Activity: WT2 – Impact of irrigation on leaf lettuce and green onion safety: combining the irrigation-harvest delay and the content of *Escherichia coli* in water.

Objectives:
The aim of this study was to evaluate the impact of irrigation water *E. coli* content and delay between irrigation and harvest on the presence of indicator and pathogenic microorganisms on green onion and leaf lettuce.

Actions completed
Field operations, sampling, microbiological analyses and data analysis and interpretation are completed.

Methods
Experimental design
Green onion and leaf lettuce experimental plots were set in 2011 and 2012 on a muck soil at the Research and Development Institute for the Agri-Environment (IRDA) research farm located in St-Bruno-de-Montarville (Quebec). Treatments were disposed in a randomized complete block design including 9 irrigation treatments repeated 4 times (Table 1). Two irrigations were done per season, referred as "Irrigation 1" and "Irrigation 2". In leaf lettuce experiment, irrigation 1 was performed 9 and 14 days before the first lettuce sampling in 2011 and 2012, respectively. In green onion experiment, irrigation 1 was performed 14 and 13 days before the first onion sampling in 2011 and 2012, respectively. Irrigation 2 was performed one day before the first vegetables sampling. Table 2 presents irrigation dates for lettuce and onion experiments. Three levels of *E. coli* in water were tested for both irrigation times:

- Low *E. coli* level : < 100 CFU /100 ml;
- Moderate *E. coli* level : between 100 and 500 CFU/ 100 ml;
- High *E. coli* level: > 500 CFU / 100 ml.

Plots were 4 m long and 4 m wide. Lettuce plots contained 8 rows of organic certified Tropicana green leaf lettuce. Plants were set 30 cm apart in the row, and rows were 50 cm apart, for a total of 112 plants per plot. Onion plots contained 5 rows of organic certified Parade bunching onion. Plants were 2.5 cm apart in the row, and rows were 38 cm apart, for a total of 800 green onions per plot. Lettuce and onion plots were 8 m apart, and replicates were 8 m apart.

Table 1. Irrigation treatments evaluated in green onion and leaf lettuce production.

Treatment	*E. coli* levels in water	
	Irrigation 1	Irrigation 2
1	low	low
2	low	moderate
3	low	high
4	moderate	low
5	moderate	moderate
6	moderate	high
7	high	low
8	high	moderate
9	high	high

Table 2. Irrigation dates for green onion and leaf lettuce experiments for both seasons.

	2011		2012	
	Irrigation 1	Irrigation 2	Irrigation 1	Irrigation 2
Leaf lettuce experiment	June 21	June 29	June 20	July 3
Green onion experiment	August 10	August 23	September 5	September 17

Soil was sampled before lettuce transplantation and green onion sowing in order to apply adequate fertilization for both productions. This sample was composed of fifteen subsamples (0-20 cm layer) taken in the whole field. Fertilizers application was based on soil analysis and plants requirements according to the "Centre de référence en agriculture et agroalimentaire du Québec" usually used by Quebec's agronomists and growers (CRAAQ, 2010). Soil physical and chemical analyses are presented in Tables 3 and 4 for lettuce and onion fields, respectively. Organic poultry manure pellets were used as fertilizer. A sample was taken in the spreader during land application for microbiological analyses.

Table 3. Soil physical and chemical analyses for the leaf lettuce field.[1]

	pH	buffer pH	OM[2]	P	K	Ca	Mg	Al	B	Cu	Fe	Mn	Zn	Na	N_{tot}	C/N
			%	Meh III (mg /kg)											%	
2011	5.5	5.4	80.1	18	240	8595	1209	738	0.686	1.16	659	15	20	14	1.37	31.2
2012	5.7	5.7	62.4	62	325	10120	1283	1202	0.983	2.27	825	13	26	21	1.41	-

[1]sample was taken in the the 0-20 cm surface layer.
[2] Organic matter.

Table 4. Soil physical and chemical analyses for the green onion field.[1]

	pH	buffer pH	OM[2]	P	K	Ca	Mg	Al	B	Cu	Fe	Mn	Zn	Na	N_{tot}	C/N
			%	Meh III (mg /kg)											%	
2011	5.8	5.7	74.3	46	243	9484	1176	813	0.868	1.43	751	11	18	15.1	1.33	32.4
2012	5.6	5.4	78.2	50	184	11025	1419	1227	0.935	1.51	1017	16	31	15.8	1.58	-

[1] sample was taken in the 0-20 cm surface layer.
[2] Organic matter.

