, although carrots can also be grown on flat soil. Beds are commonly formed to prevent soil compaction (which can impact on carrot size) and aid harvesting.

3.14.3 DRILLING/PLANTING AND TIMINGS

Seeds coated with pesticide and antimicrobial chemicals (to prevent the establishment of plant pathogens) is drilled with a precision drill i.e. Stanhay or Mini Air into a pre-moistened seed bed. First early harvest crops are sown under polythene in the UK, typically in

October. Second early harvest crops are also sown under polythene from December to February. Main crop carrots are sown in the open from March to early July.

3.14.4 IRRIGATION

Irrigation is essential in most of the carrot growing regions particularly on well-drained sandy soils and for early crops. Irrigation is typically scheduled to be frequent, but with small volumes to avoid wet-dry cycles, which can cause the carrot roots to split and crack.

3.14.5 HARVEST AND STORAGE

Carrot crops are harvested from June onwards. The roots are easily damaged and careful handling is required at all stages. Early crops can be lifted using top-lifters, which pull the roots up by the leaves, and then top the roots to remove foliage. Top-lifters work well during the summer until the end of October, when the tops become too weak. From late October onwards, share-lifters are used which require the tops to be flailed-off first. To maintain freshness, carrots are often lifted at night, when it is cooler and the road network is clear allowing rapid transport to a pack house.

Carrots can be lifted and held in long term cold stores, but after storage of more than a few weeks they lack the fresh appearance associated with newly lifted carrots. Most stored crop is left in the ground during the winter and harvested as required. Carrot crops are either earthed-up or more commonly covered black polythene sheets with a layer of straw 30 cm deep (approximately 100t/ha). Straw or earth protects the crop from frost and helps to stop regrowth in the spring. Lifting a crop can continue until the crop becomes too woody or otherwise unsaleable, usually by early May. Yield can vary from 20 t/ha for early crops to well over 60 t/ha for main crops, however rejects in grading can be 30% or more.

Maximum utilisation of the crop is considered by growers to be important. Carrots are often washed and polished (using brushes to scrub the surface) to remove light blemishes of scab and cavity spot. Polishing is an economic necessity for carrot growers because it increases the proportion of high value class 1 crop.

3.15 LEEK PRODUCTION

Leeks are alliums but do not bulb and are less pungent than Spanish onions. The crop is grown throughout the UK is seasonally available from July to May. Leeks are sold with the leaves trimmed into an inverted V, straight or stripped back. Leeks sold as pre-pack are usually smaller than those sold loose. Leeks can be harvested from the field over winter as they are tolerant of frosts.

The varieties change over the growing season with more rapid maturing varieties providing the early crops. Varieties that can persist in the field are suited to overwintering.

3.15.1 SOILS AND CLIMATE

Leeks can be grown on a wide range of soil types, but the most suitable are sandy loam to sandy clay loam, silts and some peat based soils. It was considered best to avoid very light soils because these can devalue the crop if soil gets blown down into the leaves. Heavy soils retain water and may restrict harvest access in winter.

3.15.2 CULTIVATIONS

For leeks, the land is typically ploughed, stones are removed from loam soils and clay soils are de-clodded and beds are commonly formed before planting. A number of growers mentioned that soils with a tendency to cap should be avoided if the leeks are drilled rather than sown as plantlets.

3.15.3 DRILLING/PLANTING AND TIMINGS

Leeks can be raised from direct drilled seed or small plants organised in blocks or modules. Leeks are planted from January until July and harvested from July to May. In general, crops drilled from January to April give crops before Christmas and May drillings give crops after Christmas. Early crops can be grown under plastic/fleece covers until mid-May to accelerate the harvest by two weeks.

3.15.4 IRRIGATION

Leeks require adequate soil moisture for yield and a lack of water during crop development can lead to bolting (the formation of seeds that renders crops tough and inedible) in the crop. Care is needed with maintaining adequate soil moisture following drilling as the seeds and seedlings are sensitive to dry conditions. Irrigating newly planted modules can help wash soil around the modules improving root contact with the soil.

3.15.5 HARVEST AND STORAGE

Leeks can be harvested by hand or machine. Specialist leek harvesters are available that undercut the leeks and trim the leaves before placing them in bulk boxes. Leeks for pre-pack can be manually trimmed and outer leaves stripped back. Roots need trimming back but care is needed not to damage the base plate of the leek. The leeks should be cooled and held between 2°C and 10°C prior to despatch. Leeks should not be harvested when frozen as they can be damaged by handling. The gap in harvest in May and June is typically supplied by stored leeks. These require refrigerated and controlled atmosphere stores.

3.16 POTATO PRODUCTION

Potatoes are tubers grown for retail (often called pre-pack) or for processing (i.e. crisps, chips/fries, mashed potato, canning etc.) prior to retail. Potatoes are widely grown in the UK and can be stored for nearly a year in dark, cool conditions before being sold. Potatoes are mainly sold as a washed product formultiple retail, but a proportion of the crop is sold with soil on in bulk for both retail and wholesale.

3.16.1 SOILS AND CLIMATE

Light, well-drained soils are used for early potatoes because these soil types warm up more quickly. Main crops utilise deep, fertile loam soils to allow the maximum yield to be achieved.

3.16.2 CULTIVATIONS

The soils used to cultivate potato are heavily worked. Ideally, the land is ploughed before winter to allow frost action to help form a tilth structure (soil that has desirable particle size, texture and organic content). Prior to planting in Spring, the soil is tilled by deep cultivations, discing and power harrowing. Stones are removed from the soil, and if the soil depth requires it, soil is raised into beds which will either be directly planted for some baby crops. Some growers form beds with longitudinal ridges prior to planting.

3.16.3 DRILLING/PLANTING AND TIMINGS

Potatoes are planted by machine using seed potatoes (i.e. chitted mini tubers [chitting is the process of removing all but the strongest shoots from a seed potato]). Early crops are planted from February to March for harvesting late May to July. Main crops are planted in April/May for harvesting August to October. First early crops may be planted under plastic to prevent frost damage and also hasten the early development of the crop.

3.16.4 IRRIGATION

Potatoes can benefit from irrigation in drier seasons. Irrigation is applied using rain guns, booms and in specialist crops through trickle tape. Dry soil during tuber formation can lead to increased common scab and it is usual for the crop to be irrigated unless there is sufficient rainfall. Yield can be increased through irrigation but excessive late irrigation reduces the dry matter content of the crop. Consequently, irrigation typically stops 4-5 weeks before harvest. In some soils the ground is irrigated 1-2 days prior to lifting to soften the soil.

3.16.5 HARVEST AND STORAGE

Early crops are commonly sold with loose skin (i.e. new potatoes with immature tubers) and are harvested from a growing crop. The tops are flailed before lifting the crop. Main crops have set skins and are ready to be lifted about three weeks after the tops (haulm) have died back or been desiccated. Crops are typically harvested by machine. The tubers are dug up from the ground and passed over a series of belts (webs) to allow soil and stones to fall

away. Potatoes are either harvested into bulk trailers or bins before being graded and stored. A high proportion of main crop potatoes are stored in the UK. Storage is in boxes or in bulk using on-floor systems. Potatoes are 'cured' before long term storage. Curing is a process where tubers are warmed to suberize (form skin) or heal wounds and curing reduces disease, rot development and dehydration during storage. Potatoes are held at ~12°C for 10 days and ventilated daily with "dry" air for several hours to reduce humidity in the store. The store will then be cooled using refrigeration or cold 'ambient' external air drawn in through controlled louvers.

A summary of the harvesting and handling processes derived from the survey are shown below for each crop (Figure 1 - Figure 3).

3.16.6 CARROTS

3.16.6.1 IRRIGATION

Irrigation requirements depend on soil type and season. Carrots can be heavily irrigated on sandy soils. However, late irrigation to soften soils is only used by two businesses and only on heavy land in a dry period. Opinion on the timing of the last irrigation application varied between businesses and ranged from 24 hours to 3-4 weeks before harvesting. A water source risk assessment for microbiological contamination is developed by each business and water used for irrigating crops is compliant with regard to laboratory testing and sample collection frequency with customer requirements. Growers are audited by their customers and required to produce evidence in the form of laboratory test certificates and microbiological risk assessments.

3.16.6.2 HARVESTING PROCESSES

All carrots produced by the businesses interviewed were mechanically harvested into bulk trailers and transported dirty to the pack house.

3.16.6.3 WASHING

On arrival to the pack house, some crops are washed out of the trailer with water (to reduce breakages) and flumed into a wash tank. Other businesses delivered the carrots dry to the pack house and they were then elevated into a wash tank. The initial wash is termed a 'pre-wash' and is to remove gross debris and soiling. All businesses discussed the fact that the water is dirty following the first batch of carrots being delivered. This involves a large volume of water in the process and the pre-wash water is usually sourced from recycled clean water used in the final wash/rinse stage. The pre-wash water is topped up and the excess runs to waste. The pre-wash water is changed at regular intervals depending on the volume of material being processed. Some businesses were recycling the water through treatment units and settlement tanks.

The carrots were then washed either through a barrel washer or hydrocyclone using potable water from mains or borehole and polished. The polishing process involved a barrel washer with counter rotating brush rollers that abraded the surface of the carrot. The carrots were also rinsed during this process with potable water.

3.16.6.4 COOLING

The water used in the process is typically cooled. Some businesses have an additional hydro-cooling stage after the final wash/rinse others rely on the use of cold water throughout the washing processes. Target temperature for the finished product ranges from 1.5°C to 5°C. The retail supply chain functions at 5-8°C. The businesses routinely monitor the temperature of wash water.

3.16.6.5 STORAGE

Carrots are packed into plastic bags (prepack) or sealed tray liners (loose) and held in a cold store before dispatching to retail depots on refrigerated lorries. Product is typically held for not more than three days before dispatch. Most product is packed for dispatch on the same day. The temperatures of cold stores and supply chain lorries are routinely monitored. Most of the retail customers (with the exception of M&S) also had temperature checks on arrival at retail distribution centres (RDC).

3.16.6.6 WHOLESALE V MULTIPLE RETAIL SUPPLY

Of the five businesses surveyed no business supplied unwashed carrots. All but one business also supplied carrots to the wholesale sector, although the volumes were relatively low (20-30% of a harvest does not meet the supply criteria). No processes differed for the wholesale product, although larger pack sizes were commonly used.

Figure 1 Processes in harvesting and handling carrots from harvest to dispatch.

3.16.7 LEEKS

3.16.7.1 IRRIGATION

Irrigation requirements depended on soil type and season and one business said that only 5% of the crop was irrigated. Four businesses said that leeks may be irrigated close to the harvest ranging from three days before harvest to day of harvest. The other business said that crops would not be irrigated from four weeks before harvest. Late irrigation was to maintain a turgid crop at harvest, rather than for soil softening. A water risk assessment was developed by each business and the water used for irrigating crops was compliant (and audited) with customer requirements.

