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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

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ABSTRACT

Eight Belgian lettuce farms located in the West Flanders were sampled to establish the relationships between levels of indicator bacteria, detection of enteric zoonotic pathogens and the temperature and precipitation during primary production. Pathogenic bacteria (PCR EHEC positives, *Salmonella* spp. or *Campylobacter* spp.) and indicator bacteria (total psychrotrophic aerobic plate count (TPAC), total coliforms, *Escherichia coli*, enterococci) were determined over a period of one and a half year from seedling leaves, peat-soil of the seedling, lettuce crops, field soil and irrigation water. Neither *Salmonella* isolates nor PCR EHEC signals were detected from lettuce although one out of 92 field soil samples contained *Salmonella* spp. and five soil samples provided PCR positives for EHEC virulence factors (*vt1* or *vt2* and *eae* gene). A low prevalence of *Campylobacter* (8/88) was noted in lettuce. It was shown that irrigation water is a major risk factor with regard to the bacterial contamination of the fresh produce as the water samples showed on a regular basis *E. coli* presence (59.2% of samples ≥ 1 CFU/100 ml) and occasionally detection of pathogens (25%, n = 30/120), in particular *Campylobacter* spp. The highest correlations between indicator bacteria, pathogens, temperature and the amount of precipitation were observed for the water samples in contrast to the soil or lettuce samples where no correlations were observed. The high correlations between *E. coli*, total coliforms and enterococci in the water implicated redundancy between analyses. Presence of elevated levels of *E. coli* increased the probability for the presence of pathogens (*Campylobacter* spp., EHEC and *Salmonella* spp.), but had a low to moderate predictive value on the actual presence of pathogens. The presence of pathogens and indicator bacteria in the water samples showed a seasonal effect as they tend to be more present during the months with higher temperature.

1. Introduction

Severe foodborne disease outbreaks can be caused by pathogenic microorganisms associated with fresh produce (Delaquis et al., 2007). Foods of non-animal origin were associated with 10% of the outbreaks, 26% of the cases, 35% of the hospitalizations and 46% of the deaths in reported foodborne outbreaks in EU in the period 2007–2011 (EFSA, 2013a). Several publications showed that enteric diseases linked to consumption of fresh produce have dramatically increased in the last several decades (Mukherjee et al., 2007; Sivapalasingam et al., 2004). For this reason, fresh produce has been well recognized as a potential vehicle for transmission of pathogenic microorganisms known to cause human disease. Outbreaks associated with fresh produce result in considerable economic losses to farmers, distributors and the food industry (Golberg et al., 2011).

Primary production is probably the main concern in terms of introduction of hazards as pre-harvest contamination of vegetables can occur directly or indirectly via (wild) animals, insects, water, soil, dirty equipment and human handling. The contamination of produce can occur in the field by contaminated soil (such as the use of insufficiently composted manure), by the use of contaminated water for irrigation or pesticide application or by deposition of feces by wild animals (Ingham et al., 2005; Johannessen et al., 2005). Fecal bacteria (including enteric pathogens) are in particular in wet conditions and clouded weather (limited UV irradiation) able to survive for extended periods in soils (Islam et al., 2004), manure (Nicholson et al., 2005) and water (Chalmers et al., 2000; Steele and Odumeru, 2004) and thereby provide potential inoculum for contamination of the fresh produce.

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Enteric diseases have a seasonal pattern, with the highest incidence of illness during the summer months (Amin, 2002; Barwick et al., 2000). Warmer ambient temperature may contribute to the increased incidence of enteric diseases (Fleury et al., 2006). Changes in temperature and precipitation can influence environmentally mediated pathogen transmission pathways, playing an important role in driving seasonality in these diseases (Lal et al., 2012; Liu et al., 2013).

Intensive precipitation may increase surface and subsurface runoff, which might be an intermediate contamination pathway of pathogens from manure at livestock farms and from grazing pastures (Donnison and Ross, 2009; Parker et al., 2010). Rainfall is able to release fecal coliform and a variety of pathogenic microorganisms, which may be released into the environment in large numbers (Guber et al., 2006; Parker et al., 2010). Flooding or runoff can affect the microbial contamination of leafy vegetables through the spread of fecal waste onto the growing area, or through contaminated water. Fecal contamination of agricultural soils has been shown to increase after environmental flooding (Casteel et al., 2006).

To determine the microbial quality and hygiene, in particular in the framework of verification of good agricultural practices in lettuce crop production it is common practice to monitor the presence and levels of indicator bacteria such as total coliforms, enterococci and *Escherichia coli* in irrigation water or *E. coli* in the lettuce crops from the field. These microbial parameters are often used to indicate insufficient sanitary quality or potential fecal pollution. In addition, having *E. coli* of fecal (human or animal) origin has also been established as an “index” or “marker” organism (Mossel, 1978, 1982) to provide evidence of an increased likelihood of potential contamination of food or water by ecologically closely related pathogens such as Gram negative enteric pathogens encompassing human pathogenic EHEC, *Salmonella* spp. and *Campylobacter* spp. The detection of pathogens is expensive, time consuming, and complex due to pathogen variability (Savichtcheva and Okabe, 2006). Consequently, pathogens are most of the time not directly monitored in plant production areas or well or borehole waters. However, the extent of correlation among themselves and predictive value of these hygiene indicators for pathogen's presence has not been thoroughly established or quantified.

