Article

Occurrence of *Escherichia coli, Campylobcter, Salmonella* and Shiga-Toxin Producing *E. coli* in Norwegian Primary Strawberry Production

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Abstract: The aim of this study was to investigate the bacteriological quality of strawberries at harvest and to study risk factors such as irrigation water, soil and picker's hand cleanliness. Four farms were visited during the harvest season in 2012. Samples of strawberries, irrigation water, soil and hand swabs were collected and analyzed for *E. coli*, *Campylobacter*, *Salmonella* and STEC Although fecal indicators and pathogens were found in environmental samples, only one of 80 samples of strawberries was positive for *E. coli* (1.0 $\log_{10}$ cfu/g) and pathogens were not detected in any of the strawberry samples. The water samples from all irrigation sources were contaminated with *E. coli* in numbers ranging from 0 to 3.3 $\log_{10}$ cfu/g. *Campylobacter* (8/16 samples) and *Salmonella* (1/16 samples) were isolated from samples with high numbers of *E. coli*. The water samples collected from a lake had lower numbers of *E. coli* than the samples from rivers and a

stream. The present study indicated continuous background contamination in the primary production environment. Although the background contamination was not reflected on the strawberries tested here, the results must be interpreted with caution due to the limited number of samples.

1. Introduction

Fresh produce, including fresh berries, is being increasingly involved as a vehicle of foodborne disease [1,2]. This may be related to the large increase in global trade of fresh produce necessary to ensure year-round availability, the increasing consumption, and the fact that these products are often consumed raw or after only a mild risk-reducing treatment. The majority of the outbreaks of human disease associated with berries, often frozen, has been caused by viruses such as Norovirus and Hepatitis A [1,2]. Bacterial gastroenteritis associated with the consumption of fresh strawberries is rarely reported. To the best of our knowledge we have found only one outbreak reported, namely an outbreak of *E. coli* O157:H7 infections traced to locally grown strawberries contaminated by deer [3]. However, Hundy and Cameron [4] reported in a small case control study of sporadic human infection with STEC in South-Australia that people infected with STEC were more likely to have eaten berries (including strawberries) in the 10 days before illness.

Microbial food safety of fresh produce starts at the primary production stage on the farms, whether it is open field, green houses, tunnel production, soilless systems or others. Nevertheless, most surveys that have been carried out on fresh produce and in particular fresh berries have been done at processing, distribution and retail level. Studies by Bohaychuk *et al.* [5] and Johannessen *et al.* [6] indicate that the occurrence of fecal indicators and enteric pathogens in fresh berries is low. In a survey carried out by the US Food and Drug Administration (FDA), *Salmonella* was detected in one of a total of 143 samples of imported strawberries [7]. Only a few studies have been found in the literature regarding primary production of strawberries, and the results from these surveys also indicate low occurrence of fecal indicators and typical pathogens on strawberries [8–10].

Known risk factors in primary production of fresh produce are water, use of manure, soil or other growth substrates, access of wild life to the fields, livestock in the proximity of fields and water sources, equipment and human handling [1].

Strawberry production is a vulnerable type of production, especially when it is open field production. The harvesting season is short, depending on the variety, and production conditions will affect the quality of the product. The fruits are delicate, and a wet growing season will shorten their shelf-life. In Norway, the strawberry production area is 1521 ha [11]. Strawberries are mainly cultivated in open fields, and the production in tunnels and greenhouses is still small, but increasing. In normal seasons, irrigation is usually required, and in Norway overhead irrigation with sprinklers or portable water reel irrigation systems using surface water sources such as streams, rivers and lakes is common.

In the European FP7 project Veg-i-Trade [12], strawberries were selected as one of the case studies for the work package on microbial risks based on economic relevance, vulnerability to food safety

hazards and climate change, and consumption patterns and trends. There is a large global trade of strawberries, and in countries like Norway with a very short domestic harvesting season, there is a considerable import of strawberries. The aim of this study was to investigate the bacteriological quality of strawberries in primary production and to study risk factors such as irrigation water, soil and picker's hands in the primary production environment.

2. Experimental Section

2.1. Strawberry Producers and Sampling Period

Four strawberry producers were selected for participating in the described study. Details of the farms are listed in Table 1. The sampling period was from 18 June 2012 (first visit on farm 1) to 8 August 2012 (fourth sampling on farm 4). All farms used surface water for irrigation. However, farm 3 used water from a large lake where the water was pumped from a few meters depth about 20 m from the shore.