3.16.7.2 HARVESTING PROCESSES

Leeks can be machine-harvested using a toplifter, however the majority of crops are hand harvested and trimmed in the field. Harvested leeks may be packed in the field rig, which was common for loose product i.e. where packed into a plastic tray sometimes within a plastic tray liner. Whilst one business was flow wrapping pre-packed leeks in the field rig, the majority of businesses were harvesting leeks into trays before transporting to a pack house for flow wrapping.

3.16.7.3 WASHING

All businesses were washing the leeks on the field rigs. The systems were similar for all business; an initial wash followed by a clean rinse. The initial wash was either in a tank or through a spray system and removed gross debris and soiling. Initial wash water was typically run to waste and where tanks were used; these were emptied at the end of each day or more frequently if the water became heavily soiled. A second wash/rinse typically used a spray or jet hose. All businesses interviewed were using potable water. Some businesses were recycling the second rinse water into the initial wash tank.

3.16.7.4 COOLING

The leeks may be initially held in the field in a refrigerated lorry to remove some field heat whilst waiting for a full lorry load which is transported to a pack house. All businesses either cooled leeks using a blast chiller (i.e. forced air system) or through storage in a cooled room. Target temperature for the businesses ranged from 0-8°C. Retail requirements were 5-8°C as leeks were distributed in a standard retail cool chain.

3.16.7.5 STORAGE

Leeks were stored for up to 4 weeks at the end of the season or during frost periods (when they can't be harvested) and were routinely stored close to freezing to prevent 'telescoping'. However, during the main season leeks were rarely stored for longer than 1-2 days as they were harvested to customer orders. The temperatures of cold stores and supply chain lorries are routinely monitored. Most of the retail customers (with the exception of M&S) also had temperature checks on arrival at retail distribution centres (RDC).

3.16.7.6 WHOLESALE VERSUS MULTIPLE RETAIL SUPPLY

Of the five businesses surveyed, none supplied unwashed leeks to any market. Two of the businesses supplied small volumes of leeks to wholesale. Leeks destined for wholesale are handled identically to those destined for multiple retailers. However, leeks were packed in different formats, with one business using cardboard outers instead of plastic trays for wholesale.

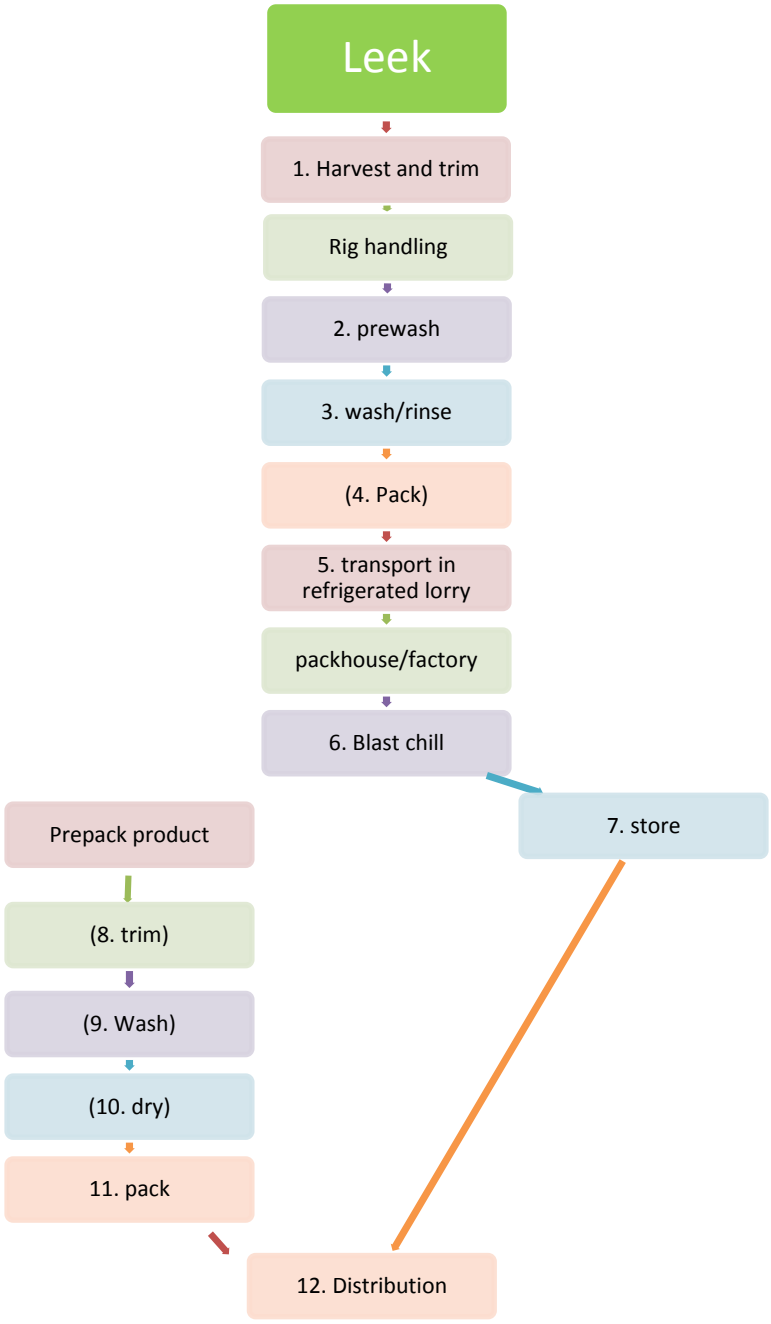

Figure 2 Processes in harvesting and handling leeks from harvest to dispatch.

3.16.8 POTATO

3.16.8.1 IRRIGATION

Irrigation requirements depended on soil type and season. All businesses said that crops could be irrigated up to 1-2 days before harvest. Although a few days from harvest was unusual, some soils and some seasons would require irrigation to soften soil to avoid damage to the crop during harvest. All businesses stated that they developed a water risk assessment for each site and water used for irrigating crops is compliant with customer requirements.

3.16.8.2 HARVESTING PROCESSES

All potatoes produced by the businesses are mechanically harvested.

3.16.8.3 WASHING

After harvesting the potatoes they were transferred into either bulk trailers or 1-3 tonne boxes and transported dirty to the pack house. Unless immediately packed (which was unusual) potatoes were stored for up to ten months (see section below).

Before packing, potatoes were warmed. Warming was particularly important if the potatoes were from a store as a protection against bruising. Some businesses graded potatoes before washing, others after washing. The wash process was similar for all businesses. As with carrots, all businesses discussed the fact that the water was dirty following the first batch of potatoes delivered. As for carrots, the solution of soil involved using a large volume of water for the pre-wash process and the pre-wash water was usually sourced from recycled clean water used in the final wash/rinse stage.

An initial pre-wash in a barrel washer removed gross soiling and was typically followed by a wash/rinse. The prewash water was topped up with recycled wash water and either cycled into waste or was recycled through treatment units (four businesses) or was completely changed approximately two times a day and filled with new clean water (one business). The final wash/rinse water used chilled potable water.

3.16.8.4 COOLING

New potatoes may also be hydro-cooled before packing but main crop is not and relied on refrigerated wash water to maintain low temperatures. Packed products were held in cold stores prior to dispatch and the target temperature for the businesses ranged from 3-8°C to 12°C depending on their customers.

3.16.8.5 STORAGE

Potatoes destined for storage were dry cured by holding at approximately 12°C for ten days before cooling to 2-3°C.

The temperatures of dispatch cold stores and supply chain lorries were routinely monitored. Most of the retail customers (with the exception of M&S) also had temperature checks on arrival at retail distribution centres (RDC).

3.16.8.6 WHOLESALE V MULTIPLE RETAIL SUPPLY

All businesses supplied potatoes to the wholesale sector although volumes were relatively low. One business supplied unwashed potatoes in small volumes to wholesale. The other businesses stated that no processes differed for the wholesale product but that different pack formats were used.

Figure 3 Processes in harvesting and handling potatoes from harvest to dispatch.

3.17 SUMMARY

The findings of the survey are summarised in Table 4.1.

Table 4 Summary of harvesting and handling processes from the businesses surveyed

	Carrot	Leek	Potato
Preharvest			
Use of excreta	No raw or composted or treated excreta was applied to cropping areas with growing crop.	No raw or composted or treated excreta applied to cropping areas with growing crop.	No raw or composted or treated excreta is applied to cropping areas with growing crop.
Irrigation	Water RA completed and compliant to RA	Water RA completed and compliant to RA	Water RA completed and compliant to RA
Latest irrigation	1 day before harvest*	Day of harvest*	1 day before harvest*
Postharvest			
Harvesting processes	Mechanical	Mechanical (rare) hand harvested (common)	Mechanical
Washing	Pre-wash, wash, polish, rinse	Pre-wash and wash/rinse	Pre-wash and wash/rinse
Cooling	Hydrocool	Room cool or blast chiller	Room cool
Target temperature on dispatch	1.5-5°C	0-8°C	3-12°C
Distribution temperature	5-8°C	5-8°C	5-8°C
Storing	1-2 days before dispatch	1-2 days before dispatch. Max 3-4 weeks at end of season and during frosts	Max 10 months before packing. 1-2 days before dispatch
Wholesale v MR (Multiple Retail)	All businesses sold washed and polished carrots to multiple retailers. 4/5 businesses sold washed and polished carrots to wholesale. Processes identical.	All businesses sold washed leeks to multiple retailers. 2/5 businesses sold washed leeks to wholesale. Processes identical.	All businesses sold washed potatoes to wholesale and multiple retailers. 1/5 businesses also sold small volumes of unwashed potatoes to wholesale.

*Not routine and only occurred only in drier periods on susceptible soils

3.18 CONCLUSION

It was apparent from a review of the literature that outbreaks associated with root vegetables have been quite rare in recent years. Twenty years ago, outbreaks were far more common. For these historical outbreaks, a number of reports concluded that excreta used as a soil fertiliser was the ultimate source. However, the evidence supporting such suggestions tended to be circumstantial and speculative. We note that over the last 10-15 years excreta usage for soil conditioning and fertilisation had declined. Furthermore the management of excreta applications in terms of treatment to reduce microbial load and the application to harvest interval have improved. It is tempting to speculate reduced outbreaks and improved excreta management are linked, although there is presently only circumstantial evidence to support such a hypothesis.

For the few recent outbreaks that have been linked with vegetables, the majority have concluded that the contamination source was an infected food handler. The evidence supporting infected staff as the source includes very low incubation periods for some outbreaks with identical strains being isolated from food handlers and the suspected food. Contaminated food stored without adequate refrigeration was also implicated as a contributory factor for some outbreaks.

