Assessment of foodborne pathogen presence in the peach supply chain and its potential risk to the end consumer

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Highlights

•No *Salmonella* Typhimurium was detected from samples (n = 428).

•Livestock that frequented on farm water sources led to *E. coli* O157:H7 contamination.

•Produce contamination was associated with poorly enforced hygiene and sanitation.

•A resultant low-to-medium consumer risk was calculated.

•Proper implementation of food safety management systems prevents contamination.

ABSTRACT

Peaches are popular, nutritious and widely consumed. Being a tree crop, it is considered a low risk fruit, with no direct water contact, and no previous foodborne disease outbreaks associated with its consumption. However, in 2014 the pioneer association between stone fruit and a foodborne illness was reported, linking *Listeria monocytogenes* to stone fruit. This highlights the need for better understanding of risk associated with contaminated fresh stone fruit, in order to implement adequate preventative measures. No information is available on the presence of foodborne pathogens on peaches in the supply chain. A case study approach was therefore followed to assess foodborne pathogen presence on the farm, focusing on the impact of irrigation water, facility sanitation and hygiene by collecting various fruit and environmental samples (n=428). This study demonstrates the effectiveness of integrating basic microbial testing with safety management and risk assessment tools that can be collectively used to improve the food safety management system. No *Salmonella* Typhimurium was detected from samples, however, *Escherichia coli* O157:H7, *Listeria* spp. and *Staphylococcus aureus* were detected on fruit and environmental samples. Despite the GlobalG.A.P. certification status of the farm, livestock frequented water sources which lead to *E. coli* O157:H7 contamination. This conclusion was based on positive detection of foodborne pathogens from the water sources and subsequent removal of livestock which resulted in a definite decrease in pathogen detection. A number of *E. coli* O157:H7 and *S. aureus* were detected during the second year of monitoring from environmental samples and it was observed that the personal hygiene and facility sanitation was not adequately enforced. Based on feedback given to the farmer, enforcement was improved and a definite decrease in foodborne pathogens was observed in the following sampling cycle. Areas of risk that were still identified following the fourth year of monitoring included the water source used for irrigation and poor sanitation in the production and processing facilities. Limited foodborne pathogen prevalence on peaches over the full study period as well as the extended export supply chain at controlled temperatures resulted in low-to-medium calculated consumer risk. The correct and meticulous implementation of integrated and holistic pre- and post-harvest food safety management systems is therefore essential to prevent produce contamination, reduce the consumer risk and therefore ensure overall product safety.

ABBREVIATIONS:

CDC – Centre for Disease Control and Prevention

EFSA – European Food Safety Authority

EU – European Union

HSMS – Horticultural safety management system

RRR – Risk Ranger ranking

SA – South Africa

UK – United Kingdom

USA - United States of America

WHO – World Health Organisation

1. INTRODUCTION

Escherichia coli O157:H7, *Listeria monocytogenes* and *Salmonella* spp. are well described foodborne pathogens, having been associated with several disease outbreaks on fresh produce. Stone fruit are not traditionally considered a high-risk product due to production practices. However, in recent years' commodities previously not associated with foodborne disease outbreaks are becoming implicated, as was the case with the recent illnesses associated with *L. monocytogenes* on stone fruit (Jackson et al., 2015) and caramel apples [Centre for Disease Control and Prevention (CDC), 2015]. Due to extensive global distribution of fresh produce, outbreaks are not confined to the country of origin, as was the case in the June 2011 *E. coli* O104:H4 outbreak associated with contaminated sprouts, imported from Egypt, which affected 16 countries including 14 countries in the European Union (EU) as well as the United States of America (USA) and Canada [European Food Safety Authority (EFSA), 2011; World Health Organisation (WHO), 2011]. Food safety assurance is therefore of global importance.

Peaches are packed and consumed raw without any decontamination, it is therefore essential to prevent contamination. Preharvest contamination can occur through contact with contaminated soil, irrigation water and improperly composted manure (Beuchat, 2002). The presence of animal farming in fields adjacent to cultivation areas (Gruszynski et al., 2014; Kilonzo et al., 2013) or cultivation in fields which are historically used for animal rearing (Tauxe et al., 1997) could lead to the spread of persistent foodborne pathogens. Postharvest

contamination usually occurs through contact with contaminated harvesting equipment, handlers and contact surfaces (Beuchat, 2002; Warriner et al., 2009).

Food safety standards and systems that target zero microbial contamination have been developed specifically for the food processing industries. However, the general philosophy is that zero tolerance is not realistic in a preharvest environment. Since microbiological analysis of food is time consuming, the International Commission on Microbiological Specification for Foods stated that *"Good Agricultural Practices and acceptable hygienic farming practices are more important than microbiological testing of food samples before selling"* (Food Science Australia, 2000). Good agricultural practices have been standardly adopted by the fresh produce industry and GlobalG.A.P. has become a global benchmark for exported produce. The use and implementation of effective food safety management systems should therefore provide additional food safety confidence.

The overall aim of this study was to determine 1) hazard presence, 2) on-farm risk areas and 3) end-consumers risk. The presence of *E. coli* O157:H7, *Listeria* spp*., Salmonella* Typhimurium and *Staphylococcus aureus* in water, on pre- and post-harvest fruit and pre- and post-harvest contact surfaces was determined. The overall risk and risk areas as well as control were assessed using a self-diagnostic tool (Kirezieva et al., 2013; Kirezieva, Luning, Jacxsens, & Uyttendaele, 2015) and an easy-to-use semi-quantitative risk assessment tool (Ross & Sumner, 2002).