2.2. Climatic Conditions during Sampling

The summer of 2012 was generally cold and wet, and the strawberry harvesting season was later and shorter than normal. A normal open field harvesting season is usually around 4–5 weeks, but in 2012 it was only three weeks. Data on mean daily temperature and cumulative day precipitation were collected from VIPS (Forecasts of plant diseases, pest and weeds) [13] for farms 1–3 and from eKlima (database of weather and climate data from Norwegian Meteorological Institute) [14], for farm 4. At the 'Lier' weather station (farm 1–3) the mean daily temperature from 15 June 2012–31 July 2012 was 14.9 °C and there was a cumulative precipitation in the same period of 196.4 mm. For the Buran weather station (farm 4), only precipitation was recorded. During the period 15 July 2012–15 August 2012, the precipitation was 73.5 mm. For this farm, the temperature was recorded at sampling by the farmer and was between 13 and 15 °C at the four sampling occasions.

Table 1. Information on the farms participating in the present study.

Farm	Location	No of Employees during Season	Other Production	Open Field/Tunnel	Source of Irrigation Water	Irrigation Method	Fertilizer
Farm 1	Lier valley, Buskerud county, south-east Norway	45	Vegetables, grains	Open field	River	Overhead sprinkler	Mineral
Farm 2	Lier valley, Buskerud county, south-east Norway	Approx. 200	Fruit, grains	Open field	River	Overhead sprinkler	Mineral
Farm 3	Lier valley, Buskerud county, south-east Norway	50–249	Fruit, lettuce, grains	Open field and tunnel	Large lake	Overhead sprinkler	Mineral
Farm 4	Skogn, Nord-Trøndelag county, mid-Norway	10–49	Vegetables, grains, sheep	Open field	Stream	Drip	Cattle manure

2.3. Sampling of Strawberries, Soil, Water and Hands

Samples were collected four times at each farm during the harvesting season. Farms 1–3 were visited by the authors for sample collection, while farm 4 collected the samples themselves and sent the samples refrigerated by courier service to the laboratory within 24 h. Each time, five samples of strawberries in the field, five samples of soil, one sample of irrigation water, and five swabs of hands were collected. The strawberry samples were collected from the field being harvested at the time, and consisted of 10 strawberries each. Soil samples (50–100 g) were taken from around the area the plants from which the strawberries were picked. Water samples consisted of 5 L collected either from the water source (farms 1 and 4), at pump houses (farms 2 and 3), or from the irrigation equipment when irrigation took place during sampling. Since harvesting of strawberries is manual work, it was considered important to sample the hands of the pickers. This was done by swabbing one hand of five different pickers. Each swab was kept in 2.5 mL of peptone saline water. All samples were transported under chilled conditions to the laboratory and analyses were commenced within 24 h of sample collection.

2.4. Bacteriological Analyses

For the analysis of *E. coli* in strawberries, soil and swab samples, Petrifilm Select *E. coli* (3M™ Petrifilm™ Select *E. coli*, St. Paul, MN, USA) was used as described by the manufacturer. Briefly, five berries (approximately 10 g) was mixed by shaking by hand for 30 s with 9× volume of peptone saline (0.1% peptone (Difco,Sparks, MD, USA), 0.85% NaCl (Merck,Darmstadt, Germany)), and serially diluted. A volume of 1 mL of the appropriate dilutions was placed on the Petrifilm prior to incubation. The detection limit was 1.0 $\log_{10}$ cfu/g. For analysis of water for *E. coli* and enterococci, Colilert-18 (Colilert®-18/Quanti-Tray/2000, IDEXX Laboratories, Wilmington, DE, USA) were used according to the manufacturer's instructions. A total of 100 mL of water was used for each analysis.

For the detection of pathogens, NMKL No. 71 [15] was used for *Salmonella*, NMKL no 119 [16] was used for *Campylobacter* and the then draft method of ISO TS 13136 [17] was used for the detection of STEC. Briefly, 25 g of strawberries or soil were mixed with the appropriate enrichment broth (Buffered Peptone Water, BPW (Oxoid Ltd., Basingstoke, Hampshire, UK), for *Salmonella* and STEC, and Bolton-broth (Oxoid) for *Campylobacter*) and incubated at 37 °C for *Salmonella* and STEC and 41.5 °C for *Campylobacter* prior to mixing with glycerol and freezing at −80 °C. For analysis of water, 1 L was filtered through a 0.45 µm filter (Millipore, Billerica, MA, USA). If the filter clogged, more than one filter was used per sample. The filter(s) was (were) transferred to 225 mL of the appropriate enrichment broths and incubated as described above. For all analyses of the pathogens, 6.55 mL of the primary enrichment were frozen with 3.45 mL 85% glycerol (Merck) at −80 °C awaiting further analysis. When ready to start the appropriate analysis, the frozen enrichment broths were placed immediately in a water bath at 50 °C and thawed for approximately 2.5 min until the ice disappeared. The tubes were removed and inverted 2–3 times during thawing. After thawing, the tubes were inverted five times. For *Salmonella* and STEC, one mL of the primary enrichment broth was transferred to 9 mL of BPW and incubated at 37 °C for three h. For further analysis of *Salmonella,* 0.1 mL of the BPW was transferred to 10 mL Rappaport-Vassiliadis-Soya Peptone Broth (Oxoid) and incubated at 41.5 °C for 24 ± 3 h, followed by plating on XLD (Oxoid) and Brilliance™Salmonella

agar (Oxoid). The plates were incubated at 37 °C for 24 h and examined for typical colonies. The presence of *Salmonella* was confirmed by testing the colonies on Triple Sugar Iron agar (Difco) and Urea agar (agar base: Oxoid, with 40% urea (Sigma-Aldrich, St. Louis, MO, USA), and subsequently using API 20E for biochemical characterization (bioMerieux, Marcy l'Etoile, France).