Three separate carrot-associated outbreaks were caused by a single strain of *Yersinia*. It was suggested that probable routes of contamination were direct contact with wildlife faeces during storage (Yalava et al., 2006); or contamination from shrews picked up with the carrots by harvesting machinery and held in storage bins along with the carrots (Kangas et al., 2008). Further speculation was made that cross-contamination may have occurred during the post-harvest processes of washing and peeling. The current proposal includes work to assess contamination of crops with slurry close to harvest and cross-contamination between contaminated and uncontaminated batches of crops during washing and polishing. Thus, it is apparent that the contamination routes originally proposed will provide quantitative information on the fate of enteric human pathogens under the conditions suspected of causing at least one of the carrot-associated outbreaks. The opinion of the project team is that there is no need to alter the original proposal to provide information on different contamination scenarios identified by the literature review.

As part of this study, five sets of carrot, potato and leek growers were contacted and asked about their sowing, harvest, post-harvest processes and storage conditions. There was relatively little variation between companies, and a summary of typical commercial practices is provided as Table 4. No difficulties were envisaged in mimicking the commercial cultivation conditions and practices.

In terms of sowing crops and irrigation, land was chosen that complied with the condition of six months between drilling and the last untreated excreta application in the Red Tractor standard. For the experimental cultivation plots, the most recent excreta application was composted FYM applied more than six months before planting/drilling and thus the preparation met both that of typical commercial practice and the safe use criteria described by the FSA manure guidance. Closer to harvest, and prior to the application of slurry, soil samples will be tested to confirm that there are no background enteric bacteria.

The dates for planting/drilling the crops were also compliant with typical agricultural practices and appropriate soil types. The leeks were drilled on the 5th May 2014, and the carrots and potatoes on the 3rd June 2014. In order to ensure as faithful a model as possible of a commercial planting, the staff and equipment of a specialist commercial grower for each of the crop types was used for each of the plantings/drillings.

4 HAND HYGIENE AND CROSS CONTAMINATION TO CROPS AFTER THE USE OF A FIELD LATRINE

4.1 ABSTRACT

A study was undertaken to simulate the likely effects of a field worker with poor hygienic practices that had returned to work too soon after recovering from an infection by an enteric pathogen. The studies simulated a variety of hand-washing practices from no washing to washing with soap and water followed by an application of alcohol gel after using a field latrine. The effect of handwashing on the numbers of *E. coli* on hand and glove surfaces

was quantified. In addition, the transfer of *E. coli* to a carrot through handling by a worker following hand-washing was also determined. The numbers of *E.coli* isolated from workers' hands declined with increasing thoroughness of handwashing treatments with unwashed hands > water > water and soap > water, soap and alcohol gel. Where gloves were worn the counts obtained for the treatments were significantly reduced but it was observed that unwashed hands contaminated gloves during the process of putting them on. Hand contamination following the use of a field toilet transferred contamination to carrots. These results suggest that if no gloves are worn it would be best practice to wash hands with water and soap and apply alcohol gel after using a field toilet. Wearing gloves reduced the risk of contaminating handled produce but workers should still wash their hands after using a field toilet and before applying gloves.

4.2 BACKGROUND

Fresh produce is associated with 3% of the total cases of foodborne illness in the UK (Adak et al. 2002). This percentage equates to roughly 50,000 cases of illness per year and consequently this class of food is of particular concern to regulatory authorities. A key factor is that some fruits and vegetables are likely to be consumed after minimal processing and without cooking; and so any human pathogens present on produce have an increased likelihood of causing foodborne illness compared with food that is cooked. Consequently, the management of risk factors is a particular focus in the production of ready-to-eat (RTE) crops such as leafy vegetables that are eaten without further preparation (Gil et al., 2015).

In contrast to leafy salads, there are relatively few outbreaks associated with root vegetables contaminated with enteric human pathogenic bacteria (EFSA, 2013). Between 2007 and 2011, there was one outbreak each linked with onions, leeks and potatoes, and two outbreaks associated with carrots. In contrast, leafy greens that are eaten raw were linked to eight outbreaks and sprouted seeds to 15 (EFSA, 2013). Although the number of outbreaks was low, outbreaks linked to the supply of root vegetables can affect large numbers of consumers, with 250 cases of human illness caused by verocytotoxic *E. coli* linked to the handling of raw leeks and potatoes in 2011 (HPA, 2011). This latter case was of particular interest as it was associated with loose leeks and potatoes sold in independent stores, suggesting that production may have come from a smaller-scale operation. Root crops such as carrots and potatoes are generally produced on a large-scale in Europe with machine harvesting and automatic grading giving little opportunity for direct contact between worker's hands and the harvested crop (Monaghan, 2014). However, there are still small-scale growers that manually lift and hand-grade root crops for subsequent sale in farmers' and local markets.

There have been a number of foodborne outbreak investigations that have implicated the contaminated hands of food workers as the source of human pathogens (Todd et al., 2010). Overall, the percentage of total foodborne disease outbreaks across thirty countries involving infected food workers was assessed by Todd et al., (2007) to be as high as 11.4%. However, the majority of investigations have centred on food handlers in restaurants or other high-throughput food preparation areas. Examples of outbreaks where food handlers have been implicated include a large outbreak caused by *Shigella* (Reller et al., 2006). The outbreak was linked to hand-sorted bruised and overripe tomatoes from a single distributor. Workers at the restaurant handled the tomatoes and other foods with bare hands without washing in between the handling of different foods. In addition, distributor staff had

previously sorted the tomatoes by hand (without gloves) into the overripe and less-old batches. It was speculated that contamination occurred during or after the sorting by the distributor's staff and the route of initial contamination was by the hands of an infected sorter at the distributor's premises. The importance of correctly-implemented hand hygiene has been demonstrated in a study of spinach farms in the USA where produce contamination with generic *E. coli* was significantly reduced when workers used hand-washing stations and the farm provided portable toilets and trained the workers in their use (Park et al., 2013). Recent work has highlighted the transfer from contaminated workers hands of *Salmonella enterica* serotype enteritidis on to living lettuce at harvest (Waitt et al., 2014) and *E. coli* O157:H7 being transferred from contaminated hands to strawberries during harvesting (Shaw et al., 2015). However, no previous work has reported microbial transfer from contaminated hands to hand-harvested root vegetables.

The aim of this study is to provide information on the risks associated with manual harvesting if a worker was shedding an enteric pathogen and had poor hygienic practices after using a field toilet without washing their hands properly, or using a field toilet not equipped with hand washing facilities. This research provides new information about post - harvest cross-contamination risks associated with hand hygiene procedures following the use of field toilets and highlights the need for the development of safe back to work and produce handling practices for the hand harvesting of vegetables.

4.3 MATERIALS AND METHODS

4.3.1 HAND WASHING TREATMENTS

The experiments took place at five holdings in the UK that commercially cultivated fresh vegetables. At each site, subjects were briefed on the purpose of the experiment and randomly assigned a hand wash procedure to be followed after using a field toilet. The subject used the latrine to defecate and utilised toilet paper provided; immediately afterwards the subject undertook the pre-assigned hand washing treatment. The treatments that were undertaken are listed in Table 5. The detergent used for hand washing was Seraman Sensitive Foam (Ecolabs, Cheshire, UK) and the alcohol gel was Spirigel (Ecolabs). Hand drying was achieved using paper towel (400cm^2 area - 20cm x 20cm). The gloves used were Simply Blue Nitrile Gloves (Glove Club Ltd, Perivale, UK). The experiment was repeated on five occasions at five different field sites. On each occasion, three replicates were undertaken for each hand washing treatment (n=15). On those occasions when there were insufficient workers available to cover the number of samples required to be collected, single workers were sampled twice. Each sample was obtained from a separate use of the latrine.

Table 5 The treatments undertaken for hand washing effectiveness assessments after harvest workers had used a field latrine. Hands were washed according to each treatment listed. After washing gloves were worn or not worn before the worker handled a baby carrot. Both the vegetable and sample of diluent derived from the worker's hands or gloves were examined for numbers of generic *E. coli*.

Hand wash treatment.	Gloves worn for produce handling (Yes/No).
No hand washing	No
Hand washing using water	No
Hand washing using water and soap	No
Hand washing using soap, water, alcohol gel	No
No hand washing	Yes
Hand washing using water	Yes
Hand washing using water and soap	Yes
Hand washing using soap, water, alcohol gel	Yes

After undertaking a hand wash treatment, the subject picked up a commercially-purchased baby carrot, closed their hand around it and deposited it into in a stomacher bag for testing. The carrots were purchased on the morning of the experiment for each location. The hand that had been used to pick up the carrot was massaged for two minutes inside a stomacher bag containing 20ml of maximum recovery diluent (MRD, Oxoid, Basingstoke, UK) to generate a rinse sample. Each treatment produced a hand or glove rinse and a carrot sample for analysis. Carrots that were not handled were included as controls for each trial. After the hand washing treatments and sample collections, the test subjects were instructed to wash their hands properly under supervision.

4.3.2 SWABBING OF LATCHES AND LATRINE DOOR HANDLES

Jumbo head cotton swabs (Sterilab, Harrogate, England) were used for wet-dry swab sampling. Samples were collected from a 10 cm^2 area. Each swab was moistened in maximum recovery diluent [MRD; Bacteriological Peptone (Oxoid L37) 1 g, sodium chloride 8.5 g to 1000 ml], and rolled between the thumb and index finger as it was rubbed across the surface of the latrine flush or door handle. Immediately after rubbing with the MRD-moistened swab, the procedure was repeated over the same area using a dry swab.

4.3.3 MICROBIOLOGICAL EXAMINATIONS

4.3.3.1 ENUMERATION OF *E. COLI*

Samples were stomached (Colworth 400; Seward, Thetford, UK) for 2 minutes in mesh filter bags (6041/STR, Seward) and the homogenates were diluted decimally in MRD. Aliquots (10ml) of each homogenate and derived decimal dilution series were vacuum-filtered through 0.45μm nitrocellulose filters (Sartorius, Epsom, UK). *E. coli* numbers were estimated by placing the filters on chromogenic tryptone bile X-glucuronide agar (TBX, Oxoid, Basingstoke**,** England) to each dish. Initial incubation was for 4h at 37^{o}C followed by 20h at 44^{o}C. The theoretical detection limit for the method was 0.1 cells per ml of homogenate for hand rinse samples.

4.3.4 STATISTICAL ANALYSES OF RESULTS

For samples where no colonies were visible on the filters, a value of half the limit of detection of the test method was substituted to allow log transformation of results. The theoretical detection limit for the method was 0.1 cells per ml of homogenate for hand rinse samples. Geometric means, associated standard deviations and standard errors were calculated from $\log_{10}$ transformed counts. Statistical comparisons were also undertaken using $\log_{10}$ transformed counts. Analyses of variance, Tukey's honest significance test (HSD) and t-tests were performed using Statplus 2009 Professional (Analystsoft Inc. Walnut, CA, USA).

4.4 RESULTS AND DISCUSSION

4.4.1 EFFECT OF HANDWASHING PRACTICE ON LEVELS OF *E. COLI* ON WORKERS HANDS AND SUBSEQUENT CROSS CONTAMINATION ON TO A HANDLED CARROT

Workers unwashed hands were contaminated with *E. coli* at up to 2.8 log cfu/hand with a geometric mean of 1.65 log cfu/hand (Figure 4). This range was similar to that reported in a study of lettuce producers in Brazil where field workers' hands were contaminated with *E. coli* ranging from less than 1.0 to 1.9 log cfu/hand (de Quadros Rodrigues et al., 2014). In contrast, a study of Spanish baby-leaf production isolated no coliforms on workers' hands (n=15) but all hand samples showed presence of *Enterobacteriaceae* at a mean of 3.4 log cfu/surface (Castro-Ibanez et al., 2015).