2. EXPERIMENTAL
2.1. Study site and sampling strategy

An integrated commercial export farm managed according to industry guidelines and GlobalG.A.P. certified in the Limpopo Province, South Africa (SA) was selected as the site. This farm cultivated peaches, maize and citrus, in addition, the farm engaged in compost production, game and cattle livestock farming. Peaches (*Prunus persica* L.) were grown in uncovered fields, drip irrigated and conventional pesticide application with water sourced from on-farm collection dams filled with water from the Lephalala River. The farm's packhouse was located near the orchards (within 15km). Peaches were mainly exported to the UK and EU markets. Precipitation data was gathered from the South African Weather Services (2012).

A total of 428 samples were collected and analyzed during four consecutive growing seasons (Table 1; Table 2). During seasons 1 and 4 the farm was visited once during the peak harvesting period, and during seasons 2 and 3 the farm was visited twice, once during

Table 1: Weather data summary (South African Weather Services, 2012).

Season	1	2			3			4
Period	At harvest	Last spray	Between last spray and at harvest	At harvest	Last spray	Between last spray and at harvest	At harvest	At harvest
Date	04/01/2007	29/09/2007		10/10/2007	05/11/2008		09/12/2008	04/12/2009
Rainfall	24.4 mm	11.7 mm	43 mm	43 mm	5.6 mm	89.6 mm	8 mm	29.8 mm
Day temperature	32.4°C	23.9°C		25.9°C	27.5°C		34.6°C	32°C

the last spray period (one month prior to harvest) and once during the harvest period (Table 2).

Table 2: Sample collection summary

Time of Season	Type of sample	Total number of samples take per sample type	Location	Season collected		
Spray	Water	30	Holding Dam 1 (n=10)	2	3	
			Holding Dam 2 (n=10)	2	3	
			River (n=10)	2	3	
Harvest		65	Holding Dam 1 (n=15)	1	2	4
			Holding Dam 2 (n=15)	1	2	4
			River (n=15)	1	2	4
			Pesticide fill point (n=10)	2	4	
			Washwater (n=10)	2	4	
	Fruit	60	Orchard (n=40)	1	2	
			Before Pack (n=10)	2	4	
			After Pack (n=10)	2	4	
	Contact Surfaces	273	Hands (n=57); Environmental (n=216) *	2	4	

* Hands: pickers (n=20), sorters (n=17), packers (n=20); Environmental: crates (n=26), taps (n=32), sortline (n=18), packline (n=20), floors of packhouse (n=60), walls and floors coldroom (n=60)

Water samples (n=90; 5 x 1l per site) were collected using a telescopic water sampling arm (1.5 m) (Table 2). Water was collected from holding dams, river, pesticide fill point and at the packhouse. *Prunus persica* L. cv. Oom Sarel samples (n=60) were collected from the orchard (preharvest) and from the packhouse (postharvest). In the orchard, fruit were collected from five trees from a single orchard block, at four points per tree and three fruit per point. Location of the trees were recorded and visited in subsequent seasons. Five fruit samples of three fruit were randomly collected before and after packing. Transport swabs with Amies medium (Lasec, Johannesburg, SA) were wetted in the transport medium and used to sample a 25 cm^2 area of all contact surfaces according to standard procedures for environmental swab sampling (Public Health England, 2013). Preharvest samples in the orchard included; hands of pickers and crates. Postharvest samples in the packhouse included, hands of workers, processing line (sort- and pack-line), taps (bathroom and wash station) and floors. In the cold room; floors and walls were sampled. All packhouse equipment was recorded as being cleaned daily with water and soap. All samples were transported on ice, stored refrigerated and processed within 24 h (water) to 48 h (fruit) and swabs were processed within one week after collection.

2.2. Hazard characterization

Water samples (100 ml) were processed for Colilert-18[®] (Dehteq, SA) analysis as per manufacturer's instructions and incubated at 37°C. Results were recorded and the most probable number (MPN) of coliforms and *E. coli* were determined.

Further to this water samples (1000ml), fruit sample rinsates and swab samples were analysed for the presence of *E. coli* O157:H7, *Listeria* spp., *Salmonella* Typhimurium and *S. aureus* using molecular PCR detection. Water samples (1000 ml) used for molecular detection were filtered through a 0.45 µm nitrocellulose filter. Fruit samples were washed in 500 ml quarter strength Ringer's solution (Merck, SA) amended with 0.02% Tween-80 in an ultrasonic bath for 5 min and subsequently filtered through a 0.45 µm nitrocellulose filter.