Irrigation water was pumped from an aerated pond located on the experimental farm. Water was transferred into three 10 000 litres tanks. Hog and bovine liquid manures were added to the two tanks involving contaminated water (moderate and high levels). They were from a growing-finishing hog production, and a dairy farm. Liquid hog manure taken in this farm will be referred as “Liquid hog manure A”. In 2012, a mix of liquid hog manure coming from two farms was used to contaminate water. Liquid manure coming from the second farm (also from a growing-finishing production) will be referred as “Liquid hog manure B”. Liquid manure was used to contaminate water because it contains many strains of indicator microorganisms like *E. coli*, which have different survival capacity in the environment. Also, the ratio of pathogenic and indicator microorganisms counts is representative of a faecal contamination. Irrigations were performed using the Rain Jet system (Harnois industries) in order to irrigate each plot individually. A water height of 25 mm was applied at each irrigation, with a flow of 4.1 US gallons per minute (gpm).

Sampling

Liquid manures used to contaminate irrigation water were sampled two days before irrigation to determine required volumes for water contamination. Liquid hog manure samples were taken in sterile 1-liter polypropylene bottles directly in the underground holding tank. Liquid bovine manure samples were also taken in sterile 1-liter bottles near the pump installed in the storage tank while mixing.

Irrigation water samples were taken 3 times during irrigation in sterile 1-liter polypropylene bottles. While water was mixed in tanks using pumps, water samples were taken at the exit of the pump bringing water to plots.

Lettuce and onions were sampled 1, 3 and 7 days after the last irrigation, and will be referred as “sampling day 1”, “sampling day 3” and “sampling day 7”. Disposable gloves and boots were changed before entering each plot. Each produce sample consisted of three lettuce and fifteen onion plants aseptically taken in each plot paying particular attention to leave external rows and a 50-cm buffer zone at the end of each row untouched to minimize edge effect. Vegetables were cut into one gram pieces in the laboratory and homogenized to form a composite sample. These composite samples were used for microbiological analyses.

Soil samples were taken in each plot on sampling day 7. Each soil sample consisted of ten 20 cm-depth subsamples taken in each plot and homogenized to form composite samples. Subsamples were taken with an open ended soil probe. In order to reduce cross contamination between plots, probes were changed between each plot and new disposable plastic boots were also worn before entering a new plot.

All samples were kept at 4°C until analyses which were performed less than 48 hours after sampling.

Microbiological analyses
Samples were analyzed to determine:

1- generic *Escherichia coli* counts in poultry manure pellets, liquid manure, vegetables and soil samples using Petrifilm *E. coli* / coliforms count plate according to manufacturer's instructions and Health Canada MFHPB-34 method. *E. coli* counts in water were determined by the membrane filtration method using the modified mTEC agar according to the "Centre d'expertise en analyse environnementale du Québec" (CEAEQ) MA.700-Ec-mTEC 1.0 protocol.

An enrichment procedure was also performed on produce and soil samples with the Colilert medium. Briefly, peptone water 0,1 % (225 ml) was added to 25 g of sample and incubated overnight at 35° C. One millilitre of the culture was transferred in 9 ml of Colilert medium and tubes were bath-incubated 24 h at 35 ºC. Yellow and fluorescent tubes under a 6-watt, 365-nm UV light were considered as *E. coli*-positive. For each sample lot, negative (peptone water and colilert) and positive controls for *E. coli* (*E. coli* ATCC 25922) and total coliforms (*Klebsiella pneumoniae* ATCC 31488) were processed.
2- presence of *rfbE* gene (associated to *Escherichia coli* O157), and *stx1, stx2* and *eae* genes (associated to pathogenic *E. coli)* in poultry manure pellets, liquid manure, vegetables and soil samples using PCR procedures as previously described (Wang G. et al. 2002, J. Clin Microbiol 40:3613-3619; Beaudry et al. 1996 J Clin Microbiol 34:144-148; Woodward et al. 1992 Vet Micro 31:251-261) and following EcL laboratory procedures (http://www.apzec.ca/en/Protocols#). This analysis was performed in EcL laboratory at the veterinary medicine faculty from Université de Montréal, in St-Hyacinthe (Quebec), by Dr John. M. Fairbrother's team. Briefly, 25 g of sample were added to 225 ml of modified tryptic soy broth (mTSB) and incubated 24 h at 37 °C. For the irrigation water samples, 6 volumes of 50 or 100 mL, according to the turbidity of the samples, were filtrated, and then the membranes were placed in 75 mL of mTSB. After incubation, 1 ml of each enrichment was pelleted by centrifugation, washed twice with phosphate-buffered saline (PBS), resuspended in 0.5 ml of sterile deionized water and boiled for 10 minutes. The boiled cell suspensions were centrifuged and the resulting cell lysates were used as DNA template for PCR.