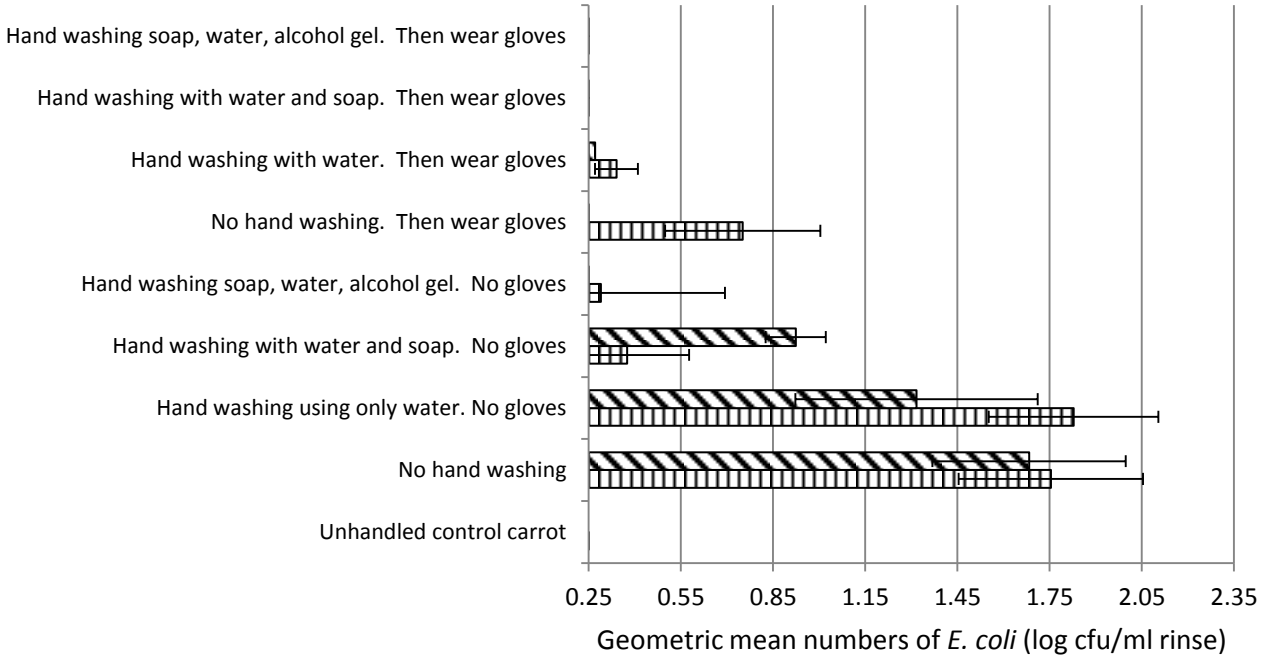

Figure 4 The impact of different hand washing treatments after using a field latrine on the numbers of E. coli transferred to carrots (vertical hatch) and then rinsed from hands or gloves (diagonal hatch). Error bars are ± the standard error of the mean. Data are the mean of 15 replicates.

Table 6 The results of analyses of variance (ANOVA) of the test results of diluent used to wash the hands of harvest worker after using a field latrine and undertaking one hand wash

treatment. Treatments were no handwashing (NW) handwashing using water (HW – W), hand washing using soap and water (HW - SW) and hand washing using soap, water and alcohol gel (HW - SWG). An unhandled (Control) carrot was included. Gloves were either applied after washing (G) or not (NG). Differences between treatments were determined to be significant (Accepted) by a Tukey *ad hoc* honest significant difference (HSD) test.

Treatment	p-level	Significance
Control vs G HW - W	0.007	Accepted
Control vs G HW SW	0.946	Rejected
Control vs G HW SWG	0.436	Rejected
Control vs NG HW - SW	0.185	Rejected
Control vs NG HW - SWG	0.216	Rejected
Control vs NG HW - W	0.000	Accepted
Control vs NG No HW	0.000	Accepted
G HW - W vs G HW SW	0.006	Accepted
G HW - W vs G HW SWG	0.054	Rejected
G HW - W vs NG HW - SW	0.167	Rejected
G HW - W vs NG HW - SWG	0.141	Rejected
G HW - W vs NG HW - W	0.005	Accepted
G HW - W vs NG No HW	0.029	Rejected
G HW SW vs G HW SWG	0.397	Rejected
G HW SW vs NG HW - SW	0.164	Rejected
G HW SW vs NG HW - SWG	0.192	Rejected
G HW SW vs NG HW - W	0.000	Accepted
G HW SW vs NG No HW	0.000	Accepted
G HW SWG vs NG HW - SW	0.582	Rejected
G HW SWG vs NG HW - SWG	0.644	Rejected
G HW SWG vs NG HW - W	0.000	Accepted
G HW SWG vs NG No HW	0.000	Accepted
NG HW - SW vs NG HW - SWG	0.930	Rejected
NG HW - SW vs NG HW - W	0.000	Accepted
NG HW - SW vs NG No HW	0.000	Accepted
NG HW - SWG vs NG HW - W	0.000	Accepted
NG HW - SWG vs NG No HW	0.000	Accepted
NG HW - W vs NG No HW	0.506	Rejected

There was a wide range of counts obtained from the hand rinses and contaminated carrots (Figure 4). The range resulted in sizeable errors associated with some results, despite the robust replication of fifteen examinations for each data point. It was likely that some workers used the facilities more hygienically than other workers and that the variation observed was typical of that found at most field sites. There are other factors with the potential to cause variation in bacterial transfer between different workers that include skin dryness and the composition of indigenous skin surface microbiota (Spellberg, 2000).

The numbers of *E. coli* isolated from workers' hands declined with increasing thoroughness of handwashing treatments with unwashed hands > water > water and soap > water, soap and alcohol gel (Figure 4). Unwashed hands had an average of 1.65 log cfu/hand compared with few (2/15) detections from hands washed with water, soap and alcohol. Similar results with field workers have been reported by de Aceituno et al., (2016) where hand washing alone did not decrease microbes as effectively as the use of alcohol-based hand sanitizer.

There were no *E. coli* isolations from any of the unhandled control carrots, therefore there was a high degree of confidence that the *E. coli* isolated from the handled carrots were from the test subjects. All of the treatments, where *E. coli* was counted on the hands of workers and gloves were not worn resulted in the transfer of generic *E. coli* to the carrots. There were small numbers of *E. coli* transferred when hands were washed with soap and alcohol gel, with four of the 15 carrots containing numbers of *E. coli* high enough to be counted. The maximum amount of cross-contamination was observed for the unwashed and water-only washed treatments, each of which transferred around 60 *E. coli* cells to each carrot on average. Washing using water-only resulted in a slightly greater transfer of *E. coli* compared with no washing, but the increase was not significant (Table 6). For the other treatments, a typical transfer was between two and 30 cells per carrot. As might be expected, there was a general trend that the numbers of *E. coli* transferred to the carrot decreased as the thoroughness of the hand washing increased.

4.4.2 EFFECT OF WEARING GLOVES FOLLOWING HANDWASHING ON LEVELS OF *E. COLI* ON WORKERS GLOVES AND SUBSEQUENT CROSS CONTAMINATION ON TO A HANDLED CARROT

Gloves can function as an effective barrier to bacterial cross-contamination between hands to food (Montville et al., 2001; Todd et al., 2010). As with un-gloved hands, there was a general trend that the numbers of *E. coli* transferred to the carrot decreased as the

thoroughness of the preceding hand washing increased (Figure 1). Overall, the counts obtained for the treatments where gloves were worn were significantly reduced compared with the counts from the treatments where no gloves were worn (Paired t-test, $P<0.01$). The greatest transfer to the gloves and the carrots was when gloves were worn without any preceding hand washing. The result suggests that the outside of the gloves were contaminated by the hands whilst they were pulled on to the workers' hands. The observation that contamination can transfer from unwashed hands to gloves to crops is a contamination concern for growers. Previous studies have demonstrated that *Salmonella* can also be transferred from latex gloves to lettuce leaf tissue after a contamination event (Waitt et al., 2014). The 'hand washing – water only' treatment also facilitated the transfer of small numbers of *E. coli* onto the gloves and consequently to the carrots. However, the 'no hand washing' treatment transferred nearly three times the number of *E. coli* cells to the carrots compared with the 'hand washing – water only' treatment. There were no isolations from the glove treatments that involved either the use of soap or soap and alcohol gel.

4.4.3 CONTAMINATION OF FIELD TOILET FLUSH HANDLE AND DOOR LATCH

The field latrines used for these studies had sinks that were outside of the cubicle containing the toilet (Figure 5). Workers were therefore required to operate the toilet flush and the door latch before washing their hands. At three of the sites, the flush handle and door latch were swabbed after the completion of the hand washing experiments and the mean contamination of the latch was 3±0.87 log cfu/swab and the flush handle 2.8±0.63 log cfu/swab. Although better replication would be required to unequivocally establish a range for typical contaminations, our initial investigations suggest that flush handles and door latches have the potential to act as fomites, transferring bacteria between the hands of different workers.

It is well understood that worker hygiene should be considered as a potential route of microbial contamination of produce at harvest and facilities should be provided for workers to manage hand hygiene (Gil et al., 2015). We have demonstrated that hand contamination following the use of a field toilet could lead to contamination of root crops where they are routinely handled at harvest.

Figure 5 A field latrine of the type used for this study with hand wash facilities that were external to the toilet cubicle

There was a general trend that the numbers of *E. coli* transferred to the carrot decreased as the thoroughness of the hand washing increased. These results suggest that if no gloves are worn, it would be best practice to wash hands with water, soap and alcohol after using a field toilet. Wearing gloves will reduce the risk of contaminating handled produce but workers should still wash their hands after using a field toilet before wearing gloves. There is an important role for on-farm training to ensure that hand hygiene practices are maintained by field workers (Soon and Baines, 2012). In addition, hygiene schedules should include hand contact surfaces that have potential to act as fomites such as flush handles and door latches.

5 FATE OF *ESCHERICHIA COLI* O145 PRESENT NATURALLY IN BOVINE SLURRY APPLIED TO VEGETABLES BEFORE HARVEST, AFTER WASHING AND SIMULATED WHOLESALE AND RETAIL DISTRIBUTION.

5.1 ABSTRACT

Bovine slurry that was naturally contaminated with *Escherichia coli* O145 was applied without dilution or diluted 1/10 in borehole water to experimental plots growing potatoes, leeks or carrots. The application of slurry was one week prior to harvest to simulate a near-harvest contamination event by direct excreta deposition or an application of contaminated water to simulate a flooding event or irrigation by contaminated water. The fate of the bacteria was assessed as the crops were harvested, processed through a commercially-relevant wash treatment and distributed through either a retail chain at 4^{o}C or a wholesale chain at ambient temperature.