Filters and swabs were analyzed by placing each into 9 ml tryptone soy broth, shake incubated (100 rpm) at 37°C for 48 h followed by DNA extraction and PCR with negative control, as outlined Standing et al. (2013) targeting the *UidA* gene of *E. coli* O157:H7 (F: 5'-GCG AAA ACT GTG GAA TTG GG-3'; R: 5'-TGA TGC TCC ATA ACT TCC TG-3'; 252bp amplicon) (Cebula, Payne, & Feng, 1995), the listeriolysin O gene of *Listeria* spp. (F: 5'-AGC TCT TAG CTC CAT GAG TT-3'; R: 5'-ACA TTG TAG CTA AGG CGA CT-3'; 450bp amplicon) (Thomas, King, Burchak, & Gannon, 1991), the long polar fimbriae D gene for the detection of *Salmonella* Typhimurium (F: 5'-TTG CCG GTG GTA CTG ATA GG-3'; R: 5'-TTG CCG GTG GTA CTG ATA GG-3'; 787 bp amplicon) as well as *Staphylococcus aureus* nuclease gene (F: 5'-TTG CAT ATG TAT GGC AAT TGT T-3'; R: 5'-TTT TGC TTG TGC TTC ACT TTT TC-3'; 655 bp amplicon) (Standing et al., 2013). For positive control purposes, PCR reaction mixtures containing DNA extracted from artificially inoculated peaches were used, peaches were artificially inoculated with 10^2 cfu/fruit of *E. coli* O157:H7 (ATCC 35150), *L. monocytogenes* (ATCC 19115), *Salmonella* Typhimurium (ATCC 14028) and *S. aureus* (ATCC 12600). PCR amplicons were visualized following gel electrophoresis on a 2% (w/v) agarose gel containing 0.1 % (v/v) Ethidium Bromide.

Statistical analysis was performed on the most probable numbers, using GenStat for Windows Discovery Edition 2 Software (VSN International Ltd., Lawes Agricultural Trust, UK). A one-way analysis of variance (no blocking) was used to determine the significant differences between times of sampling as well as between sampling location. Means were analyzed using Fischer's protected t-test least significant difference at a 1% level of significance.

Table 3: Information used for the Semi-Quantitative Risk Assessment

	Risk ranger Question	Details		References
1.	Hazard severity	*Escherichia coli* O157:H7 classified as a moderate hazard *Listeria monocytogenes* classified as a moderate hazard *Staphylococcus aureus* classified as a minor hazard		Mataragas, Skandamis, & Drosinos, 2008; Sumner, Ross, Jenson, & Pointon, 2005
2.	Population susceptibility	General population [European Union (EU) and United Kingdom (UK)] Susceptible population (EU and UK)		
3.	Frequency of consumption	South Africa exported 12,187 tonnes of peaches in 2011, 5,427 into UK and 2,446 tonnes peaches in the EU		Department of Agriculutre Forestry and Fisheries, 2012
		European regulations (EU 543/2011) state peaches between 65 and 300g (average: 182.5g) can be sold in the EU and UK		The European Comission, 2011
		Estimated South African peaches entering UK: 29.82 million (5,427 tonnes) Estimated South African peaches entering EU: 13.44 million (2,446 tonnes)		Department of Agriculture Forestry and Fisheries, 2012
		Therefore 5% of EU population consume one South African grown peach per year Therefore, 25% of UK population consume 2.5 South African grown peaches per year		Calculated based on current study
4.	Portion of population consuming the product	Therefore 5% of EU population consume one South African grown peach per year Therefore, 25% of UK population consume 2.5 South African grown peaches per year		Refer to question 3
5.	Size of the population	General population	United Kingdom: 62.3 million European Union: 425.25 million	Office of National Statistics, 2010
		Susceptible population	United Kingdom: 103 700 (0.25% of general population) European Union: 850 838 (0.2% of general population)	
6.	Probability of contamination	Current study data used		Current study
7.	Effect of processing	Effect of international supply chain: *E. coli* O157:H7 demonstrated a 3 log reduction which was considered as a "usually eliminates" *L. monocytogenes* and *S. aureus* demonstrated a 2 log reduction which was considered "slight reduction"		Collignon and Korsten, 2010
8.	Potential for recontamination	*E. coli* O157:H7 and *L. monocytogenes* were considered as no post-processing contamination. *S. aureus* due to handling was considered to be 5% based on the environmental sample hazard characterisation in this study.		Current study

	Risk ranger Question	Details	References
9.	Effectiveness of post-processing control system	Well-controlled	Current study
10.	Increase in post-processing contamination level to cause infection	*E. coli* O157:H7 infectious dose: 10 to 100 cfu. Therefore, for the general population 100 cfu and for the extremely susceptible 10 cfu. *L. monocytogenes* infectious dose ranges between 100 and 1000, therefore for susceptible persons the infectious dose was considered 100 cfu and for the general population was 1 000 cfu. T Toxin production occurs if *S. aureus* population exceeds 100 000 cells	Feng, Weagant, & Jinneman, 2011
11.	Effect of food preparation before eating	Due to lack of data: assumed a 2 log as in the reduction in *L. innocua* titres on apples and tomatoes following a rinse and rub under cold running water. Therefore, it was assumed that all pathogens would be "usually eliminated" as contaminating titres were assumed to be low.	Kilonzo-Nthenge, Chen, & Godwin, 2006