For further analysis of STEC, DNA extractions, using a gradient-centrifugation method [18] using 1 mL of sample were performed prior to real time PCR as described in ISO TS 13136 [17]. If a sample was positive for *stx1* and/or *stx2* and *eae*, serotype specific PCR analyses for O26, O103, O111, O145 and O157 were carried out. Subsequently, if the sample was positive for one or more serotypes by PCR, isolation was attempted using automated immunomagnetic separation (AIMS) for the specific serotypes detected by the PCR followed by plating on CHROMagar™ O157 (CHROMagar, Paris, France) and sorbitol-MacConkey agar (SMAC, Oxoid) for serotypes O103, O111 and O145, CHROMagar™ O157 and R-Mac (MacConkey with rhamnose) (MacConkey Agar base, Difco, Rhamnose, Sigma-Aldrich) for O26 and CHROMagar™ O157 and CT-SMAC (SMAC with cefixime and tellurite, Oxoid) for O157. A total of 10 colonies from each plate were pre-agglutinated with the appropriate antiserum. If a colony agglutinated, it was isolated as a pure culture on blood agar, checked if it was presumptive *E. coli* by indole testing, re-agglutinated and tested for auto-agglutination. A presumptive *E. coli* of the aforementioned serotypes was then submitted to real-time PCR in order to confirm serotype and determine virulence factors.

For *Campylobacter*, 1 mL of of the frozen enriched Bolton broth was after thawing, transferred to 9 mL of Bolton broth containing 5% Laked horse blood (Oxoid), but without the selective antibiotic supplement (Oxoid), and incubated at 41.5 °C for 4–5 h. GENbox microaer (bioMerieux) was used to achieve the correct micro-aerobic atmosphere. After incubation, the samples were plated on mCCDA (Oxoid) and CampyFood Agar (bioMerieux) and incubated at 41.5 °C for 48 h using jars with GENbox microaer (bioMerieux). Typical presumptive positive colonies were isolated on blood agar and determined to *Campylobacter* spp. by testing motility and microaerophilic growth at 25 °C. *Campylobacter* spp. were further identified by a multiplex PCR [19].

Pure cultures of *Campylobacter*, *Salmonella* and STEC as positive controls were inoculated into their respective enrichment broths and submitted to the same treatment (enrichment and freezing) as the samples and analyzed in parallel after the samples were thawed and analyses continued.

2.5. Statistical Analyses

Proportions of positive samples were compared using Fisher's exact test. Counts of bacteria in different groups were compared using Wilcoxon/Kruskal-Wallis tests (Rank-Sums). Significance level was set at 0.05.

3. Results

The overall results (Table 2) indicated that the bacteriological quality of the strawberries sampled and tested at harvest was good, whereas the results for water, soil and hands were more variable.

Table 2. Frequency of occurrence (positive/tested) and counts ($\log_{10}$ cfu/g, $\log_{10}$ cfu/hand or $\log_{10}$ MPN/100 mL water) of selected bacteria in strawberries, soil, irrigation water and picking hand swabs obtained from four Norwegian strawberry producers.

	Sampling Period	Sample Type	*E. coli*		*Salmonella*	*Campylobacter*	STEC *
			Positive/Tested	Median Count (min–max)	Positive/Tested	Positive/Tested	Presumptive Positive by PCR
Farm 1	18 June–4 July 2012	Strawberries	0/20	<1	0/20	0/20	0/20
		Soil	6/20	<1 (<1–3.3)	0/20	ND	0/20
		Hands	8/20	<1 (<1–2.2)	ND**	ND	ND
		Water	4/4	2.8 (2.5–3.3)	1/4	3/4	4/4
Farm 2	3 July–19 July 2012	Strawberries	1/20	<1 (<1–1)	0/20	0/20	0/20
		Soil	11/20	1 (<1–3.3)	0/20	ND	0/20
		Hands	1/20	<1 (<1–1.8)	ND	ND	ND
		Water	4/4	1.71 (1.4–3.3)	0/4	2/4	3/4
Farm 3	3 July–23 July 2012	Strawberries	0/20	<1	0/20	0/20	0/20
		Soil	10/20	1 (<1–2.4)	0/20	ND	0/20
		Hands	7/20	<1 (<1–2.6)	ND	ND	ND
		Water	4/4	0.5 (0–1.2)	0/4	0/4	0/4
Farm 4	16 July–7 August 2012	Strawberries	0/20	<1	0/20	0/20	0/20
		Soil	4/20	<1 (<1–3.1)	0/20	ND	1/20
		Hands	2/20	<1 (<1–1.7)	ND	ND	ND
		Water	4/4	2.8 (2.3–2.9)	0/4	3/4	3/4
Total			66/256		1/176	8/96	11/176

* Samples positive by PCR on stx1 and/or stx2, eae and one or more serotypes of O26, O103, O111, O145 and O157; ** ND = not done.