At harvest, crops were contaminated at up to two log cfu/g. Washing caused a transfer of *E. coli* into the wash water of a flotation tank used to wash potatoes and did not completely remove all traces of contamination from the crop. A second batch of uncontaminated potatoes washed immediately after a contaminated batch consequently acquired small amounts of contamination from the wash water. There was no cross-contamination when leeks were sprayed with water. Carrots washed in an abrasive, brush-lined drum that removed the outer surface of the carrots effectively decontaminated the vegetable. Leeks were contaminated with a small number of O145 cells after retail or wholesale simulated distribution. For potato, *E. coli* O145 was isolated after retail but not wholesale distribution. There were no post-distribution isolations from carrots. These findings indicate it is plausible a recent UK outbreak may have been caused by soil on vegetables cross contaminating food preparation environments.

5.2 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 co-factors (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 (Taylor et al., 2010; 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 - 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 UK. The first reported outbreak of haemorrhagic colitis associated with potatoes was most likely contaminated with *Escherichia coli* O157:H7 and occurred in East Anglia (Morgan et al., 1988). Eleven patients were hospitalised 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 are of particular concern to regulatory authorities because it is important that reduced consumer confidence in ready-to-eat fruits and vegetables does not change eating habits and reduce the consumption of nutritious fresh produce. 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 times of pathogens capable of causing human illness. Traditionally,

these survival estimates have involved the use of laboratory cultured cells (Hutchison et al., 2004; 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., 2004; Hutchison et al., 2005; Visvalingam et al., 2013) can cause up and down regulation 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 optimising our estimates of the fate of human pathogens (Maks and Fu, 2013).

This project 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 non-toxigenic *E. coli* O145, as a marker for human-pathogenic *E. coli*. The marker was a natural component of the microbiota and contamination was one week prior to harvest. The crops were processed by washing and held under simulated commercial distribution conditions, typical of those used in Western Europe and North America.

5.3 MATERIALS AND METHODS

5.3.1 IDENTIFICATION OF EXCRETA NATURALLY-CONTAMINATED WITH A VEROTOXIC *E. COLI* SURROGATE.

A composite of bovine fecal deposits in a single slaughter batch was collected in the lairage from animals presented for slaughter at the University of Bristol teaching slaughterhouse. Excreta (5g) was enriched in an equal volume of modified tryptone soya broth (mTSB; Oxoid, Basingstoke, UK) supplemented with 20 mg/l novobiocin (Sigma, Poole, UK), 1.5 g/l bile salts (Oxoid) and 1.5 g/l K_2HPO_4 (Sigma) with incubation at 41.5^{o}C for 12 h. Cells from 1 ml of enriched broth were pelleted (10,400 *g* for 5 min) and re-suspended in sterile distilled water (1 ml). The re-suspended pellet was boiled (2 min) to generate a crude DNA template.

5.3.2 PCR CHARACTERISATION 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 the primers and reaction conditions described by Paton and Paton (2002). The primer sequences used for the multiplex PCR are listed in Table 7.

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

Table 7 Primer sequences used to characterise *E. coli* virulence genes contained within enriched samples of cattle manure. Reproduced from Paton and Paton (2002).

Primer	Primer sequence (5'–3')	Specificity	Amplicon size (bp)
stx1F	ATAAATCGCCATTCGTTGACTAC	nt 454–633 of the A subunit coding region of stx_1	180
stx1R	AGAACGCCCACTGAGATCATC		
stx2F	GGCACTGTCTGAAACTGCTCC	nt 603–857 of A subunit coding region of stx_2 (including stx_2 variants)	255
stx2R	TCGCCAGTTATCTGACATTCTG		
eaeAF	GACCCGGCACAAGCATAAGC	nt 27–410 of *eaeA* (this region is conserved between EPEC and STEC)	384
eaeAR	CCACCTGCAGCAACAAGAGG		
hlyAF	GCATCATCAAGCGTACGTTCC	nt 70–603 of EHEC *hlyA*	534
hlyAR	AATGAGCCAAGCTGGTTAAGCT		

Samples were subjected to 35 amplification cycles. Denaturation was for 1 min at 95°C followed by 2 min of annealing at 65°C for the first 10 cycles, ramping down to 60°C by cycle 15. 1.5 min was allowed for elongation at 72°C, increasing to 2.5 min from cycles 25 to 35. PCR reaction mixtures were electrophoresed on 1.5% (w/v) agarose gels (Bio Rad, Hemel Hempstead, Herts) and stained with ethidium bromide before visualisation under ultraviolet light of 220 nm wavelength.

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

Molecular determination of serotype and the presence of loci encoding the H7 antigen (only) was by PCR using a previously-described methodology (Perelle et al., 2004). The primer sequences used for the individually-determined serovars are shown in Table 8.

Table 8 Primer sequences used to determine serotypes in confirmed *E. coli* isolatef from cattle manure. Reproduced from (Perelle et al., 2004).

Target gene (serotype)	Primer sequence (5'–3')	Amplicon size (bp)
rfbE (O157)	TTTCACACTTATTGGATGGTCTCAA CGATGAGTTTATCTGCAAGGTGAT	88
wbdI (O111)	CGAGGCAACACATTATATAGTGCTTT TTTTTGAATAGTTATGAACATCTTGTTTAGC	146
wzx (O26)	CGCGACGGCAGAGAAAATT AGCAGGCTTTTATATTCTCCAACTTT	135
wzy (O113)	GAGCGTTTCTGACATATGGAGTGA TTGCTATAAATGGAAGCCATTCTTT	107
wzy (O91)	CGATTTTCTGGAATGCTTGATG CAATACATAGTTTGATTTGTGTTTAAAGTTTAAT	105
wbgN (O55)	TGTAATTCGATGCACCAATTCAG CGCTTCGACGTTCGATACATAA	70
ihp1 (O145)	CGATAATATTTACCCCACCAGTACAG GCCGCCGCAATGCTT	132
fliC H7 (H7)	CCACGACAGGTCTTTATGATCTGA CAACTGTGACTTTATCGCCATTCC	96

Amplification was performed using the buffers and polymerase described above with a reaction-specific concentration of primer and $MgCl_2$, as shown in Table 9.

Table 9 Primer and $MgCl_2$ concentrations used for each serotype determination

Amplification	[Primers] (nm)	[$MgCl_2$] (mM)
rfbE (O157)	500	5
wbdI (O111)	1000	5
wzx (O26)	500	5
wzy (O113)	1000	4
wzy (O91)	500	5
wbgN (O55)	500	5
ihp1 (O145)	500	5
fliC H7 (H7)	500	5

Visualisation of the amplicons was as described above.

5.3.3 ON FARM SLURRY COLLECTION

Slurry samples (1kg) from the previously identified herd were collected for 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^{o}C during shipping to the laboratory and testing commenced within 24 h.

Slurry for the inoculation of crops was pumped directly from the storage lagoon into 1000 liter 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 around one hour and without refrigeration.

5.3.4 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 sub-lethally stressed cells. (Hutchison et al., 2004). In brief, vegetables were chopped using sterile knives into 1cm^3 blocks. All samples were diluted decimally in mTSB supplemented with 40 μg/ml novobiocin and stomached (Colworth 400, Seward, Thetford, UK) for 1 minute in mesh bags (6041/STR; Seward) . A 10ml volume for each liquid homogenate was filtered for all samples, except carrots; where a 20ml 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 five hours at 37^{o}C was allowed by placing the filters onto a sterile felt pad soaked in mTSB supplemented with 40 μg/ml novobiocin. After recovery, the filters were placed onto a chromogenic selective agar and incubated for 16-20h at 41^{o}C (Posse et al., 2008) before counting. The limit of detection of the test method was 1 cfu/g for potatoes and leek; and 0.5 cfu/g for carrots.

5.3.5 MOTILITY

The agar used was motility test agar, (Becton Dickson, Franklyn Lake NJ product number 211436). Test cultures were stabbed into the agar and checked after a 24h incubation (37^{o}C) for a diffuse growth indicative of swarming.

5.3.6 CROP CULTIVATION

Crops were field-grown at Harper Adams University, Shropshire in the west of England (geo: 52.777404, -2.429197). Prior to planting each crop the field area 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 approximately 400-600 potatoes, leeks and carrots over a 5 m length of bed at harvest.

The potato (Figure 6) and carrot (Figure 7) 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. The leek experimental plots (Figure 8) were planted in a 4 x 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 4 plots

with an untreated plot of leeks separating them from the next treated plot to minimise the risk of cross-contamination through run-off.

5.3.7 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 60l of slurry or a 60l contaminated irrigation or flood event in a section of field containing produce. The contamination was applied one 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.

Figure 6 The experimental plots used to cultivate commercially-relevant quantities of potatoes

Figure 7 The experimental plots used to cultivate commercially-relevant quantities of carrots

Figure 8 The experimental plots used to cultivate commercially-relevant quantities of leeks

5.3.8 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 (Figure 9). 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 (Figure 10). 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 (Figure 11).

Figure 9 Potatoes were harvested mechanically using a tractor-pulled potato harvester

Figure 10 Leeks were harvested by manually trimming the roots and leaves using a knife followed by stripping back the flag leaves to expose the shank

Five samples were collected from each of the three independent treatment 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 25g of randomly-selected, chopped sample.

Figure 11 Carrots were harvested by using a fork to lift the roots to the surface and manual lifting

Potatoes were washed in unchlorinated rainwater by immersion in a 200l flotation tank with a 10l/min water overflow and 10l/min air sparge applied from the bottom of the tank. The bottoms of the leek shank were spray washed (McGeary Spray System Solutions, Dungannon, Ireland; 6x Nozzle DNN114) with rainwater. A pilot-scale brush washer (Niagri Engineering, Norfolk, UK; model Cleanwash 25; Figure 12) was used to clean the carrots

and remove their 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.

Figure 12 A pilot-scale abrasive brush washer was used to clean carrots in 25kg batches and remove the outer surface of the crop

5.3.9 SIMULATED CROP DISTRIBUTION

For simulated wholesale distribution, crops were stored at ambient temperature. For retail distribution storage was at 4^{o}C. For carrots and potatoes, the storage duration was two weeks. For leeks, it was one week. 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 UK. For potatoes, only the post-wash, 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.

5.3.10 CHEMICAL CHARACTERISATION OF SLURRY.

Dry matter was determined by drying in an oven until no further weight loss was observed and ammonia concentration was estimated by chemical titration with 0.05 M sulphuric acid (Hutchison et al., 2005). The pH and conductivity of the slurry were determined directly using a pH and conductivity meter respectively.

5.3.11 RECORDING OF CLIMATIC CONDITIONS

Weather conditions for the field plots were recorded at the University Meteorological Station located 200m 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.