2.3. Horticultural safety management system assessment

Possible risk areas and control activities on the farm were determined using a self-diagnostic tool (Kirezieva et al., 2013). Briefly, the tool was designed to assess the riskiness of a production system's output by taking the system design, operation and specific context in which the system operates into account. The tool assessed the contextual factors affecting the design and operational activities (Kirezieva et al., 2013) and the effectiveness of the core control and assurance activities (Kirezieva et al., 2015). Contextual factors were assigned a rating from 1 (lowest risk) to 3 (highest risk). Control activities and assurance activities were assessed and rated from 1 (lowest performance) to 4 (highest performance). The final system output gave an indication of the level of the system as well as the company's understanding of its own performance (Kirezieva et al., 2013). The information was gathered by conducting an individual interview with the farm managing director.
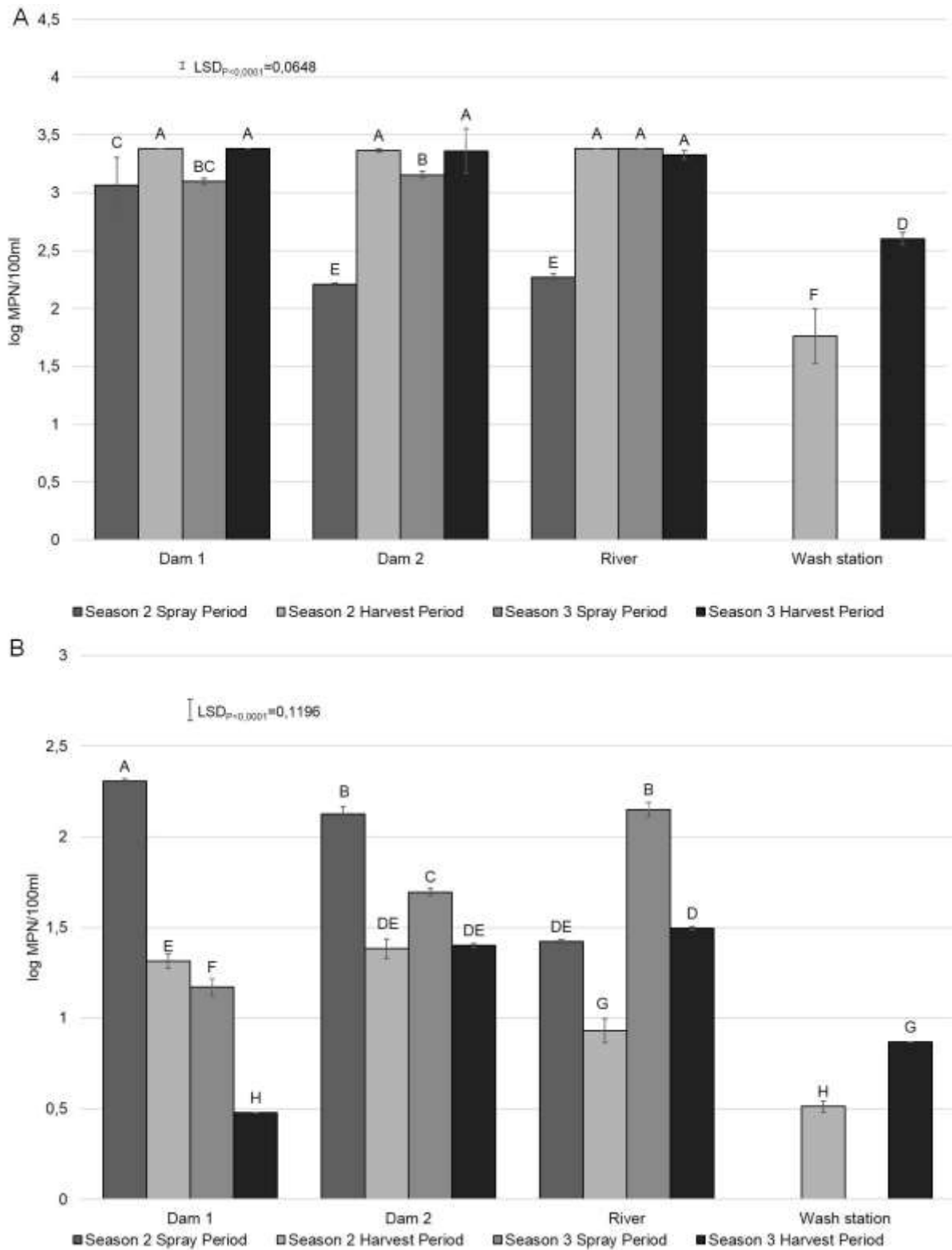
2.4. Risk characterization

Salmonella Typhimurium was not included in the semi-quantitative risk assessment, as no samples tested positive. The Risk Ranger (Ross and Sumner, 2002), selected as an instrument due to its availability and ease of use, classifies different variables to determine risk. Factors used for the analysis are summarized in Table 3. Risk was characterized as low when the Risk Ranger ranking (RRR) was less than 32, medium when the RRR was between 32 and 48, high when the RRR was between 48 and 60 and very high if the RRR exceeded 60.

3. RESULTS

3.1. Observations, recommendations and sequence of events

Each field visit was followed by a report outlining hot spots and areas of improvement for implementation. Livestock were observed to be roaming freely on the farm, with Holding Dam 1 being frequented as a watering hole. Upon sampling in season 2, during the pesticide spray period it was observed that the banks of Holding Dam 1 were littered with livestock feces. Following that visit 43 mm of rain fell (Table 1) which resulted in the rise of the dam's water, subsequently the previously exposed and contaminated banks were covered, resulting in the feces mixing with water in the dam. Following season 2's observations and analysis, improvements to the food safety management system and the farm's agricultural practices were implemented. Resulting in the removal of all livestock from the peach production and packaging areas as well as increased cleaning efficiency and properly enforcement of hygiene and sanitation programs, which included food safety follow-up

training for workers. Following season 4, the HSMS was assessed and risk characterization conducted.