3- *Salmonella* spp. presence in poultry manure pellets, liquid manure, produce and soil samples using Health Canada MFLP-75 method and CEAEQ MA.700-Sal-PA 1.0 method for irrigation water samples. Briefly, an enrichment procedure was performed by adding 25 g of samples in 225 ml of tryptic soy broth (TSB) that was incubated at 35 °C for 18-24 h. For the irrigation water samples, 6 volumes of 50 or 100 ml, according to the turbidity of the samples, were filtrated, and then the membranes were placed in 75 ml of TSB. Following this pre-enrichment step, 6 drops of 30 µl of all TSB were transferred on a Modified Semi-solid Rappaport-Vassiliadis (MSRV) plate agar; and incubated 24h at 42 °C. If no growth was observed after 24 h, incubation was pursued up to 72 h. When the migration zone was higher than 2 cm, result was presumed to be positive. For all positive samples, inoculation of a Brilliance Salmonella chromogenic agar plate was performed an incubated 24 h at 35 °C. After incubation, purple colonies were considered as *Salmonella* spp. All the positive strains was confirmed by further biochemical testing, using the RapID ONE system and the serotype analysis of the sample with a polyvalent serum, *Salmonella* O Antiserum Poly A-1& vi. Positive samples were sent to the Laboratory for Foodborne Zoonoses of the Public Health Agency of Canada (PHAC) in Guelph (Ontario) for serotyping.

4- *Listeria monocytogenes* presence in poultry manure pellets, liquid manure, produce, soil and water samples using Health Canada MFHPB-30 slightly modified method. Briefly, 25 g of samples were aseptically transferred into 250 ml of UVM Modified *Listeria* Enrichment Broth (UVM) and incubated for 24 h at 30 °C. For irrigation water samples, 6 volumes of 50 or 100 ml of water, according to the turbidity, were filtrated and membranes were placed into 50 ml of UVM. After 24 h, 200 µl of all UVM enrichment broths were transferred into 10 ml of Fraser Broth and incubated for 24-48 h at 37 °C. Fraser selective enrichment was streaked onto Rapid 'L mono (RLM) plates when positive (brown-black color). RLM plates were incubated for 24-48 h at 37 °C. For each sample, *L. monocytogenes* presumptive blue without a yellow halo colonies were isolated and their identities were confirmed by PCR and PFGE procedures. PCR serovar identification procedure was performed in the laboratory of Dr Philippe Fravalo at the veterinary medicine faculty from Université de Montréal, in St-Hyacinthe (Quebec). PFGE pulsovars were determined in the « Laboratoire de santé publique du Québec » (LSPQ) in Quebec city.

For each analytical method, negative, positive and matrix controls were properly performed at each sample lot.

Statistical analysis
A generalized linear mixed model using SAS GLIMMIX procedure was performed to evaluate the impact of the treatments on *E. coli* prevalence. This model takes into account the Poisson distribution of the observations and the random effects of years, plots, subplots and subsamples within subplots, as described in Littell *et al.* (2006). Fixed effects of irrigation 1 and irrigation 2, and the sampling day were tested for significance with F tests and treatment mean counts were calculated and compared using contrasts.

Results
For 2011 and 2012 in lettuce and onion experiments, organic poultry manure pellets presented no generic *E. coli* and no pathogenic bacteria.

A. Leaf lettuce trial
Liquid manures used for water contamination

Microbial content of liquid manures used for water contamination of irrigated lettuce are shown in Tables 5 and 6 for 2011 and 2012 trials, respectively.

Salmonella was detected in liquid hog manure from farm A (2011) and farm B (2012), as well as in liquid bovine manure in 2011.

Listeria monocytogenes serovar IIa, PFGE pulsovar 23 and 380 were detected in liquid bovine manure used in 2012 for irrigation 1 and 2, respectively. Pulsovar 23 matched with human and food isolates previously described, while pulsovar 380 matched with human isolates but not food isolates, and is rarely found in Quebec.

In 2011, *rfbE* gene was detected in liquid hog manure A, and *eae* and *stx2* virulence genes were detected in liquid bovine manure. In 2012, the presence of *rfbE*, *eae* and *stx2* genes was detected in liquid hog manure A.

Table 5. Indicator and pathogenic microorganisms found in liquid manures used to contaminate irrigation water in leaf lettuce experiment in 2011.

	Liquid hog manure A		Liquid bovine manure	
	Irrigation 1	Irrigation 2	Irrigation 1	Irrigation 2
generic *E. coli* (CFU/g)	125 750	128 500	78 000	69 500
***Salmonella* presence**	√		√	√
***E. coli* O157 presence (*rfbE* gene)**		√		
***eae* + *stx1* or *stx2* genes presence**			√	
***L. monocytogenes* presence**			√	

Table 6. Indicator and pathogenic microorganisms found in liquid manures used to contaminate irrigation water in leaf lettuce experiment in 2012.