In the present field study, pathogenic bacteria (PCR enterohaemorrhagic (EHEC) positives (or *Salmonella* spp. or *Campylobacter* spp. isolates)) and indicator bacteria (total psychrotrophic aerobic plate count (TPAC), total coliforms, *E. coli*, enterococci) were determined in samples from the lettuce crops and primary production environment. Samples were taken from seedling leaves, peat-soil of the seedling, lettuce crop, field soil and irrigation water and analyzed for this broad scope of microbiological parameters. In parallel also climatic parameters (i.e., temperature, precipitation) close to the crop production fields were collected. The objectives of the study were to (i) determine risk factors for lettuce contamination with pathogenic bacteria and (ii) to establish correlations between the type and the levels of indicator bacteria, the detection of enteric zoonotic pathogenic bacteria and the effect of temperature, precipitation and seasonality on the bacterial contamination during lettuce primary production and this in order to check the extent of the parameters in correlation to the presence of pathogens.

2. Materials and methods

2.1. Selection of lettuce production farms

Eight Belgian lettuce producing farms comprising four greenhouse farms and four open field farms, all located in West-Flanders' region, were selected and agreed upon to take part in this study. All the farms were certified for the Belgian quality assurance standards Flandria and IKKB Standard (Integral Chain of Quality Management) (IKKB, 2010). In order to tackle problems during export of vegetables, IKKB Standard was benchmarked to GLOBALGAP (GlobalGap, 2012). Six farms were using open well water for irrigation and two farms used bore hole water. All farms used overhead sprinklers for irrigation of crops. The dimensions of the farms ranged from 1 ha up to 120 ha.

2.2. Sampling plan

The greenhouse farms having year-round production were each sampled during three lettuce crop production cycles distributed over the whole year. Because of temperate climate, the open field farms only had lettuce production going on from May to the end of September. In that restricted time period for each of the open field farms also three lettuce crop production cycles were monitored. This resulted in an accumulation of obtained results in the summer period. The complete study took place in two phases, the start-up with one crop cycle at open field farm in April–May 2011 whereas the remainder of the open field and greenhouse farms was taken up from September 2011 with the last sampling occurring (at a greenhouse farm) in December 2012. A production cycle is the time required to follow a lettuce crop from seedling at the start until its harvest and takes approximately 5–14 weeks depending upon the season. During a production cycle, 4 visits (and thus sampling times) were included: at the start during the planting of the lettuce seedling, next two weeks before harvest, one week before harvest and at harvest of the lettuce crop. Samples were collected from seedling or lettuce crop, soil and irrigation water and if applicable (at harvest) from food handler's hands or crates. All the samples were stored and transported in the dark at <4 °C to the lab for further handling (cutting/pooling) and subsequent microbial analysis. Samples were analyzed within 4 to 24 h.

2.3. Sampling of seedlings, soil, lettuce crop and irrigation water

The first sampling time for a lettuce crop production cycle took place at the start during the planting of the lettuce seedling. Before planting, nine samples were taken from the potting peat-soil of the seedling ($9 \times \pm$ 300 g), the seedlings ($9 \times$ one seedling) itself and soil of the planting field ($9 \times \pm$ 300 g). During the next three sampling visits in the lettuce crop production cycle (two and one week before harvest and at harvest) sampling of lettuce crops (9×1 crop), planting field soil and irrigation water (5 l) occurred. Upon each visit, nine crops of lettuce were cut by a sterile knife and put directly into a sterile bag using disinfected gloves. At harvest in addition, nine samples were also taken from the ready to market rinsed lettuce crops.

For enumeration purposes, the nine peat-soil samples, planting field soil samples and the nine lettuce crops s were randomly pooled by three in the lab. The three crops were pooled as follows: each crop was cut in two, three halves were discarded, the remaining three halves were cut in pieces of 3 cm and the pieces were mixed thoroughly. The seedling samples were all nine pooled together due to the low mass of the seedlings. Next 10 g of each pooled sample was weighed in a stomacher bag and homogenized (for the lettuce and seedling by using a stomacher) for 1 min in 90 ml peptone physiological salt water (PPS) as a starting point for serial tenfold dilution in PPS and plating for enumeration of indicator organisms. This resulted in three enumerations of indicator organisms for the peat-soil, planting field soil and lettuce per sampling visit and one result for the seedlings. For detection of pathogens in the peat-soil of the seedlings, in the field soil and for the lettuce crops, all nine samples were joined to one sample resulting in a single detection result per visit per sample type. 25 g was taken from the pooled samples and put in a stomacher bag with the respective enrichment media.

Water samples were collected during the lettuce crop production cycle. Samples were taken from the water source and if possible at the water tap (outlet of the irrigation sprinkling system to the crops). Rinsing water was collected as well when sampling during harvest. Five liter samples were collected into sterile bottles according to ISO 19458:2006 (ISO, 2006b). The pH and temperature of the water were measured directly after sampling at the farm.