Figure 1: Total coliform (A) and *Escherichia coli* (B) log MPN/100ml counts of water samples

Error bars represent the Standard Error. Letters above bars that are the same indicate no significant difference (*P<0.0001*). LSD represents the least significant difference.

3.2. Hazard characterization

Coliform concentrations were higher during the harvest period when compared to the spray period for Holding Dam 1 and 2 for both season 2 and 3, and coliform counts of the River higher in season 2 with no significant difference in season 3 (Figure 1A) ($p<0.0001$). Given that the WHO (2006) and Department of Water Affairs and Forestry (1996) recommend that *E. coli* counts must not exceed 1,000 counts per 100 ml for the safe use of grey water for unrestricted drip irrigation, all *E. coli* counts in water samples were within both guideline limits (Figure 1B). In contrast to coliform counts, *E. coli* counts were highest during season 2's spray period for Holding Dam 1 and 2, with *E. coli* counts for the river highest during season 3's spray period (Figure 1B).

Table 4: Sample result summary of *Escherichia coli* O157:H7, *Listeria* spp and *Staphylococcus aureus* present in or on water, fruit or contact surfaces.

Pathogen detected	Point of Contamination		No. samples contaminated	Season of contamination
Escherichia coli O157	Water	Holding dam 1	3	Season 2
		River	2	Season 2
	Fruit	Before packing	1	Season 2
	Contact Surfaces	Pickers crates	4	Season 2
		Washstation Taps	1	Season 2
		Sortline	1	Season 2
		Packers' Hands	1	Season 2
		Packhouse Floors	10	Season 2
		Cold Room Walls	1	Season 2
		Cold Room Floors	1	Season 4
Listeria spp.	Fruit	Fruit on trees	1	Season 1
	Contact Surfaces	Pickers' hands	1	Season 2
		Cold room floors	1	Season 4
Staphylococcus aureus	Fruit	Fruit in packhouse	1	Season 2
	Contact Surfaces	Pickers' hands	3	Season 2
		Washstation Taps	1	Season 2
		Cold room floors	1	Season 2
		Packers' hands	1	Season 4

Samples that tested positive for *E. coli* O157:H7, *Listeria* spp. and *S. aureus* are outlined in Table 4. *Salmonella* Typhimurium was not detected from water, fruit or contact surfaces (n= 404 samples). No *Listeria* spp. and *S. aureus* were detected from the 65 water samples. A
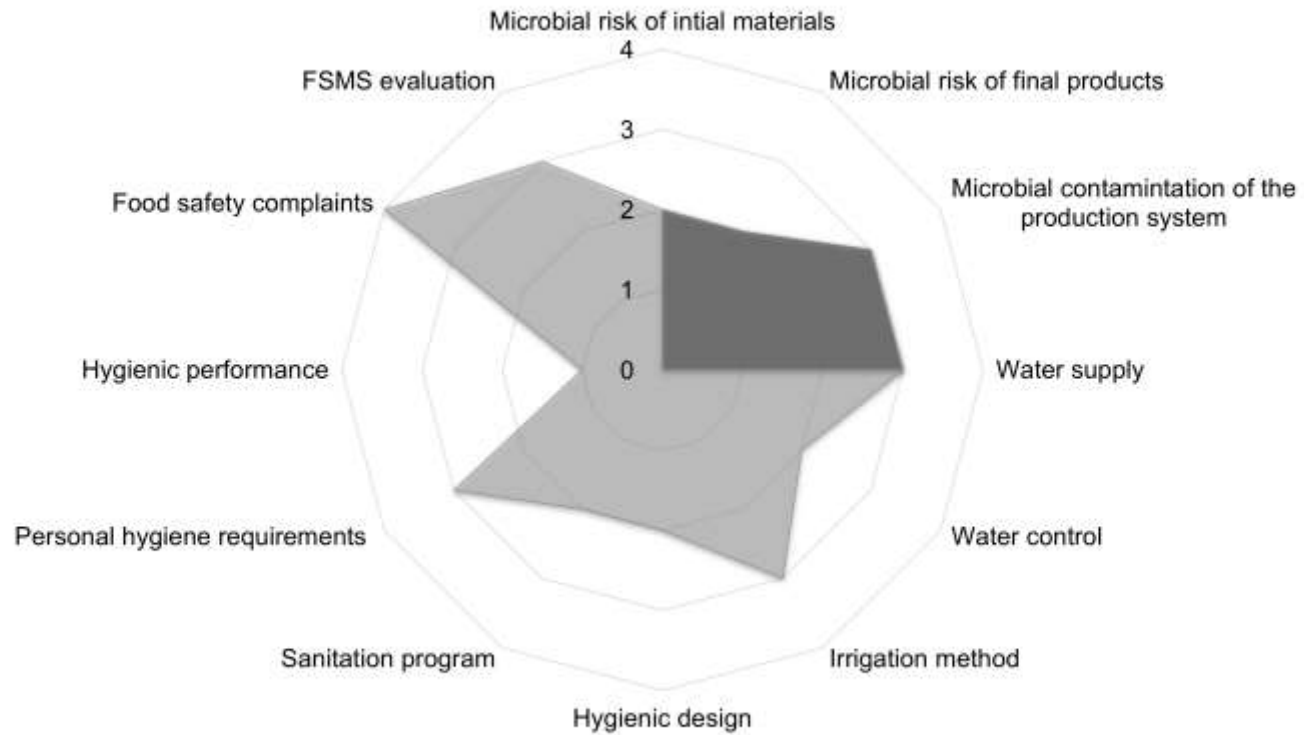
total of 25 samples tested positive for *E. coli* O157:H7. During season 2 the water contamination rate was 12.5%, with 11.3% fruit and contact surfaces contamination rate. *Listeria* spp. were detected three times over the three seasons, with one fruit (season 1), one packer's hand (season 2) and one cold room floor (season 4) sample testing positive. Seven samples tested positive for *S. aureus* with an overall contamination rate of 1.7%. Six of the seven positive *S. aureus* samples were detected in season 2, mainly from the packhouse (n=6). No isolations were made and no characterisations was therefore done.