3.1. Strawberries

No pathogens were detected in any of the 80 strawberry samples, and only one sample was positive for *E. coli* in very low numbers (1 $\log_{10}$ cfu/g).

3.2. Irrigation Water

E. coli was enumerated in all the 16 water samples in varying numbers, ranging from 0 to 3.3 $\log_{10}$ MPN/100 mL. There was a clear difference between the numbers of *E. coli* enumerated from the different farms, where farm 3, which used a large lake as irrigation water source, had significantly lower numbers of *E. coli* compared to the other three farms that used water originating from rivers and streams (Figure 1).
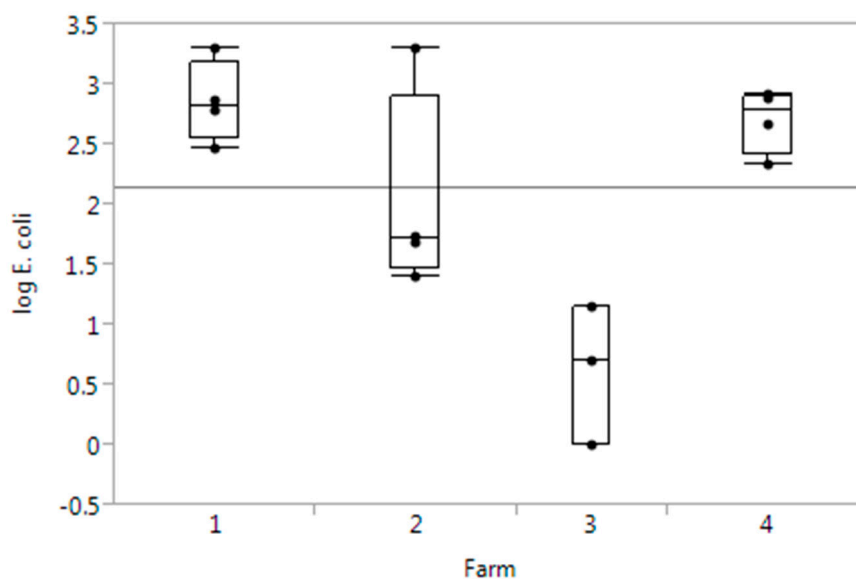
Figure 1. Boxplots of *E. coli* counts in water ($\log_{10}$ MPN/100mL water) by farm. The horizontal line shows the overall mean value.