	Liquid hog manure A		Liquid hog manure B		Liquid bovine manure	
	Irrigation 1	Irrigation 2	Irrigation 1	Irrigation 2	Irrigation 1	Irrigation 2
generic *E. coli* (CFU/g)	180 000	167 000	1 150	2 500	7 300	7 400
***Salmonella* presence**			√	√		
***E. coli* O157 presence (*rfbE* gene)**		√				
***eae* + *stx1* or *stx2* genes presence**		√				
***L. monocytogenes* presence**					√	√

Irrigation water
Microbial content of irrigation water samples are shown in Tables 7 and 8 for 2011 and 2012 trials, respectively.

In 2011, *Salmonella* was detected in water containing moderate and high *E. coli* levels from irrigation 2. In 2012, this bacterium was recovered in water samples containing moderate and high *E. coli* levels from irrigation 1, and in water containing high *E. coli* levels from irrigation 2.

L. monocytogenes was detected in water from the 1st irrigation of both years, in water containing high *E. coli* levels. Serovar IIa, PFGE pulsovars 5 and 104 were detected in water (high *E. coli* level) from the 1st irrigation in 2012. Both pulsovars matched with human and food isolates previously described.

rfbE, eae, stx1 and *stx2* genes were not detected in water sampled in 2011. In 2012, *rfbE, eae* and *stx2* genes were isolated in water from the 2nd irrigation (high *E. coli* level).

Table 7. Indicator and pathogenic microorganisms found in irrigation water used in leaf lettuce experiment in 2011.

	Irrigation 1			Irrigation 2		
	low	**moderate**	**high**	**low**	**moderate**	**high**
generic *E. coli* (CFU/ 100 ml)	4	120	1 961	3	411	3 533
***Salmonella* presence**					√	√
***L. monocytogenes* presence**			√			

Table 8. Indicator and pathogenic microorganisms found in irrigation water used in leaf lettuce experiment in 2012.

	Irrigation 1			Irrigation 2		
	low	**moderate**	**high**	**low**	**moderate**	**high**
generic *E. coli* (CFU/ 100 ml)	10	437	3 167	2	248	3 767
***Salmonella* presence**		√	√			√
***E. coli* O157 presence (*rfbE* gene)**						√
***eae* + *stx1* or *stx2* genes presence**						√
***L. monocytogenes* presence**			√			

Lettuce

Over 216 lettuce samples taken in 2011 and 2012, *E. coli* counts could be determined in 12 samples, other samples being under the method detection limit, which is evaluated at 10 CFU/ g. Counts varied between 10 and 30 CFU/ g. These samples mostly came from plots that received moderate and high *E. coli* levels at irrigation 1 and 2, respectively. All samples met the maximum acceptable *E. coli* according to Health Canada's standards and guidelines for microbiological safety of food, i.e 100 CFU/ g of generic *E. coli* in fresh produce.

For both seasons combined, enrichment procedure revealed an *E. coli* prevalence of 54 % in lettuce samples. Statistical analysis on *E. coli* prevalence showed a statistically significant impact of sampling day according to *E. coli* levels in water for irrigation 2 (Tables 9 and 10). Distribution of these positive samples according to the *E. coli* levels in water on the last irrigation is shown in figure 1 for both seasons combined. For a low level of *E. coli* in water for irrigation 2, the probability to detect *E. coli* on lettuce was not influenced by the sampling day. For a moderate *E. coli* level in water, contrasts showed a statistically significant difference between sampling day 1 vs. 7 (P=0.0034, odds ratio = 13.3), and between sampling day 3 vs 7 (P=0.0092, odds ratio = 5.8). For a high *E. coli* level in water on irrigation 2, a statistically significant difference was established between sampling day 3 vs 7 (P= 0.0045, odds ratio = 24.4). *E. coli* prevalence in lettuce samples according to *E. coli* levels in water used for irrigation 2 for 2011 and 2012 is presented in figure 2.