3.3. Horticultural Safety Management System Assessment

The diagnostic tool assessment (Figure 2) demonstrated the microbiological risks identified for the production of peaches (Table 5). Contextual factors that led to higher (negative) risk scoring included the subtropical climate and open cultivation as well as the contaminated nature of the source water. Water control and method of irrigation therefore was an important control strategy. Drip irrigation mitigated the microbial contamination risk of the final product, unfortunately water was not regularly tested with no remedial action implemented on the farm. There was generalized cleaning of the facility with no formal design and no scheduled analysis was conducted in order to determine the effectiveness of hygiene implementation, as is required for a more favourable risk assessment, this resulted as a negative risk factor. The tailored handwashing facility with appropriate instructions and cleaning material was rated as being a low to medium risk (score 3). No food safety complaints were recorded and no assessment of possible microbial contamination was conducted, as is required for a more favourable risk ranking. The evaluation of the farms food safety management system was audited by a third party annually, as is required and therefore received a score of 3. Despite all the favourable attributes implemented the overall score of the food safety output of the HSMS is poor-moderate.

Table 5: Horticultural safety management system assessment of farms

Horticultural Safety Management Assessment Points	Overall Food Safety Output	
Mean product and process assessment	2.5	Medium-high risk
Mean organisation and chain	2.5	Medium-high risk
Mean control activities design	2.1	Basic Control
Mean control activities operation	1.7	Basic Control
Mean assurance activities	2.1	Basic Control
Mean food safety output	2.8	Poor-moderate Food Safety Output

Figure 2: Diagnostic tool assessment of the horticultural safety management system.

The darker areas indicate the risk of microbial contamination, and the larger areas indicate increased risk. The lighter areas indicate performance of control and assurance activities of the horticultural safety management system to mitigate risk, larger areas indicate better performance.

Table 6: Risk ranking summary of peaches following export to the United Kingdom and the European Union for the low and high susceptible consumers

Hazard	Export Destination	Susceptibility of Consumer	RISK, controlled post-processing			
			Probability of illness per day per consumer of interest (P_{inf} x P_{exp})	Total predicted illnesses/annum in population of interest	Risk Ranking Score	Ranking
Escherichia coli O157:H7	United Kingdom	General	8,22E-11	4,67E-01	28	Low
Escherichia coli O157:H7	United Kingdom	Extremely	1,64E-07	2,34E-03	29	Low
Escherichia coli O157:H7	European Union	General	8,22E-11	6,38E-01	24	Low
Escherichia coli O157:H7	European Union	Extremely	1,64E-07	2,55E-03	25	Low
Listeria monocytogenes	United Kingdom	General	4,11E-09	2,34E+01	37	Medium
Listeria monocytogenes	United Kingdom	Extremely	8,22E-06	1,17E-01	39	Medium
Listeria monocytogenes	European Union	General	4,11E-09	3,19E+01	33	Medium
Listeria monocytogenes	European Union	Extremely	8,22E-06	1,28E-01	35	Medium
Staphylococcus aureus	United Kingdom	General	4,11E-10	2,34E+00	20	Low
Staphylococcus aureus	United Kingdom	Extremely	8,22E-08	1,17E-03	16	Low
Staphylococcus aureus	European Union	General	4,11E-10	3,19E+00	16	Low
Staphylococcus aureus	European Union	Extremely	8,22E-08	1,28E-03	12	Low

Risk <32 is considered **low**, risk between 32 and 48 is considered **medium**, risk between 48 and 60 is considered **high** and risk >60 is considered **very high**.

3.4. Risk Characterization

Table 6 presents the risk ratings and estimated potential illnesses following the consumption of peaches in the international market (UK and EU). Only peaches possibly contaminated with *L. monocytogenes* were demonstrated to pose a medium risk to consumers (general and extremely susceptible) in the UK and EU (Risk ranking for UK: 37 and 39, and for EU: 33 and 35). In the UK, the total predicted illnesses per annum were calculated as 23.40 in the general population and 0.12 in the extremely susceptible population. In the EU, the total predicted illnesses in the general population were calculated as 31.9 and 0.13 in the extremely susceptible population. The calculated risk to consumers eating peaches that were potentially contaminated with *E. coli* O157:H7 and *S. aureus* was low. With a total of 0.47 and 0.64 predicted illnesses associated with *E. coli* O157:H7-peach combination in the general population in the UK and EU, respectively and 0.002 predicted illnesses in the extremely susceptible population. *Staphylococcus aureus*-peach combination resulted in higher predicted illness but the risk of the illnesses was lower and therefore resulted in lower overall risk ranking.