5.4 RESULTS

Sixty two slaughter batches of animals from 36 different farms were examined for *stx* genes over a period of four months from January to April. 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 toxin genes, plating onto the chromogenic media gave rise to two blue-green colored colony morphologies that were identified by biochemical testing to be *E. coli* and by PCR to be serotype O145. The strain was characterised as lacking stx_1, stx_2, *eae*, the H7 antigen and *hylA*. When batches of manure were re-examined immediately before the excreta was used to contaminate each crop, *E. coli* O145 was exclusively determined as the serotype from blue colonies on all three occasions.

The numbers of *E. coli* O145 in each batch of slurry used to contaminate crops was variable and decreased over time (Table 10). The physicochemical properties of the excreta remained similar throughout the course of the study (Table 10), 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 (Figure 13).

Table 10 Concentration of *E. coli* O145 and physicochemical properties of the slurry used to contaminate crops. Results are the mean of five replicates.

Crop	Geometric mean count *E. coli* O145 (log CFU/g)	Dry matter content (% w/w)	pH	Conductivity (mSi/cm)	Ammonium N (mg NH_4-N/kg slurry)
Potato	4.00	7.34	6.88	3.96	988
Leek	3.78	7.22	6.90	3.96	1090

Carrot	3.30	6.98	6.78	4.27	1006

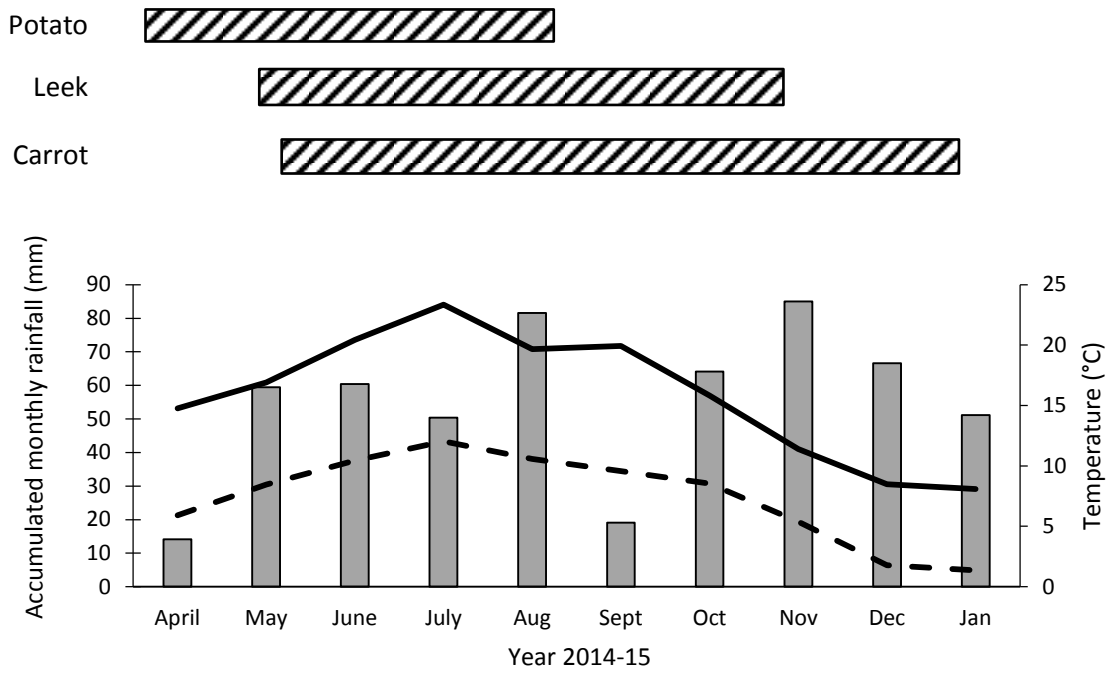

Figure 13 A summary of climactic conditions across the cultivation periods (horizontal diagonal-hatched bars) for potato, leek and carrot. Accumulated monthly rainfall is shown as grey bars and the monthly average of daily maximum and minimum temperatures are shown as solid and dashed lines respectively.

For the slurry-contaminated potatoes, *E. coli* O145 was present at around 2 log cfu/g vegetable at harvest (Figure 16). For the irrigation water treatment, the contamination was lower at around 0.35 log cfu/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=0.051). Both the irrigation water and slurry

treatments released 0.5 log cfu/g and 1 log cfu/g into the wash water respectively (Figure 16). 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/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. After simulated distribution (Figure 14, Figure 15 and Table 11), *E. coli* O145 was isolated only from 2/15 replicates (11 colonies in total) of the slurry treatment and only from the refrigerated retail storage (Figure 16).

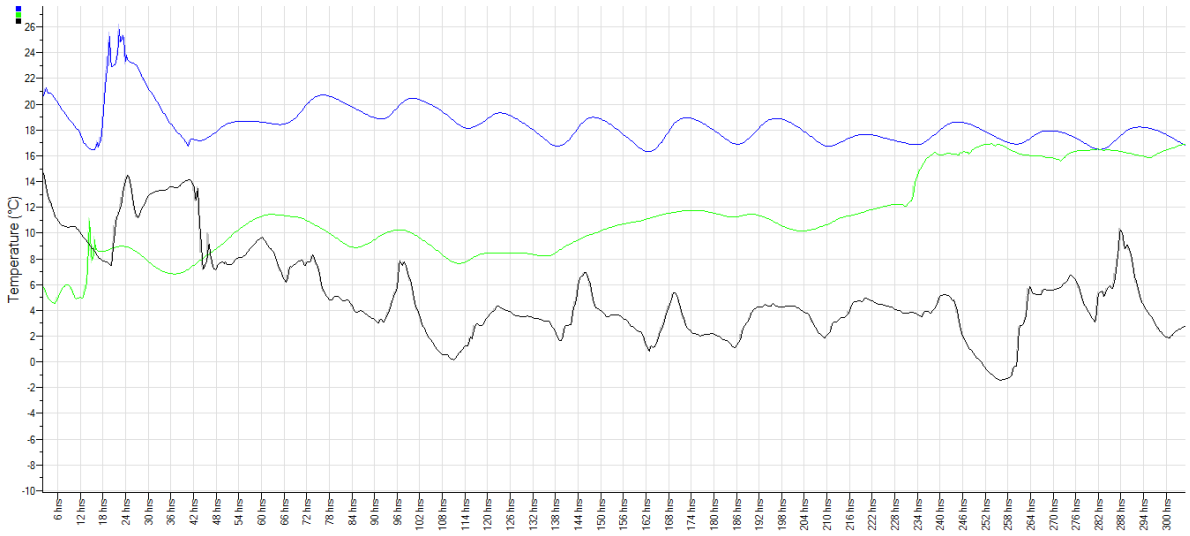

Figure 14 Temperatures experienced by crops during ~14 days simulated wholesale distribution of carrot (black trace), leek (green trace) and potato (blue trace). Time scale on the x-axis is relative and measured from time of harvest.

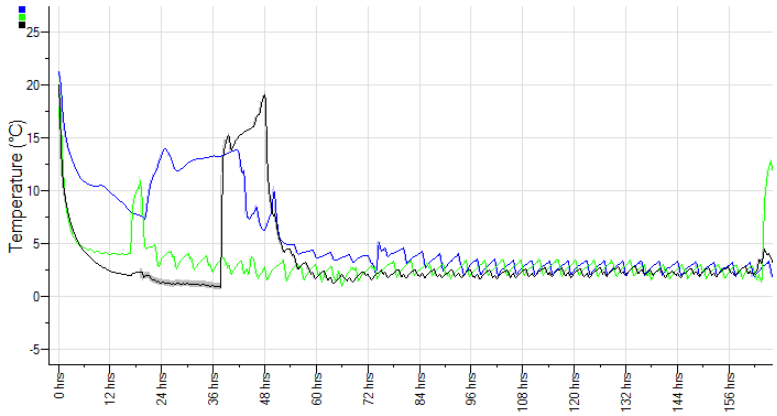

Figure 15 Temperatures experienced by crops during ~7 days simulated retail distribution of carrot (black trace), leek (green trace) and potato (blue trace). Time scale on the x-axis is relative and measured from time of harvest.

Table 11 Average relative humidities during simulated crop distribution through a retail and wholesale supply chain.

Crop	Average humidity (%) during simulated Retail distribution at 4°C	Wholesale distribution at ambient temperature as shown in Figure 14
Potato	91.07	62.23
Leek	90.52	59.93
Carrot	90.87	60.51

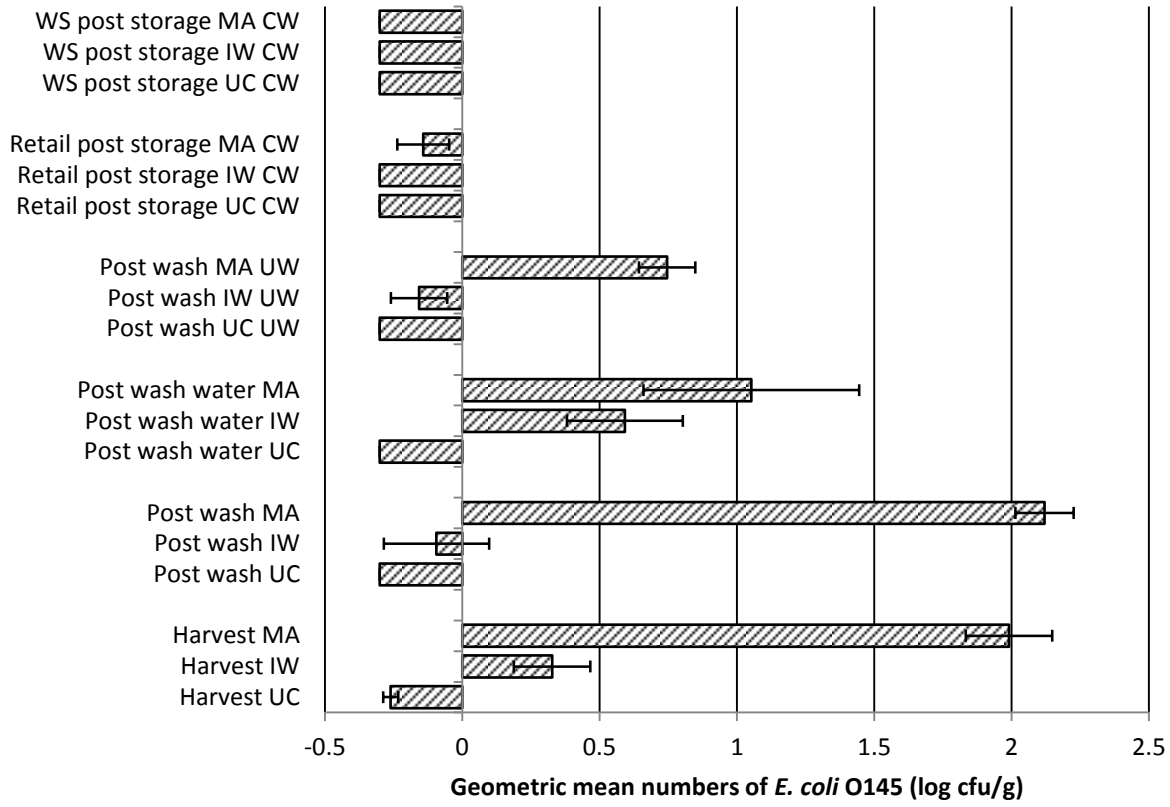

Figure 16 Numbers of *E. coli* O145 on potatoes contaminated one week before harvest with slurry (MA) or irrigation water (IW) and uncontaminated controls (UC). Contaminated potatoes were washed in uncontaminated water (CW) and previously uncontaminated potatoes subsequently washed in the same wash water (UW). The contaminated potatoes were stored under conditions to simulate retail and wholesale (WS) distribution. Error bars are the standard error of the mean log of 15 replicates per treatment.