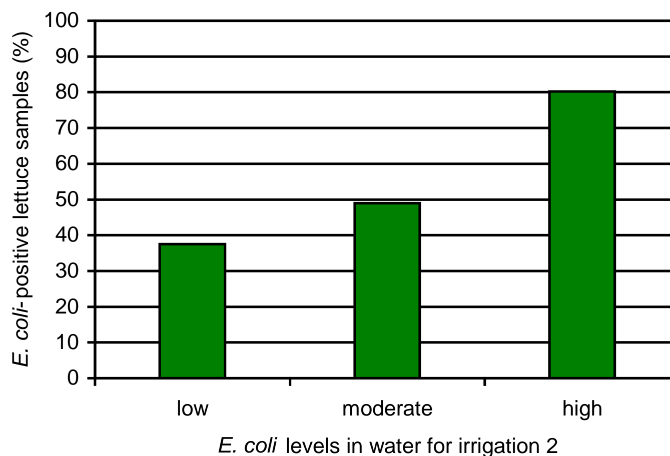

Figure 1. *E. coli* prevalence in lettuce samples according to *E. coli* levels in water on irrigation 2 for 2011 and 2012.

Table 9. Impact of *E. coli* levels in irrigation water and sampling day on *E. coli* prevalence in lettuce samples for both seasons.

Parameter	2011-2012	
	D.F. [1]	Pr>F
E. coli level in water for irrigation 1	1	0.1931
E. coli level in water for irrigation 2	2	0.2660
Sampling day	2	**0.0008**
E. coli level in water for irrigation 1 X sampling day	4	0.7316
E. coli level in water for irrigation 2 X sampling day	4	**0.0568**

[1] degrees of freedom.

Table 10. Impact of sampling day for different *E. coli* levels in water for irrigation 2 for 2011 and 2012.

Contrast label	D.F. [1]	Pr > | t |	Odds ratio
Low level, 1 day VS 3 days	165	0.2170	0.3643
Low level, 1 day VS 7 days	165	0.5976	0.6443
Low level, 3 days VS 7 days	165	0.3515	1.7687
Moderate level, 1 day VS 3 days	165	0.3106	2.2958
Moderate level, 1 day VS 7 days	165	**0.0034**	13.2888
Moderate level, 3 days VS 7 days	165	**0.0092**	5.7883
High level, 1 day VS 3 days	165	0.9776	97171
High level, 1 day VS 7 days	165	0.9714	2366137
High level, 3 days VS 7 days	165	**0.0045**	24.3501

[1] degrees of freedom.

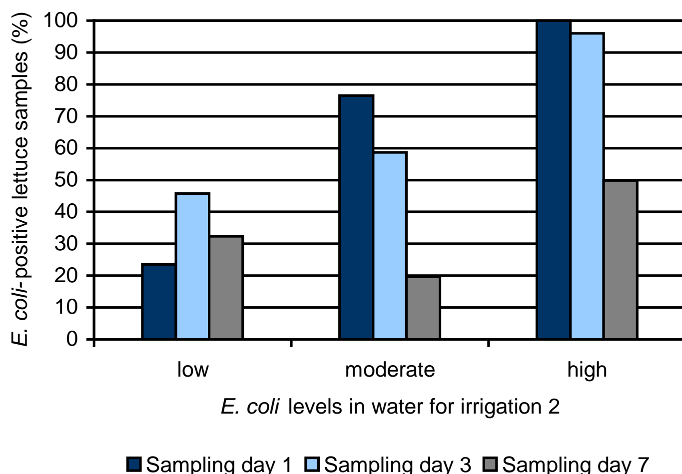

Figure 2. *E. coli* prevalence in lettuce samples according to sampling day and *E. coli* levels in water for irrigation 2 for 2011 and 2012.

All treatments combined, results showed an important impact of sampling day on *E. coli* prevalence on lettuce (*P*= 0.0008). In fact, the risk to detect *E. coli* on lettuce on sampling day 1 was 43 and 272-fold higher than on sampling day 3 and sampling day 7, respectively. Prevalence according to sampling day is shown in figure 3.

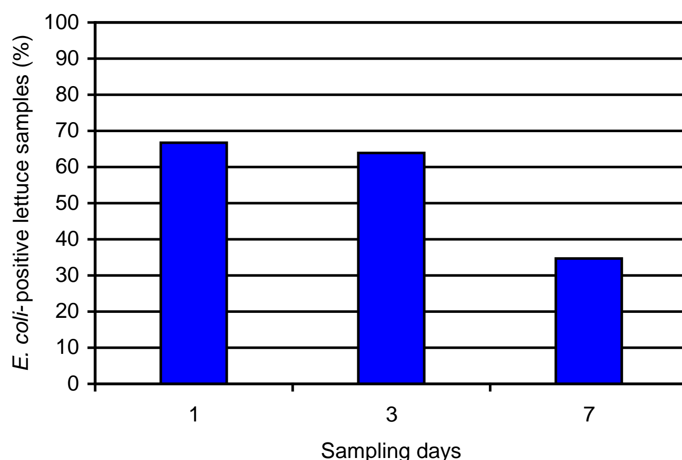

Figure 3. *E. coli* prevalence in lettuce samples according to sampling day for 2011 and 2012.