Salmonella Newport was detected in one sample of water from the irrigation source (river) at farm 1. *Campylobacter* spp. was isolated from the water source (river and stream) from three out of four producers. Fifty percent of the water samples (8/16) contained *Campylobacter*, which was significantly higher than the frequency of occurrence of Salmonella, 6% (1/16). There was significantly higher numbers of *E.coli* in *Campylobacter*-positive than in *Campylobacter*-negative samples (Figure 2). The sample, from which *Salmonella* was isolated, also harbored a high number of *E. coli* (>3.0 $\log_{10}$ MPN/100 mL). The *Campylobacter* isolates were identified as *C. jejuni* and *C. lari*, while one isolate was not identified by the method used. STEC was not isolated from any sample. However, 10 out of 12 water samples from farms 1, 2 and 4 were presumptive positive for STEC by PCR (*stx1* and/or *stx2+*, *eae+*, one or more serotype+), although no STEC isolates were obtained.
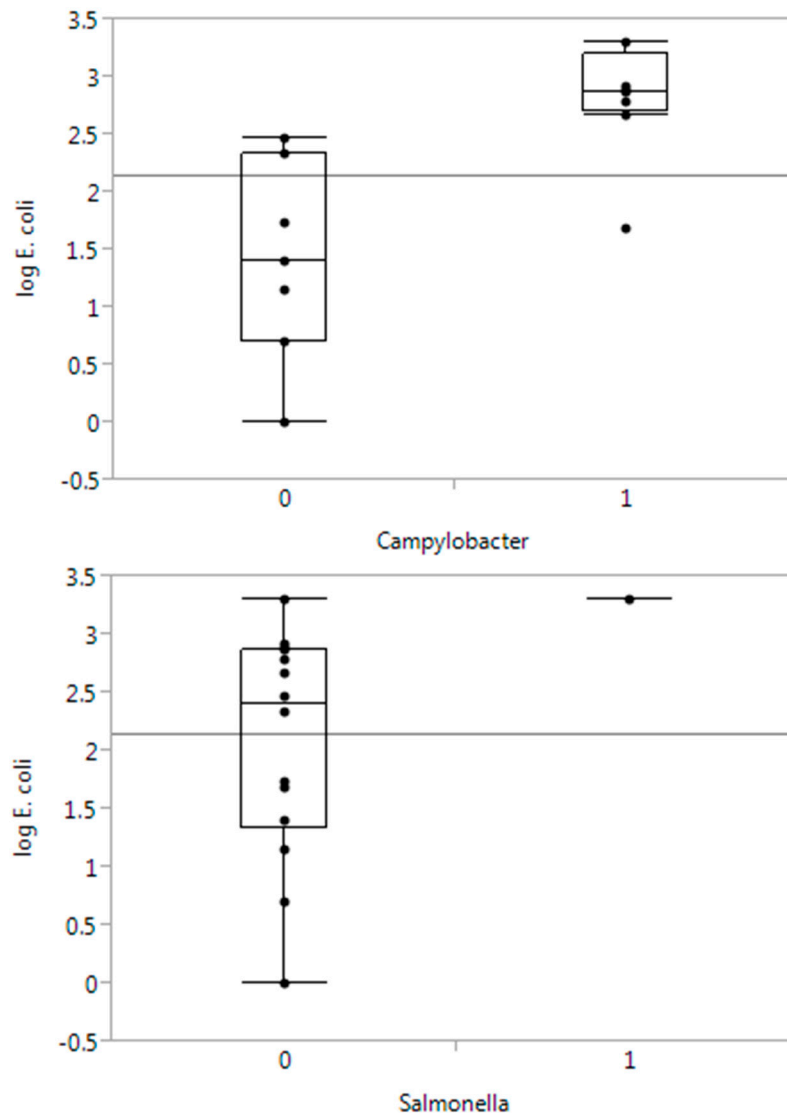
Figure 2. Boxplots of *E. coli* counts ($\log_{10}$ MPN/100 mL water) by absence (**0**) or presence (**1**) of *Campylobacter* or *Salmonella* in water samples. The horizontal line shows the overall mean value.

3.3. Soil

E. coli was detected in soil samples from all the farms visited (Table 2). The numbers in the countable samples (detection level was 1 $\log_{10}$ cfu/g) ranged from 1 to 3.3 $\log_{10}$ cfu/g, with a median of 1.6 $\log_{10}$ cfu/g, indicating that the levels of *E. coli* in the soil samples were usually low. Farms 2 and 3 had the greatest proportion of *E. coli* positive soil samples, but not the highest level of *E. coli* in the samples. *Salmonella* was not detected in any sample. One soil sample was presumptive positive for STEC by having positive PCR results for *stx*, *eae* and one or more serotypes, but no isolates were obtained. It came from farm 4, which was the only farm using manure as fertilizer. There was no statistically significant difference in *E. coli* levels in soil samples from farm 4 compared to the other farms (Figure 3).
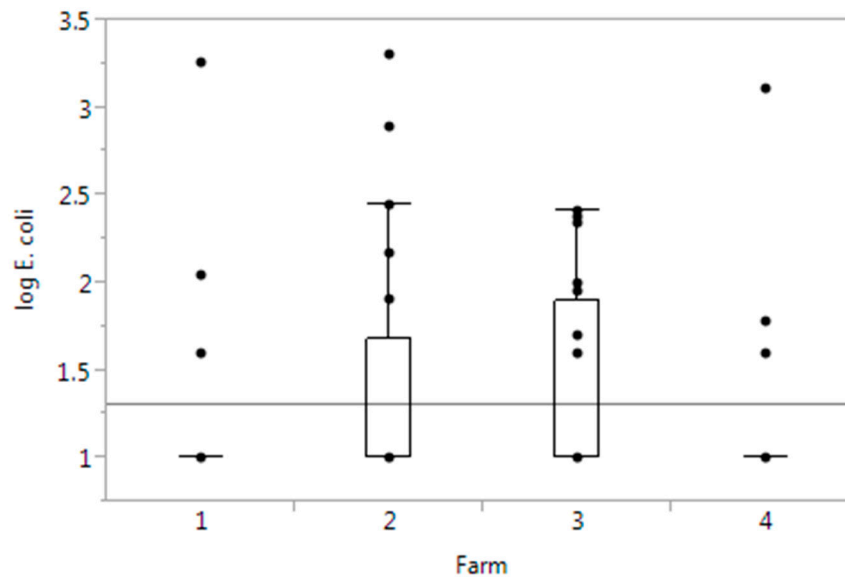
Figure 3. Boxplots of *E. coli* counts (log$_{10}$ cfu/g) in soil by farms. The horizontal line shows the overall mean value.

