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Microbial quality of irrigation water used in leafy green production in Southern Brazil and its relationship with produce safety

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ABSTRACT

Irrigation water has been recognized as an important microbial risk factor for fruits and vegetables in many production areas, but there is still a lack of information about how the microbiological quality of different irrigation water sources and climatic conditions influence the safety of vegetables produced in Brazil. This study evaluated the distribution of generic *E. coli* and the prevalence of *E. coli* O157:H7 in two different water sources (ponds and streams bordering farmlands and urban areas) used for irrigation and on commercially produced lettuces in Southern Brazil. We also evaluated the effect of agricultural factors and meteorological conditions in the potential contamination of water and produce samples. A longitudinal study was conducted on four farms during a year (July 2014 to August 2015). The results showed generic *E. coli* prevalence of 84.8% and 38.3% in irrigation water samples and on lettuces, respectively, indicating irrigation water as an important source of contamination of lettuces. No significant differences were detected in the counts of *E. coli* between the two different surface water sources. The climatic conditions, particularly rainfall and environmental temperature, have influenced the high concentration of *E. coli*. The highest loads of *E. coli* in irrigation water and on lettuces were found during the warmest time of the year. *E. coli* O157:H7 was detected by qualitative polymerase chain reaction (qPCR) in 13 water samples but only 4 were confirmed by isolation in culture media.

1. Introduction

Leafy greens have been associated with foodborne outbreaks worldwide (EFSA AND ECDC, 2015; CDC, 2014). In Brazil, following the general global tendency, almost 3% of the foodborne outbreaks have been associated with fruits and vegetables in the last years (ANVISA, 2014). In an attempt to understand the most probable sources of contamination of leafy greens, recent studies have evaluated potential risk factors during primary production (Castro-Ibañez et al., 2015a; Ceuppens et al., 2014, 2015; Benjamin et al., 2013). In most of the cases, obtained results showed a strong evidence of contamination of produce from irrigation water. Additionally, recent reviews identified contamination events where water was recognized as a risk factor in the production and harvesting of fresh produce (Allende and Monaghan, 2015; Uyttendaele et al., 2015).

Irrigation water used in growing fields originates from a variety of water sources and much knowledge is needed to relate risk factors associated with the transfer coefficients for pathogens by source, concentration and use (Leaman et al., 2014). Among the main water sources used for irrigation, it can be identified, from lower to higher contamination risk, the wells, rainwater harvesting, rivers, and reservoirs (Castro-Ibañez et al., 2015b; Ahmed et al., 2012; Ferguson et al., 2012).

Mostly due to the low prevalence of pathogens in environmental and fresh produce samples, numerous published research papers rely on the enumeration of microbial indicators as a good strategy to characterize microbial contamination (Allende and Monaghan, 2015). However, there are controversial opinions regarding the relationship between the microbial quality of irrigation water and the food safety of fresh produce (Leaman et al., 2014). While some studies reported a correlation between numbers of *E. coli* in irrigation water and irrigated leafy vegetables, other researchers did not find a significant relation between fecal

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indicators on fresh produce with those found in irrigation water (Castro-Ibañez et al., 2015a; Gayeon et al., 2013; Pahl et al., 2013; Pachepsky et al., 2011). On the other hand, recently published research papers found a higher prevalence of foodborne pathogens in water and fresh produce samples with higher loads of *E. coli* (Castro-Ibañez et al., 2015a; Ceuppens et al., 2014).

A study focused on the microbial quality and safety assessment of lettuce production in the Southern of Brazil identified contamination of vegetables with high counts of enteric microorganisms probably originating from irrigation water (Ceuppens et al., 2014). Although the recognized importance of irrigation water on the safety of vegetables, there is still a lack of information about how the microbiological quality of different irrigation water sources and climatic conditions influence the safety of vegetables produced in Brazil. This leads to the necessity of additional research to better understand microbial risks associated to irrigation water.

The present study aims to describe the distribution of generic *E. coli* and the prevalence of *E. coli* O157:H7 in irrigation water in commercial fields from the Southern Brazil. The impact of meteorological conditions on the microbial quality of irrigation and fresh produce samples was also evaluated. Moreover, the relationship between the distribution of generic *E. coli* and the presence of foodborne pathogen *E. coli* O157:H7 in samples was established.