4. DISCUSSION

Escherichia coli was detected in all agricultural water sources sampled in this case study. However, none exceeded the guideline values, therefore at the time of the study the water quality of the Lephalala River was found to be compliant (Department of Water Affairs and Forestry, 1996; WHO, 2006). Compliance is necessary for GlobalG.A.P. and to ensure an effective food safety management system. In contrast, other river systems tested in SA reflected higher contamination levels, as a result of surrounding or upstream contamination due to being densely populated with human settlement, mining or industry activities (Chigor et al., 2013; du Plessis et al., 2015; van Dyk et al., 2016; Gemmell & Schmidt, 2012, 2013). However, this case study did not demonstrate the same trend of polluted agricultural water. Even though this farm was compliant in terms of hazard prevalence (based on the HSMS contextual criteria), the water supply was characterized as high risk, due to its source. Generally, in SA water sourced from a river which is considered high risk and therefore additional control is required (Steele & Odumeru, 2004).

South African rivers have also been shown to pose a risk of transferring bacterial pathogens onto irrigated fresh produce confirming the risky context that the water supply plays in terms of fresh produce safety (Du Plessis et al., 2015; Gemmell & Schmidt, 2012; Ijabadeniyi, 2011; Olaniran et al., 2009). In our study, *E. coli* O157:H7 was detected from agricultural water samples. The presence of this pathogen demonstrated a level of risk if using

contaminated water. However, the use of drip irrigation mitigated the risk (van Dyk et al., 2016; Markland et al., 2012). A 7.7% overall *E. coli* O157:H7 presence in water samples was recorded. Surface water quality is difficult to control due to possible contamination by livestock and human activity (Kirby et al., 2003; Odumeru & Steele, 1999).

In SA, informal settlements as well as livestock upstream of river sources has been reported to cause contamination of these water sources as well as municipal pollution (Gemmell & Schmidt, 2012; Gomba et al., 2016; Müller et al., 2001; Odjadjare & Okoh, 2010). Increased contamination with *E. coli* O157:H7 can be explained by the observations of livestock frequenting the holding dam. Cattle in Africa have the highest percentage of *E. coli* O157:H7 prevalence (Islam et al., 2014). In South Africa, the prevalence of *E. coli* O157:H7 in the North West Province has been recorded between 5.4% to 20% in cattle (Ateba, Mbewe, & Bezuidenhout, 2006). Coliforms and *E. coli* O157:H7 were found to increase which could be linked to higher water levels after rain which resulted in flooding of the banks of the dam. We surmise that contamination of the holding dam was caused by the presence of livestock which led to increased presence of coliforms and *E. coli* O157:H7 in the source water. The lack of *E. coli* O157:H7 detection in the subsequent season following removal of the livestock further supports this assumption. In further support of this, Hagedorn et al. (1999) and Tanaro & Piaggio (2014) correlated the presence of bacterial pathogens with the presence of livestock. However, the generic *E. coli* counts did not increase, it is a well-known fact that *E. coli* O157:H7 is glucuronidase negative and therefore its increase was not detected using the Coli-lert. Future isolation, characterisation and source tracking studies as conducted by Du Plessis et al. (2015) and Jongman and Korsten (2016) should be done to confirm this.

The self-assessment tool revealed that the farm met basic hygiene and sanitation requirements. During season 2, a 11.5% prevalence of *E. coli* O157:H7 in environmental samples was determined which corresponded to the findings of Cooley et al. (2007) from environmental samples (water, soil and dust) for two vegetable farms. During this study, *E. coli* O157:H7 was detected on peach crates, on the sortline and on a packer's hands during season 2, all of which represent potential sources of contamination. All contact surfaces on this farm were washed using purified water. However, at the time of sampling the water used for cleaning purposes tested negative for *E. coli* O157:H7. The packhouse and cold room floors as well as cold room walls were also contaminated with *E. coli* O157:H7. Eighty percent of *E. coli* O157:H7 (n=10) detected from samples collected from packhouse floors were found to be at the entrance to the packhouse. The likelihood that *E. coli* O157:H7 was carried into the packhouse on the soles of workers' shoes entering the facility was considered as a possible explanation requiring further investigation. A previous study

conducted found that after a new pair of shoes were worn for two weeks, coliforms and *E. coli* were detected on the outside of the shoes (Cleaning Industry Research Institute Staff, 2011). The same study also demonstrated the transfer of bacteria from the source of origin to the participant's households. An important consideration is that only one peach sample was found to be contaminated with *E. coli* O157:H7. In addition, in subsequent years following feedback to the farm management and better implementation of facility sanitation there was a definite decrease in *E. coli* O157:H7 prevalence on environmental samples with none detected on fruit and water samples. In addition, Collignon and Korsten (2010) demonstrated that *E. coli* O157:H7 was unable to survive the peach export supply chain at levels that were able to cause disease. Therefore, taking the frequency of contamination, the effect of processing, the effect of the supply chain and the population consuming the potentially contaminated product into account the level of risk to the consumer was found to be low, even when the HSMS self-assessment demonstrated that the hygiene and sanitation were basic. Future research should focus on preventative measures, behavioral correction and disinfectant efficacy, to avoid the contamination with *E. coli* O157:H7 as it can indirectly lead to fruit contamination.