For the leeks, there were no isolations of *E. coli* O145 from any of the uncontaminated controls. Despite visible fecal 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/g contaminating the crop and 0.4 cfu/g for the irrigation water. 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 $P<0.05$) as a consequence of spraying (Figure 17). As

before, the water used for washing was collected and tested, but did not contain numbers of *E. coli* O145 above the detection limit of the test method. However, leeks washed in the collected water did acquire low levels of *E. coli* O145 contamination (Figure 17). 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 and retail distribution. As before, the highest numbers of cells was observed for the retail distribution, although for the indirectly-contaminated slurry treatment.

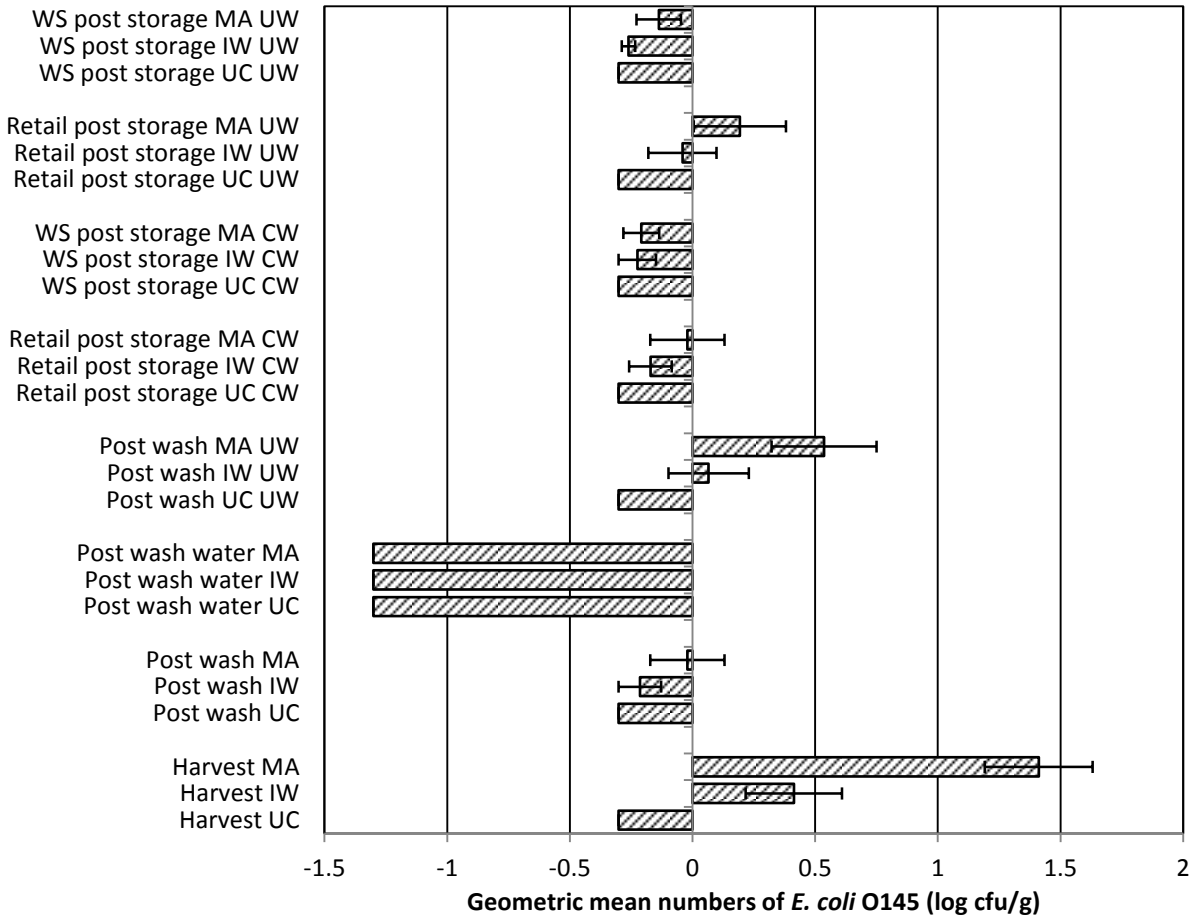

Figure 17 Numbers of *E. coli* O145 on leeks contaminated one week before harvest with slurry (MA) or irrigation water (IW) and uncontaminated controls (UC). Contaminated leeks were sprayed with uncontaminated water (CW) to remove soil adhering to the roots. Previously uncontaminated leeks were subsequently 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 standard error of the mean log of 15 replicates per treatment.

For carrots, there was exceptionally atypical elevated rainfall (Figure 13) for the three 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 one of the fifteen slurry treatment replicates and five of fifteen water treatments, when the water was applied 24h 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 hard soil crust created as a consequence of excessive rainfall followed by rapid drying from intense sunlight or wind prior to harvest.

5.5 DISCUSSION

Animals from 36 different farms were tested for the presence of *stx* genes for this study, with only a single batch of animals testing negative for *stx*2. The slurry contained *E. coli* O145 and enterohemorrhagic strains of the same serotype have been previously-implicated as the cause of foodborne disease associated with fresh produce (29,31). 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-harboring 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., 2004) as a consequence of their age, stage of infection and other factors such as diet (Hutchison et al., 2005).

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 post distribution 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^{o}C or 15^{o}C. *E. coli* O157 did not survive on uncut sprouts at either temperature. However, an important finding of the study was bacterial growth was possible 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^{o}C compared with decline at 4^{o}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 et al., 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 for 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 the UK 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 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 increasing 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 eight log

cfu/g pathogenic *E. coli* (Hutchison et al., 2004). 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.

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7 MAIN PROJECT FINDINGS AND THE CONSEQUENT POTENTIAL FOR FURTHER STUDIES TO BETTER-PROTECT CONSUMERS

An important finding from this study was that cross-contamination between consecutively-washed batches of vegetables can occur via recycled wash water. Previously, it had been reported that cross-contamination may have occurred when rodent carcasses had contaminated washers. In order to effectively control the cross-contamination hazard from crop washing, more information is required to determine how many consecutive batches of vegetables the cross-contamination extends over. In addition, there are other considerations likely to include total tank volume and the rate of water overflow. Ideally, it would be of benefit to fit a set of equations to practically-generated results and to use such a model as the basis of advice to growers on safe washing practices. One important consideration for any advice would be that vegetable washers are rarely cleaned, even though there is commonly a build-up of detritus at the bottom of washers and wash tanks. Since this material also has the potential to re-contaminate the wash water, it would also be beneficial to determine the length of time that enteric pathogens can survive in tank detritus.

The results of this study make it apparent that current washing methods may not be optimally-suited to removing soil and microbial contamination from crops. Potentially, further studies could focus on new methods of pathogen and detritus removal. Since contaminated wash water containing pathogen is capable of cross-contaminating to uncontaminated batches of root vegetables, new cleaning protocols should make adequate account of the risks of process water reuse and ways to effectively control these hazards. A likely key focus of future efforts in this area would be water treatments that effectively remove or otherwise contain pathogenic micro-organisms.

Another interesting finding for this work was that retail refrigerated vegetables may help to preserve enteric pathogens. It would also be beneficial to establish the cause for the observed relatively higher survival of *E. coli* O145 through a simulated retail chain compared to a wholesale chain. In the discussion of the washing studies we consider a potential role for diurnal temperature cycles stressing bacteria. There are reports in the literature that storage humidity can have an impact on pathogen survival with credible reports of proliferation fuelled by nutrients leaked from injured plant tissue. Currently, the reason why there was enhanced survival as a consequence of retail distribution is not definitively known and so our discussions of it are presently quite speculative. It is likely that in addition to temperature and humidity, packaging type and during-storage/distribution changes to the gas composition inside packaging as a consequence of residual metabolism may influence the fate of enteric pathogens.

We noted a high prevalence of *stx* genes and potentially pathogenic *E. coli* in the herds surveyed. For the purposes of this work, herds were excluded on the basis of a *stx* gene amplification (because that is the primary criteria for a containment-level 3 *E. coli* as determined by the Advisory Committee for Dangerous Pathogens). Consequently, there was no information gathered by the work on the serotypes that contained the *stx* genes. A good proportion of the isolates have been stored and would be available for further serotyping by PCR were the FSS interested in horizon scanning to determine if there were emergent potentially-problematic serovars of *E. coli* in bovine populations. Alternatively, it would be relatively straightforward to repeat the slaughterhouse monitoring undertaken in Somerset at a Scottish slaughterhouse to determine if there was a similarly high prevalence North of the border. One of the contractors for the current study has exceptionally good links with the UK red meat industry that would facilitate a straightforward set up of monitoring.

This study investigated the impact of poor sanitary practices and the use of a single design of field toilet during crop harvest. However, there are other designs of field latrine, including those with internal hand washing facilities that are likely to reduce the likelihood of fomites. Thus, there is the potential for the identification of factors that improve hand hygiene in field workers on fresh produce farms. Future work could assess the hygiene of facility design, sanitisers (including active agent and concentration) and novel training methods/approaches of users to washing. Recent EU legislation has capped the concentration of some commonly-encountered antimicrobials found in hand washing solutions (e.g. quaternary ammonium salts). In addition, the information generated by this study is suitable for use in a quantitative risk assessment model. One additional approach for taking this work forward would be to use the data to determine the benefits of improved hand washing in terms of reduced transfer risk of enteric microorganisms to crops.

8 APPENDIX 1 RTFP GUIDANCE ON EXCRETA USE

8.1 EC.10 - SAFE APPLICATIONS TO LAND – 2014 UPDATE (FINALISED BUT NOT YET DEPLOYED)

All applications to land must be carried out in accordance with the 'Safe applications to land matrix' and legislation. Environmental permits or exemptions must be held where applicable. The Environment Agency website has information on spreading waste on land which may be helpful.

Note: producers should always check with buyers to ensure that any applications of sludge, compost, digestate and other materials originating outside the farm are acceptable to customers.

8.2 SEWAGE SLUDGE

Untreated sewage sludge has not been permitted on any agricultural land since 2006.