2. Materials and methods

2.1. Growing fields

Four commercial leafy green growers agreed to participate in this study. All farms were located in the "green belt" from metropolitan region of Porto Alegre city in the State of Rio Grande do Sul at the Southern of Brazil and were small family farms. The specific location was kept confidential to protect the identity of the farmers. The dimension of the farms was characteristic of the local commercial growing field and ranged between 0.1 and 0.5 ha. Two different irrigation water sources commonly used in the local commercial growing fields were involved in this study including natural ponds (Ponds) and streams bordering farmlands and urban areas (Streams). In all the cases, sprinkle irrigation was used. Irrigation was usually carried out once in the morning during autumn, winter and spring, while during summer irrigation was performed twice, early in the morning and late in the evening.

2.2. Sampling scheme

A systematic sampling plan was developed to identify potential risk factors for microbial contamination linked to irrigation water in the production of whole lettuce. The sampling sites were selected based on a previously published research paper focused on the potential risk factors that contribute to microbiological contamination on lettuce (Ceuppens et al., 2014). For each water source and growing field, the sampling plan included sample collection during a year period where samples were taken monthly from July 2014 to August 2015. Water and lettuce samples were taken in duplicate and triplicate, respectively. A total of 219 water samples were collected during the study: where 138 were water samples and 81 were lettuce samples. All samples were taken to laboratory in thermal boxes and kept for 2–12 h at <4 °C until microbiological analyses. Physicochemical parameters of the irrigation water (pH and temperature) were recorded at each sampling point.

2.3. Sampling methodology

The protocol previously described by Holvoet et al. (2014) was followed. For lettuce, 9 samples of approximately 100 g each were randomly collected from different locations in the field following a zig-zag pattern started from a randomly selected side of the field. Once in the laboratory, samples (100 g each) were randomly pooled into 3 samples (25 g each). In the case of water, samples were collected from different water sources: natural ponds (Ponds) and streams bordering farmlands and urban areas (Streams). Two liter samples were collected into sterile bottles according to ISO 19459:2006 (ISO, 2006). Microbial analyses were conducted within 2–14 h from the time of sample collection.

2.4. Microbiological analyses

The microbial quality of lettuce samples were evaluated by diluting 25 g of each sample or sample pool in 225 mL of 1% buffered peptone water. Water samples (100 mL) were filtered using a cellulose nitrate membrane filters (0.45 μM diameter, Microsart®, Sartorius, Brazil). *E. coli* was monitored as previously described (Holvoet et al., 2014). Briefly, *E. coli* were enumerated in lettuce and samples using Chromocult Agar (Merck, Brazil) after incubation for 24 h at 37 °C. The detection limits were 10 cfu/g in case of lettuce and 100 cfu/100 mL in case of water samples.

Presence or absence of *E. coli* O157:H7 were determined in water samples with more than 100 cfu/100 mL (Ceuppens et al., 2015). Screening of positive samples was analyzed using PCR technique. In this case, bacterial DNA was extracted following the protocol described by the NMKL Method nº 174 (NMKL, 2002). Briefly, 900 μl of the enrichment broth were transferred to a tube containing 600 μl of Percoll (Sigma-Aldrich®) 40%. Then, the tube was centrifuged for 1 min at 13.200 rpm. The fluid on the top of the tube was removed and 0.1 mL was leaved at the bottom. The remaining volume was transferred to a tube contain 1.2 mL sterile distilled water and vortexed by 1 min. The tube was centrifuged for 5 min at 10.000 rpm. The fluid on the top of the tube was removed, leaving 0.1 mL at the bottom, 1 mL of sterile distilled water was added and the tube vortexed by 1 min. This last step was repeated again. Then, the fluid on the top was discarded, leaving 0.2 mL in the tube, which was incubated for 20 min at 95 °C in a heating block. After incubation, the tubes were placed on ice for 5 min and centrifuged for 1 min at 10.000 rpm. The tubes were stored at −20 °C until PCR analysis. The Real-Time PCR was performed according the primers and cycling conditions described in the ISO 13136:2012 (ISO, 2012). The software StepOne Plus® (Life Technologies®, Carlsbad, United States).

2.5. Meteorological parameters

Weather data for ambient temperature, precipitation and solar radiation were obtained during the sampling period from the Brazilian National Institute of Meteorology (INMET). Temperature and solar radiation data from 24 h before the sampling point were considered while in the case of precipitation, data from 7 days prior sampling were taken and used to correlate microbiological results and climatic conditions following procedures previously described (Castro-Ibáñez et al., 2015a,b; Ceuppens et al., 2014).

2.6. Domestic animals

The presence of domestic animals was farm dependent, whit three farms performing mix crop-livestock farming while all of them had pets around the farm. To determine the agricultural practices related to domestic animals and pets a questionnaire was given to the growers (Table 1).

2.7. Statistical analysis

Non-zero microbial loads were log-transformed and stored