In this study hygiene practices and a moderate sanitation program implemented were rated as good. Despite these perceived good and moderate practices implemented, hand and contact surface contamination with *S. aureus* was found. *Staphylococcus aureus* was not detected on fruit that were harvested from trees or prior to packing, therefore the contamination of fruit is most probably taking place through human handling after harvest. Feng et al. (2014) reported detection of *S. aureus,* in their case study, 14 *S. aureus* isolates were confirmed positive from fresh fruit and environmental samples, suggesting that the postharvest contamination needed to be addressed, which was in agreement with our findings. Contamination with *S. aureus* found in these studies, was attributed too poor personal and facility hygiene. The significant detection of *S. aureus* from fruit and farm workers' hands and the wash station tap during season 2 and the fact that contamination decreased following the improvement of facility sanitation and enforcement of proper hygiene practices is evidence that the personal and facility hygiene did not comply with minimal food safety management standards in season 2. Primary producers should therefore focus their food safety management systems on training staff in the correct hygiene and sanitation practices and procedures required to assure compliance in personal hygiene and environmental sanitation. A high contamination rate of *S. aureus* resulted in a low risk to the UK and EU consumer due to the fact that the pathogen is not considered a major pathogen as the illness that can result is not severe and an infectious dose of 10^5 cfu is required in order to produce toxins (Food and Drug Administration, 2012).

In the 428 samples analyzed only three tested positive for *Listeria* spp. one from a picker's hand, one from cold room floor sample and one from a tree fruit sample. *Listeria* spp. and *L. monocytogenes* are often found as an environmental organism present due to soil or silage contamination. If peaches become contaminated with high concentrations of *L. monocytogenes* the organism has the potential to survive at cold chain conditions at levels that could exceed the minimum infectious dose (Collignon & Korsten, 2010). Due to these factors the calculated risk to the consumer in the UK and EU was determined to be medium with a predicated illness per annum of 23.40 and 31.9. Future research should determine the presence and source of the *L. monocytogenes* contamination as well as isolation and characterisation of isolates to allow source tracking.

In this study, *Salmonella* Typhimurium was not detected in water, fruit or contact surfaces sampled. In 2012, *Salmonella* spp. was reportedly the most important bacterial pathogen in terms of number of foodborne disease outbreaks (CDC, 2012b). Similarly, a number of studies have not detected *Salmonella* spp. from samples tested (Pagadala et al., 2015 with n=422; Feng et al., 2014 with n= 407; Feng et al., 2014 with n=890). In contrast, Gomba et al. (2016) found antimicrobial resistant *Salmonella* spp. isolates from fruit, water and environmental samples within a fresh fruit production system in SA as did van Dyk et al. (2016) who found two environmental samples positive. Gomba et al. (2016) concluded that *Salmonella* spp. contamination resulted due to SA municipal pollution, however this was not the case in this study. In Africa, *Salmonella* Typhimurium and *Salmonella* Enteritidis are the two most commonly reported serovars associated with human isolates (Galanis et al., 2006). In South Africa, *Salmonella* Typhimuirum was also most commonly isolated from animal origin with 917 incidents when compared to only 232 incidents of *Salmonella* Enteritidis, with 65% from poultry and 20% from cattle (Kidanemariam, Engelbrecht, & Picard, 2010). However, in the USA over the past nine years *Salmonella* Enteritidis has not been associated with a multistate outbreak associated with fruit, while *Salmonella* Typhimurium has been associated with fruit, twice. Future studies should focus on determining the prevalence and characterisation of *Salmonella* spp. in the peach supply chain systems in order to conclude that *Salmonella* spp. are not a risk factor associated with peach consumption.

The employed detection methodology could lead to an underestimation of foodborne pathogens present in the peach supply chain due to the use of a non-selective enrichment step. Future research should focus on improving this rapid screening test to include a two-phase detection system based on International Standard Operating Procedures to allow for the isolation and characterisation of foodborne pathogens possibly detected. However, this study used a unique approach of combining microbiological sampling and self-diagnostic-

and risk- assessment tools. The integration of these knowledge platforms and tools provide a more realistic assessment of actual and perceived risks. It is interesting to note that this farm was certified to GlobalG.A.P. prior to the commencement of this study and this study found it to be initially non-compliant in terms of microbiological specifications. This brings to mind the actual impact of certification versus self-improvement. The farmer feedback strategy further realized a more compliant production system with areas of self-improvement being effectively implemented.

5. CONCLUSION

In conclusion, *Salmonella* Typhimurium was not molecularly detected on any of the water, fruit or contact surface samples (n=428) analyzed in this case study. On single occasions *E. coli* O157:H7, *Listeria* spp. and *S. aureus* were detected on fruit as well as on contact surfaces and food handler's hands. It is well known that a food product can become contaminated when in contact with a contaminated surface which can lead to cross-contamination of the final product. Following proper implementation and enforcement of the food safety management system, contamination within the packhouse decreased. In spite of these improvements the assessment of the HSMS reflected poor-moderate food safety output. Despite molecular detection of *E. coli* O157:H7, *Listeria* spp. and *S. aureus* on this farm, the overall risk to the end consumer was found to be low for *E. coli* O157:H7 and *S. aureus* and medium *L. monocytogenes* using the Risk Ranger. This is mostly due to the fact that these pathogens were detected in a single season at low frequency and that the product goes through an extended period of export under controlled cold temperatures, reducing the likelihood of survival of any potential pathogens at low frequency. Future studies should focus on isolation of foodborne pathogens to allow characterisation, and ultimately source tracking and linking pathogens from various contamination areas.

6. Acknowledgements

7. REFERENCES

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