Treated sewage sludges can only be used under strictly controlled conditions. Prior to application the soil must be tested by the sludge supplier. Applications of sewage sludge to land must be in accordance with supplier's instructions (i.e. the way the sludge has been treated may affect where and when the sludge can be applied).

Two types of treated sewage sludge are permitted by the scheme:

Conventionally treated sludge - has been subjected to defined treatment processes and standards that ensure at least 99% of pathogens have been destroyed. The most common form of treatment is anaerobic digestion.

Enhanced treated sludge – - will be free from Salmonella and will have been treated so as to ensure that 99.99% of pathogens present in the original sludge have been destroyed.

8.3 EXCRETA – FRESH, STORED OR TREATED

Batch storage of solid livestock wastes and slurries for at least 6 months (that is with no additions of fresh material made to the store during this period) or 'active' treatment, are effective methods of killing pathogens. Composting of solid manure is a particularly effective method of controlling microbial pathogens, but for best results the process needs to be

actively managed. The manure should be treated as a batch and turned regularly (at least twice within the first 7 days) either with a front-end loader or preferably with a purpose-built compost turner. This should generate high temperatures over a period of time (e.g. above 55°C for 3 days) which are effective in killing pathogens and this temperature should be monitored. Allow the compost to mature as part of the treatment process. The whole process should last at least 3 months.

Lime treatment of slurry (addition of quick lime or slaked lime to raise the pH to 12 for at least 2 hours) is an effective method of inactivating bacterial pathogens. Allow the slurry to mature as part of the batch treatment process for at least 3 months prior to land spreading.

8.4 COMPOST, DIGESTATES AND OTHER RECYCLED MATERIALS

It is recommended that digestates and composts sourced from external contractors for application to land have been produced to the relevant PAS specification (PAS 110 for digestate, PAS 100 for compost) and are applied following the associated Quality Protocol. It is a requirement that anaerobic digestate is pasteurised if it is being sourced from outside your own farm. The specifications and Quality Protocols provide safeguards on the feedstock materials, the processing stages and end product quality.

For all fruit and vegetable crops information about the feedstock should be built into your risk assessment. Particular hazards might include potential foreign bodies arising from contamination of feedstocks with glass, metal or hard plastic especially when the material is used on land used for potatoes and root crops.

8.5 SAFE APPLICATIONS TO LAND MATRIX

		• Anaerobic Digestate (PAS 110 and pasteurised);	• Anaerobic Digestate (PAS 110 , not pasteurised) • Anaerobic Digestate (not assured) • Raw manure/ slurry	• Composts (including PAS100 and non-assured; green and green/food) • Treated manure/ slurry	• Conventional treated sewage sludge	• Enhanced treated sewage sludge	• Land where immediate previous use has been as grazing land
Fresh produce	Cat 1	Must be applied before drilling/ planting	Not within 12 months of drilling/planting	Any time before drilling/planting[1]	Not within 30 months of harvest	Not within 10 months of harvest	Not within 12 months of drilling/planting
	Cat 2	Must be applied before drilling/ planting	Not within 12 months of harvest and also at least 6 months before drilling/planting	Any time before drilling/planting[1,2]	Not within 30 months of harvest	Not within 10 months of harvest	Not within 12 months of harvest and at least 6 months before drilling/planting
	Cat 3	Must be applied before drilling/ planting	Must be applied before drilling/ planting	Any time before drilling/planting[3]	Not within 12 months of harvest	Not within 10 months of harvest	Any time before drilling/planting

Notes

1. Target of zero and absolute limit of <0.1% (m/m dry weight) glass must be achieved
2. Green compost (PAS100 assured) may be applied as mulch

9 APPENDIX 2. SURVEY QUESTIONS

FSA study FS101052 root crop questions Date……

Business……

FYM

1. Do you ever apply raw or composted or treated FYM to cropping areas with growing crop?
2. When?
3. Control measures?

Irrigation

4. When is the latest you would irrigate a crop (days/weeks before harvest)?
5. Do you ever irrigate to soften the soil before harvest?
6. Are there any requirements for water quality?

Supply chain conditions

7. Outline supply chain steps and target temperatures
    a. before packing
    b. after packing

Transport conditions

8. Do conditions differ for MR vs wholesale?
    a. MR
    b. Wholesale

Washing

9. Do you wash or rinse product before packing?
10. How?
11. What water do you use?
12. How do you know it is safe?

Cooling

13. Do you cool product?
14. How?
15. What target temperatures?
16. Do you log temperatures in supply chain? (where)
17. What happens after cooling
    a. RDC temperature regime?
    b. Retail display?

10 APPENDIX 3. RECORD OF THE LITERATURE IDENTIFIED BY THE SYSTEMATIC SEARCH

ID	Author	Year	Title	Database	Journal Details	Where?	When?	Crop?
1	BIOHAZ panel	2013	Scientific opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment	EFSA	EFSA Journal 11, 4, 3138-3244	Mostly Europe	2007-2010	
						UK	2011	Raw leeks and potatoes
2	BIOHAZ panel	2013	Scientific Opinion on the risk posed by pathogens in the food of non-animal origin. Part 1 (outbreak analysis and risk ranking of food/pathogen combinations)	EFSA	EFSA Journal 11, 1, 3025-3163	Mostly Europe	2007-2011	
						Sweden	2008	Carrots
						Sweden	2011	Onion
						UK	2011	Raw leeks and potatoes
						USA		Raw Carrots
						UK	(pre 2007)	Raw potatoes

3	CFIA	2007	CFIA: Health Hazard Alert - Los Angeles Salad Company Baby Carrots may Contain Shigella Bacteria	Reference lists	http://www.marketwired.com/press-release/cfia-health-hazard-alert-los-angeles-salad-company-baby-carrots-may-contain-shigella-761894.htm [Accessed 2007]	Canada	Aug 2007	Baby carrots
4	Chapman et al	1997	An outbreak of infection due to verocytotoxin-producing Escherichia coli O157 in four families: the influence of laboratory methods on the outcome of the investigation	Find it @ Harper	Epidemiology and Infection 119, 113-119	Rotherham / Bristol UK	Oct/Nov 1995	Potato
5	Cook et al.	1995	Scallions and shigellosis: a multistate outbreak traced to imported green onions	Reference lists	Proceedings of the 44th Annual Conference of the Epidemic Intelligence Service. 1995 Centers for Disease Control and Prevention Atlanta, GA p 36			

							Per a year	
6	Cooley et al	2007	Incidence and Tracking of Escherichia coli O157:H7 in a Major Produce Production Region in California	Find it @ Harper	Plos ONE 2, 11, e1159	USA	Between 1982 and 2002	
7	Doan and Davidson	2000	Microbiology of potatoes and potato products: A review	Web of Knowledge	Journal of Food Protection 63, 5, 668-683	East Anglia UK	1985	Potatoes
8	Gaynor et al.	2009	International foodborne outbreak of Shigella sonnei infection in airline passengers	Reference lists	Epidemiology and Infection 137, 3, 335-341	Hawaii	2004	Raw carrots
							Per a year	
9	Gould et al	2013	Surveillance for foodborne disease outbreaks - United States, 1998-2008	Web of Knowledge	MMWR Surveillance Summaries 62, 2, 1-34	USA	1998-2008	
						Worldwide		
10	Harris et al	2003	Outbreaks Associated with Fresh	Reference lists	Comprehensive Reviews in Food Science and Food Safety 2, 78-89	Multistate	1994	Green

			Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce			USA		Onions from Mexico
						New Mexico	1989	Onions and lettuce
						Washington USA	1997	Green Onions
						USA	1989	Lettuce, Tomatoes, Onion
						Rhode Island, New Hampshire	1993	Shredded carrot
						Midwestern USA		Green onions
11	HPA	2011	National increase in vero cytotoxin-producing E. coli O157 infection in England and Wales	HPA	Health Protection Report 5, 6 Published on: 11 February 2011	UK	Dec 2010	Unknown at this point
12	HPA	2011	National increase in VTEC O157, PT8:	HPA	Health Protection Report 5, 39 Published on: 30 September 2011	UK	Dec 2010 - Feb 2011	Raw leeks and potatoes

			conclusion of investigations					
13	HPA	2011	UK E. coli O157 outbreak associated with soil on vegetables	HPA	www.hpa.org.uk/NewsCentre/NationalPressReleases/2011PressReleases/110930Ecolioutbreakassocwithsoilonveg/ [Accessed 13/02/2014] Published on: 30 September 2011	UK	Dec 2010 - July 2011	Raw loose leeks and potatoes
14	HPA	????	Epidemiology of VTEC in England & Wales	HPA	www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/EscherichiaColiO157/EpidemiologicalData/ [Accessed 13/02/2014]	UK	Dec 2010 - July 2011	Raw loose vegetables
15	Kozak et al	2013	Foodborne outbreaks in Canada linked to produce: 2001 through 2009	Reference lists	Journal of Food Protection 76, 1, 173-183	Canada (AB, BC)	Aug 2007	Mini carrots
						Canada (AB, BC, NS, ON)	July 2009	Onion sprouts
						Canada (ON)	Oct - Nov 2008	Spanish Onions
16	Mandrell & Brandl	2004	Campylobacter species and fresh produce: outbreaks, incidence and	Reference lists	Book - In R. Beier, R. Ziprin, S. Pillai, and T. Philips (ed.), Pre-harvest and post-harvest food safety: contemporary issues and future directions. Page 59-72	Multiple	1990-1999	Produce
						US (Connecticut)	Aug 1997	Sweet potato

			biology					
17	MMWR	1994	Foodborne Outbreaks of Enterotoxigenic Escherichia coli — Rhode Island and New Hampshire, 1993	Reference lists	MMWR Morbidity and Mortality Weekly Report 1994 43, 81-88	Rhode Island, New Hampshire	March 1993	Carrots
18	Morgan et al.	1988	First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing Escherichia coli O 157. H7 in the UK	Reference lists	Epidemiology and Infection 101, 01, 695-701	East Anglia UK	July 1985	Potatoes
19	NBPSDHU	2009	Investigative Summary of the Escherichia coli Outbreak Associated With A Restaurant In North Bay, Ontario: October to November	Reference lists	North Bay: NBPSDHU	Canada (ON)	Oct - Nov 2008	Onions

			2008					
20	Pebody et al	1999	An international outbreak of Vero cytotoxin-producing Escherichia coli O157 infection amongst tourist; a challenge for the European infectious disease surveillance network	HPA	Epidemiology and Infection 123, 2, 217-223	Tourists to Fuerteventura	March 1997	
21	Rangel et al	2005	Epidemiology of Escherichia coli O157:H7 Outbreaks, United States, 1982–2002	Reference lists	Emerging Infectious Diseases 11, 4, 603-609	USA	Per year	
							1982-2002	
22	Sivapalasingam	2004	Fresh produce: A growing cause of outbreaks of foodborne	Find it @ Harper	Journal of Food Protection 67, 10, 2342-2353	USA	1973-1997	carrot

			illness in the United States, 1973 through 1997					