In 2011, no pathogenic bacteria were recovered in leaf lettuce samples. In 2012, one lettuce sample was positive to *Listeria monocytogenes.* This sample came from a plot where *E. coli* counts in irrigation water were 10 and 3 767 CFU/ 100 mL for the 1st and 2nd irrigation, respectively. This sample was taken on sampling day 3. This pathogen was not recovered in irrigation water used in this plot. However, liquid bovine manure used for the 2nd irrigation contained the bacterium. PCR characterization of isolates from lettuce and bovine manure indicated that they were both serovar IIa. PFGE results showed however different pulsovars, i.e 380 for manure and 22 for lettuce. Isolate detected in lettuce is frequently found in Quebec and matched with previously described human and food isolates. Two hypotheses can explain the difference of pulsovars encountered. First, manure can harbour many serovars that were not all recovered during manure analysis. Also, *Listeria monocytogenes* may be present in natural environments such as soil. However, analyses performed in soil samples taken in this plot did not support this hypothesis.

In 2012, another lettuce sample was found positive to *E. coli* O157 (*rfbE* gene). This sample was taken on sampling day 1 and came from a plot where *E. coli* counts in water were 437 and 3 767 CFU/100 mL for irrigation 1 and 2, respectively. *rfbE* gene was also detected in water sample from irrigation 2, as well as liquid hog manure used to contaminate water. Future characterization works will determine if these three isolates are identical.

<u>Soil</u>

Overall, generic *E. coli* populations were under the method theoretical detection limit (<10 CFU / g) in all soil samples (n= 72). In 2011, enrichment procedures revealed 26 generic *E. coli*-positive soil samples (n = 36). In 2012, 33 samples were found *E. coli*-positive (n=36). Statistical analysis showed a significant impact of *E. coli* levels in water for irrigation 2 on the probability to detect *E. coli* in soil samples (*P*=0.0199) (Table 11). In fact, *E. coli* prevalence in samples taken in plots which received the low *E. coli* level treatment on irrigation 2 was significantly different from samples taken in the moderate and high treatments (Table 12). Prevalence distribution according to *E. coli* levels in water for irrigation 2 is presented in figure 4. Pathogenic bacteria were never recovered in soil samples (n=72).

Table 11. Impact of *E. coli* levels in irrigation water on *E. coli* prevalence in soil for lettuce experiment for both seasons.

Parameter	2011-2012	
	D.F. [1]	**Pr>F**
E. coli level in water for irrigation 1	2	0.8884
E. coli level in water for irrigation 2	2	**0.0199**

[1] degrees of freedom.

Table 12. Comparison between *E. coli* levels in water for irrigation 2 on *E. coli* prevalence in soil for lettuce experiment for both seasons.

***E. coli* level in water for irrigation 2**	**D.F.** [1]	**Pr > | t |**	**Odds ratio**
Low level VS Moderate level	67	**0.0261**	0.133
Low level VS High level	67	**0.0261**	0.133
Moderate level VS High level	67	1.0000	1.000

[1] degrees of freedom.

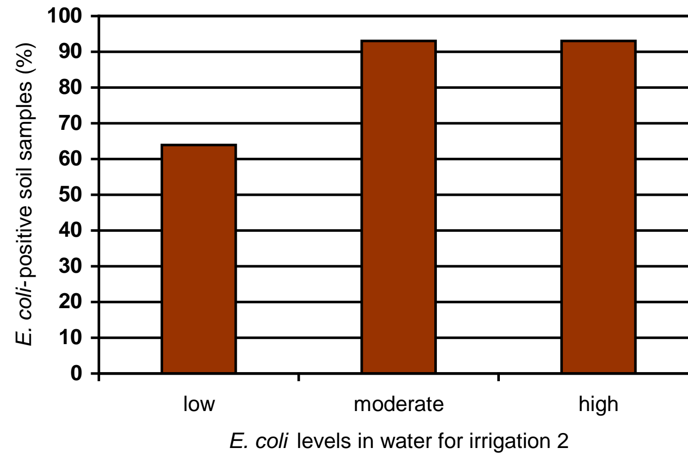

Figure 4. *E. coli* prevalence in soil samples according to *E. coli* levels in water for irrigation 2 in leaf lettuce experiment for 2011 and 2012.

B. Green onion trial
Liquid manures used for water contamination

Results for liquid manures used to contaminate irrigation water associated to green onion are shown in Tables 13 and 14 for 2011 and 2012, respectively.

Salmonella was detected in liquid hog manure from farm A in 2011 and from farm B in 2012. *rfbE, eae, stx1* and *stx2* genes were not detected in manures in 2011 and 2012. *Listeria monocytogenes* was detected in bovine manure in 2012.