3.4. Hands

The median and average levels of hand contamination in the different farms were similar. Farms 1 and 3 had higher proportions of samples from which *E. coli* was enumerated (8/20 and 7/20, respectively), although the difference was significant only compared to farm 2 (1/20). Maximal contamination level found in these farms was 2.2 log$_{10}$ cfu/hand.

4. Discussion

The results from this study indicate that levels of *E. coli* and occurrence of the pathogens *Campylobacter*, *Salmonella* and STEC in the strawberries sampled and tested are low as no pathogens were detected and only one of 80 samples harbored *E. coli* with 1.0 log$_{10}$ cfu/g. However, these results should be interpreted with caution as the number of samples tested is limited and samples were collected during only one season. Nevertheless, these results are in agreement with other studies carried out on strawberries pre-harvest. Delbeke *et al*. [10] enumerated *E. coli* in two of 72 samples in a study similar to ours, whereas in Yoon *et al*. [9] and Muherjee *et al*. [8] the levels of *E. coli* were below the detection limit.

For pathogens such as *Salmonella* and STEC or *E. coli* O157, the results were also comparable. However, the present study indicates that there is a background contamination in the primary production environment, shown by the continuous presence of *E. coli* in irrigation water and soil or growth substrate, as well as the isolation of pathogens such as *Campylobacter* and *Salmonella*. In our study *E. coli* was enumerated from 39% (31/80) of soil samples and in all the samples of irrigation water. Delbeke *et al*. [10] detected *E. coli* in approximately 30% of the substrate samples and 56% of the water samples. The higher prevalence of *E.coli* in water samples in our study can be explained by the fact that the Norwegian producers used surface water without treatment, while in Belgium borehole water and collected rainfall water stored in ponds with or without barriers were used. In contrast, Yoon

et al. [9] studied greenhouse production of strawberries and did not recover *E. coli* from irrigation water or growth substrate.

The results show large variations of *E. coli* numbers depending on water source, with the lowest levels observed in the samples from the lake. We expected that the lake had lowest numbers of *E. coli*. This is due to the volume of water and the dilution effect. In addition, the water intake for farm 3, who used lake water, was at a few meters depth about 20 m from the shore. Water intakes from the rivers and stream were neither at such depth nor distance from the shore. The high numbers of *E. coli* in the water from the rivers and stream was most likely due to the wet conditions in the 2012 growth season with subsequently little irrigation. Therefore, the majority of the water samples were collected directly from the water source (river, stream, lake) and not the water that was actually used for irrigation. Previous results from one of the rivers indicate that the numbers of *E. coli* are much more variable during periods with much precipitation, while in drier periods the levels are less variable, but still rather high [20]. Since the water is of poor bacteriological quality, measures, such as changing of irrigation method or water treatment, should be considered in order to reduce the risk for contamination. In our study there were eight *Campylobacter*-positives and one *Salmonella*-positive sample from water used in strawberry production. These samples also had high levels of *E. coli*. When pathogens were identified in water, irrigation was not in use due to rainfall. It is well known that the use of contaminated irrigation water is a risk in the production of fresh produce. We did expect the possibility of isolating both *Campylobacter* and *Salmonella* from water. We thought it likely that we would find more *Campylobacter* positive samples than *Salmonella*, which was the case. In Norway, untreated water (surface water) is a known risk factor for campylobacteriosis [21]. The prevalence of *Salmonella* in Norwegian livestock is low, but *Salmonella* Typhimurium is endemic in wild birds [22] and in hedgehogs in only particular areas in the country [23]. The impact of the irrigation water on the quality of the product depends on the irrigation method and how close to harvest irrigation is used. Among the producers visited in this project, three (farms 1, 2 and 3) used overhead spray irrigation while the fourth (farm 4) used drip irrigation. Farmer 4 also informed that he knew that the irrigation water was contaminated and had, in cooperation with the local food safety authority, decided that the use of drip irrigation was the best option for his farm. In Norway, the farmers are required to test at least one sample of irrigation water and the sample should be collected close to the first harvest regardless of which product that is irrigated.

Approximately 6% of the samples analyzed for STEC were presumptive positive by PCR (*stx*1 and/or *stx*2+, *eae*+, positive for one or more serotypes), but no isolates were recovered. Several studies have shown similar results and challenges when screening similar environmental samples; the samples are positive by PCR, but only a very few isolates are obtained [9,24]. In our study, the only presumptive positive soil sample originated from the farm using manure as a fertilizer (farm 4). In accordance with common practice in Norway, manure was only applied on the field before planting. Furthermore, the farmer used small, fresh plants as starting material in field, and such plants do not start yielding strawberries until the following year. This practice suggests that it is unlikely that the manure applied to the field is the source of the presumptive STEC-positive soil sample. Previous results from Norway indicated that *E. coli* O157:H7 survived for up to 12 weeks from soil used for production of lettuce when temperatures varied between 12 °C and 15 °C in a climate room [25]. Another source for STEC would be the irrigation water, but the farmer informed us that no irrigation