Table 13. Microbial content of liquid manures used in green onion in 2011

	Liquid hog manure A		Liquid bovine manure	
	Irrigation 1	Irrigation 2	Irrigation 1	Irrigation 2
generic *E. coli* (CFU/g)	565 000	265 000	3 650	5 150
***Salmonella* presence**		√		

Table 14. Microbial content of liquid manures used in green onion in 2012

	Liquid hog manure A		Liquid hog manure B		Liquid bovine manure	
	Irrigation 1	Irrigation 2	Irrigation 1	Irrigation 2	Irrigation 1	Irrigation 2
generic *E. coli* (CFU/g)	63 000	47 000	2 700	20 000	21 000	70 000
***Salmonella* presence**			√		√	√
***L. monocytogenes* presence**						√

Irrigation water

Results for irrigation water samples associated to green onion trials are shown in Tables 15 and 16 for 2011 and 2012, respectively. No pathogenic bacteria were recovered in 2011. In 2012, *Listeria monocytogenes* was recovered in water containing moderate and high *E. coli* levels from the 2nd irrigation. *rfbE, eae, stx1* and *stx2* genes and *Salmonella* were not detected in 2012.

Table 15. Microbial content of irrigation water used in green onion trial in 2011

	Irrigation 1			Irrigation 2		
	low	**moderate**	**high**	**low**	**moderate**	**high**
generic *E. coli* (CFU/ 100 ml)	8	490	3 022	3	91	2 407

Table 16. Microbial content of irrigation water used in green onion trial in 2012

	Irrigation 1			Irrigation 2		
	low	**moderate**	**high**	**low**	**moderate**	**high**
generic *E. coli* (CFU/ 100 ml)	6	128	602	2	289	2 433
***L. monocytogenes* presence**					√	√

Onion

E. coli counts could be determined in 8 green onion samples and varied between 10 and 115 CFU/ g (n = 216). Other samples were under the method detection limit (<10 CFU / g). No link or tendency could be established between positive samples and irrigation treatments, as samples came from several treatments (Table 17). Only one sample exceeded the maximum acceptable *E. coli* level according to Health Canada's standards and guidelines for microbiological safety of food, i.e. 100 CFU/ g of generic *E. coli* in fresh produce. It came from a plot that received low and moderate *E. coli* levels at irrigation 1 and 2, respectively.

Table 17. *E. coli* counts in onion samples according to irrigation treatments in 2011 and 2012.

	***E. coli* counts in onion sample (CFU/ g)**	***E. coli* levels in irrigation water**		**Sampling day**
		Irrigation 1	**Irrigation 2**	
2011	10	low	low	1
	10	moderate	moderate	1
	115	low	moderate	7
	10	moderate	moderate	7
	10	moderate	high	7
2012	10	high	high	1
	70	moderate	high	3
	30	high	high	3

Enrichment procedures revealed an *E. coli* prevalence of 28 % in onion samples. Statistical analysis on enrichment results showed a high significant impact of *E. coli* level in water at the 2nd irrigation on the bacterial prevalence on green onion (P<0.0001) (Table 18). Distribution of positive samples according to the *E. coli* levels in water on irrigation 2 is shown in figure 4.

Table 18. Impact of *E. coli* levels in irrigation water and sampling day on *E. coli* prevalence in green onion samples for 2011 and 2012.

Parameter	**2011-2012**	
	D.F. [1]	**Pr>F**
Contamination level in water for irrigation 1	1	0.9913
Contamination level in water for irrigation 2	2	**<0.0001**
Sampling day	2	0.1540
Contamination level in water for irrigation 1 X sampling day	4	0.4269
Contamination level in water for irrigation 2 X sampling day	4	0.1564

[1] degrees of freedom.

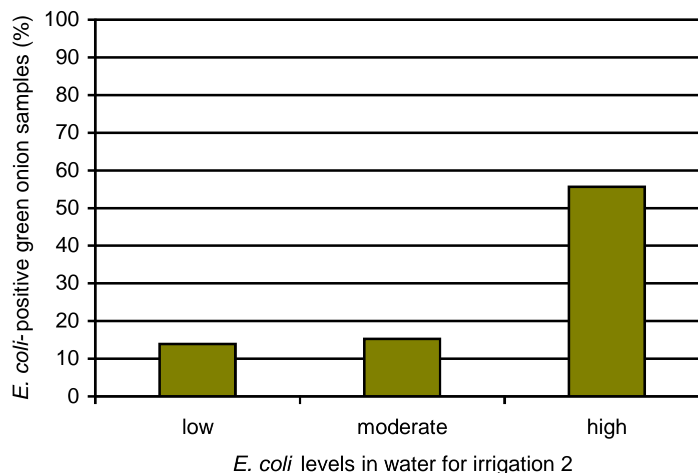

Figure 4. *E. coli* prevalence in green onion samples according to *E. coli* levels in water for irrigation 2 for 2011 and 2012.

Reduction of *E. coli* counts was observed between sampling day 3 and 7 after irrigation (figure 5), but this difference was not statistically significant at the 0.05 level (P= 0.0632). However, the risk to get onion contamination on sampling day 3 was 2.4-fold higher than on sampling day 7. *rfbE, eae, stx1* and *stx2* genes, *Salmonella* and *Listeria monocytogenes* were not recovered in green onion (n= 216).

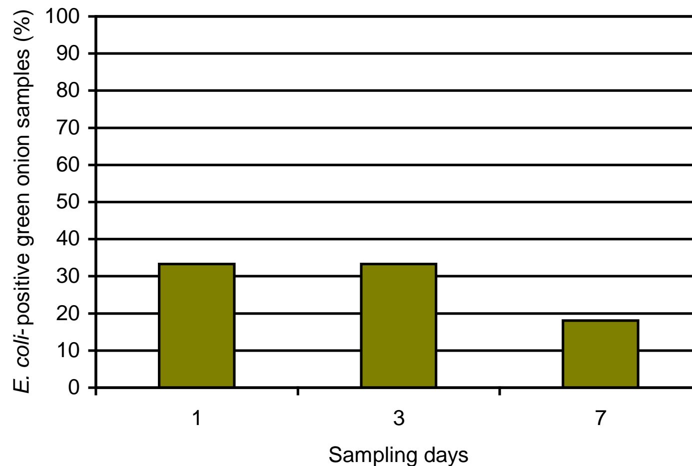

Figure 5. *E. coli* prevalence in green onion samples according to sampling day for 2011 and 2012.

<u>Soil</u>
Five soil samples presented countable *E. coli* populations, varying between 10 and 190 CFU / g (n = 72). Two of these samples came from plots that received moderate-moderate *E. coli* levels in water at irrigation 1 and 2, respectively. No link or tendency could be established between these samples and irrigation treatments. Other samples were under the method detection limit (<10 CFU / g).

Enrichment procedures revealed 51 *E. coli*-positive soil samples (n = 72). No statistical link was established between the level of *E. coli* in water on irrigation 2 and prevalence of *E. coli* in soil (P=0.1103) (Table 19). Distribution of these results is presented in figure 6. *Salmonella, rfbE, eae, stx1* and *stx2* genes and *Listeria monocytogenes* were never recovered in soil samples (n=72).

Table 19. Impact of *E. coli* level in irrigation water on *E. coli* prevalence in soil for onion experiment for both seasons.

Parameter	2011-2012	
	D.F. [1]	Pr>F
E. coli level in water for irrigation 1	2	0.8008
E. coli level in water for irrigation 2	2	0.1103

[1] degrees of freedom.

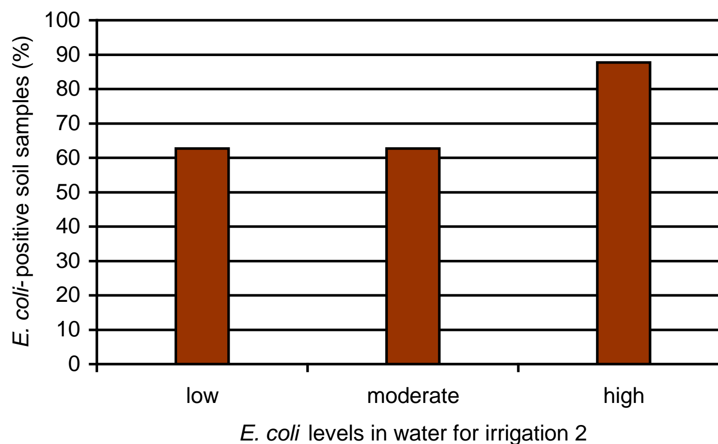

Figure 6. *E. coli* prevalence in soil samples according to *E. coli* levels in water on irrigation 2 in green onion trial for both seasons.

Summary:
Experimental plots were set in order to evaluate the impact of irrigation water *E. coli* levels and sampling day following the last irrigation on the presence of indicator and pathogenic microorganisms on green onion and leaf lettuce. Results showed that *E. coli* prevalence in leaf lettuce was affected by sampling day, while *E. coli* prevalence in green onion was highly affected by the *E. coli* level in water on the last irrigation. This study highlights that the delay between irrigation and harvest, combined to *E. coli* levels in irrigation water, should both be considered in produce safety risk management.