had been applied this season. Delbeke *et al.* [10] isolated STEC O26 from strawberry irrigation water and substrate on one farm that also had cattle. In a follow-up study on the particular farm one year later, fecal samples from the cattle were PCR positive for STEC O26, but no isolates were obtained for further comparison. The authors conclude that it was likely that the cattle were the source [10]. The presence or proximity to livestock is a known risk factor for STEC in fresh produce and in our study, farm 4 was also the only farm that had livestock (sheep). Another interesting result was the presence of the *eae*-gene in the soil samples. Out of 80 soil samples, 60% (48 samples) were positive for *eae*, with only one being presumptive positive for STEC (*eae+*/*stx+*, positive for one or more serotypes). The *eae* gene is generally not widespread, but *Citrobacter rodentium* may harbor this gene. It is also possible that atypical enteropathogenic *E. coli* (atypical EPEC) was present in the samples, but isolation was not attempted.

About 22% (18 out of 80) of the swab samples of workers' hands were positive for *E. coli*. This may suggest poor hand hygiene, but may also be a result of the wet harvesting season. None of the workers used gloves when picking strawberries as this is not required on Norway. Differences between farms were observed. All the farmers gave hygiene training to the workers in the beginning of the season, but it was variable how hygiene practices were followed up. All four farms had requirements to personal hygiene and toilets with washing stations, and two out of four farms had regular meetings with the workers. There was no effect of farm size on swab findings. Farm 1 and farm 4 were smaller farms with less than 50 workers during the season, while farms 2 and 3 were bigger with around 200 workers in the high season. When comparing our results to those from Delbeke *et al.* [9], we find a higher proportion of positive samples in Norway (22%, 18/80) compared to the Belgian results (7%, 4/57). This contrast can probably be explained by the fact that the samples in Norway were collected from open field operations in a wet harvesting season and inevitably, splashes from soil and dirt water may be transferred to the hands during picking. In Belgium the majority of the producers used either greenhouses or tunnel systems.

Although the results from the present study and others indicate that the bacteriological quality is generally good, it is important to keep in mind the limited number of samples that have been tested. A heterogeneous spread of contamination is expected in unprocessed fruits and vegetables, and no sampling scheme can guarantee the absence of pathogens. Therefore, focus on good agricultural practices and hygiene is recommended. The few samples positive for pathogens made it difficult to draw comparisons or to make inferences related to safe practices in primary production.

5. Conclusions

The results from the present study suggest that in spite of continuous background contamination in the primary production environment, the levels of *E. coli* and occurrence of *Campylobacter*, *Salmonella* and STEC on the berries is low. However, the results should be interpreted with caution as only a limited number of samples have been tested.

Author Contributions

Gro S. Johannessen was project leader. Nina Heiberg was responsible for the contact with the producers. Mumtaz Begum, Marte Monshaugen and Marianne Økland collected and analyzed the samples. Karl F. Eckner and Helga R. Høgåsen analyzed the data. Gro S. Johannessen, Karl F. Eckner and Helga R. Høgåsen wrote the paper, with specific contributions from the other coauthors.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. EFSA Panel on Biological Hazards (BIOHAZ). Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). *EFSA J.* **2013**, *11*, doi:10.2903/j.efsa.2013.3025. Available online: www.efsa.europa.eu/efsajournal (accessed on 8 January 2013).
2. EFSA Panel on Biological Hazards (BIOHAW). Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in berries). *EFSA J.* **2014**, *12*, doi:10.2903/j.efsa2014.3706. Available online: www.efsa.europa.eu/efsajournal (accessed on 18 June 2014).
3. Laidler, M.R.; Tourdjman, M.; Buser, G.L.; Hostetler, T.; Repp, K.K.;, Leman, R.; Samadpour, M.; Keene, W.E. *Escherichia coli* O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer. *Clin. Infect. Dis.* **2013**, *57*, 1129–1134.
4. Hundy, R.L.; Cameron, S. Risk factors for sporadic human infection with Shiga toxin-producin *Escherichia coli* in South Australia. *Commun. Dis. Intell. Q. Rep.* **2004**, *28*, 74–79.
5. Bohaychuk, V.M.; Bradbury, R.W.; Dimock, R.; Fehr, M.; Gensler, G.E.; King, R.K.; Riece, R.; Romero Barrios, P. A microbiological survey of selected Alberta-grown fresh produce from farmers' markets in Alberta, Canada. *J. Food. Prot.* **2009**, *72*, 415–420.
6. Johannessen, G.S.; Loncarevic, S.; Kruse, H. Bacteriological analysis of fresh produce in Norway. *Int. J. Food Microbiol.* **2002**, *77*, 199–204.
7. U.S FDA. United States Food and Drug Administration. FDA Survey of Imported Fresh Produce FY 1999 Field Assignment. 30 January 2001. Available online: http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/produceplantproducts/ucm118892.htm (accessed on 21 January 2015).
8. Mukherjee, A.; Speh, D.; Dyck, E.; Diez-Gonzalez, F. Preharvest evaluation of coliforms, *Esherichia coli*, *Salmonella*, and *Eshcerichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.* **2004**, *67*, 894–900.

9.   Yoon, Y.; Kim, K.; Nam, M.; Shim, W.B.; Ryu, J.G.; Kim, D.H.; You, O.J.; Chung, D.H. Microbiological assessment in strawberry production and recommendations to establish a good agricultural practice system. *Foodborne Path. Dis.* **2010**, *7*, 1511–1519.

10. Delbeke, S.; Ceuppens, S.; Hessel, C.T.; Castro, I.; Jacxens, L.; de Zutter, L.; Uyttendaele, M. Microbial safety and sanitary quality of strawberry primary production in Belgium: Risk factors for *Salmonella* and Shiga toxin-producing *Escherichia coli* contamination. *Appl. Environ. Microbiol.* **2015**, *81*, 2562–2570.

11. Statistics Norway. Available online: https://www.ssb.no/statistikkbanken (accessed on 5 March 2015).

12. Veg-i-Trade. Available online: www.veg-i-trade.org (accessed on 15 December 2014).

13. VIPS. Forecasts of Plant Disease, Pests and Weeds. Available online: www.vips-landbruk.no (accessed on 15 December 2014).

14. eKlima. Norwegian Meterological Institute. Available online: http://sharki.oslo.dnmi.no/portal/page?_pageid=73,39035,73_39049&_dad=portal&_schema=PORTAL (accessed on 15 December 2014).

15. NMKL. *Salmonella Detection in Foods, NMKL 71*, 5th ed.; NMKL: Oslo, Norway, 1999.

16. NMKL. *Thermotolerant Campylobacter Detection, Semi-Quantitative and Quantitative Determination in Foods and Drinking Water, NMKL 119*, 3rd ed.; NMKL: Oslo, Norway, 2007.

17. ISO. *Microbiology of Food and Animal Feed—Real-Time Polymerase Chain Reaction (PCR)-Based Method for the Detection of Food-Borne Pathogens—Horizontal Method for the Detection of Shiga Toxin-Producing Escherichia coli (STEC) and the Determination of O157, O111, O26, O103 and O145 Serogroups, ISO/TS 13136*, 1st ed.; ISO: Geneva, Switzerland, 2012.

18. NMKL. *Shigella spp. PCR Method for Detection in Foods, NMKL 174*, 2nd ed.; NMKL: Oslo, Norway, 2002.

19. Johannessen, G.S.; Johnsen, G.; Økland, M.; Cudjoe, K.S.; Hofshagen, M. Enumeration of thermotolerant *Campylobacter* spp. from poultry carcasses at the end of the slaughter-line. *Lett. Appl. Microbiol.* **2007**, *44*, 92–97.

20. Johannessen, G.S.; Wennberg, A.C.; Nesheim, I.; Tryland, I. Diverse land use and the impact of (irrigation) water quality and need for measures—A case study of a Norwegian river. *Int. J. Environ. Res. Public Health* **2015**, in press.

21. Sandberg, M.; Nygård, K.; Meldal, H.; Valle, P.S.; Kruse, H.; Skjerve, E. Incidence trend and risk factors for *Campylobacter* infections in humans in Norway. *BMC Public Health*. **2006**, *6*, Available online: http://www.biomedcentral.com/1471-2458/6/179 (accessed on 30 March 2015).

22. Refsum, T.; Handeland, K.; Baggesen, D.L.; Holstad, G.; Kapperud, G. Salmonellae in avian wildlife in Norway from 1969 to 2000. *Appl. Environ. Microbiol.* **2002**, *68*, 5595–5599.

23. Handeland, K.; Refsum, T.; Johansen, B.S.; Holstad, G.; Knutsen, G.; Solberg, I.; Schultze, J.; Kapperud, G. Prevalence of *Salmonella* Typhimurium infection in Norwegian hedgehog populations associated with two human disease outbreaks. *Epidemiol. Infect.* **2002**, *128*, 523–527.

24. Holvoet, K.; Sampers, I.; Seynnaeve, M.; Uyttendaele, M. Relationships among hygiene indicators and enteric pathogens in irrigation water, soil and lettuce and the impact of climatic conditions on contamination in the lettuce primary production. *Int. J. Food Microbiol.* **2014**, *171*, 21–31.

25. Johannessen, G.S.; Bengtsson, G.B.; Heier, B.T.; Bredholt, S.; Wasteson, Y.; Rørvik, L.M. Potential uptake *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. *Appl. Environ. Microbiol.* **2005**, *71*, 2221–2225.