

Enteric bacterial contamination and survival on produce during irrigation with dairy wastewater in the field

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ABSTRACT

The goals of this study were to quantify enteric bacterial contamination and survival on several different types of produce during irrigation with wastewater from a dairy operation. Dairy wastewater was used to irrigate three different types of vegetable crops: lettuce, carrot, and bell pepper. This study was conducted over two consecutive growing seasons. Irrigation water and vegetable samples were examined for *Escherichia coli* and *Clostridium perfringens*. In the dairy wastewater, *E. coli* and *C. perfringens* concentrations averaged 8.2×10^7 MPN/100 mL and 5.0×10^4 CFU per 100 mL, respectively. Analysis of variance test results indicated that *E. coli* and *C. perfringens* concentrations detected on the three crops after irrigation were statistically different ($p < 0.0001$). The greatest contamination occurred on the carrots followed by lettuce and bell peppers. *E. coli* and *C. perfringens* were recovered from the carrots, bell peppers, and soil 49 days after wastewater irrigation of the plots had ceased. Moisture content of the soil was statistically significantly related to survival of the organisms in the soil.

Key words | *Clostridium perfringens*, dairy wastewater, *Escherichia coli*, irrigation water, lettuce, produce

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INTRODUCTION

It is estimated that approximately 10% of the irrigated crops in the world are irrigated with wastewater or sewage polluted surface waters (Jimenez *et al.* 2010). In some countries, 80% of the vegetable production is irrigated with wastewater (Pedrero *et al.* 2010). Plants might become contaminated in the field through the use of contaminated irrigation water (Beuchat & Ryu 1997; Horby *et al.* 2003; Brandl 2006). There have been several outbreaks associated with consumption of fresh produce that were traced back to the contaminated water used for irrigation in the USA (Ackers *et al.* 1998; Hilborn *et al.* 1999). Application of quantitative microbial risk assessment has been promoted as an approach to estimate risks from irrigation of food crops eaten raw when reclaimed wastewater is used (Bos *et al.* 2010; Mara & Bos 2010). This approach requires data on the transfer of

pathogens from the irrigation water to the crop and survival on the crop. Previous studies to obtain these data have largely relied upon laboratory studies and laboratory strains of indicator bacteria (*Escherichia coli*) or pathogens (Shuval *et al.* 1997). Other analysis has relied simply on theoretical estimates (Bos *et al.* 2010).

The goal of this research was to obtain data on transfer of two naturally occurring bacteria found in dairy wastewater, *E. coli* and *Clostridium perfringens*, onto three different vegetables whose edible parts were located in the soil, on the soil surface and above the soil surface. Dairy wastewater is currently used in Arizona to irrigate crops for animal consumption (Karpiscak *et al.* 2001). An additional purpose was to obtain data on survival of these organisms on plants growing in the field and in the soil. *E. coli* was selected because of its common use as an

indicator for enteric bacterial pathogens and *C. perfringens* because of its longer survival in the environment.

METHODS

This study was conducted at the University of Arizona Campus Agricultural Center in Tucson, AZ, USA. Three 156 m² plots parallel to each other were established for three vegetables. Dairy wastewater was collected from the Dairy Research Center, also located at the Campus Agricultural Center. The Dairy Research Center has a total of 200 milking cattle and 200 heads of young stock. The wastewater passed through a solid separator as the only treatment system. Wastewater was transported by a tanker truck to the field plots and then diluted 1:1 in a second tanker truck containing non-disinfected well water (producing diluted dairy wastewater) before application to the field. Water was applied by furrow irrigation. Dairy wastewater, diluted dairy wastewater, and well water samples were taken weekly during the irrigation of the vegetables and tested for *E. coli* and *C. perfringens*. Samples were transported in ice-packed coolers to the laboratory. Three vegetable varieties were chosen for this project: lettuce (*Lactuca sativa*), carrot (*Daucus carota*), and bell pepper (*Capsicum annuum*). This study was conducted over 2 consecutive years. In the first year growing season, diluted dairy wastewater was applied to vegetable plots weekly from May through July. Two harvests were made from each vegetable plot during this time, 2 weeks apart from late May to early July depending upon the crop. In the second year the growing season was extended an additional 5 weeks to assess survival of the bacteria on the crops and soil after irrigation with wastewater was terminated. During this part of the study, the fields were irrigated only with well water. Samples of produce and soil were placed in plastic bags and transported to the laboratory in ice-packed coolers. A total of 12 vegetable (four vegetables selected from each location) and soil (two soil samples selected from each location) samples were randomly collected from the plots for each harvest period from areas not previously sampled.

Irrigation water analysis

Dairy wastewater, diluted dairy wastewater, and well water were analyzed for the presence of *E. coli* and *C. perfringens*. Wastewater was serially diluted in Tris-buffered saline (Trizma base; Sigma, St Louis, MO, USA) and according to the manufacturer's instructions SimPlate (Biocontrol, Bellevue, WA, USA) in conjunction with Quanti-Tray™ (IDEXX Laboratories, Inc., Westbrook, MA, USA) was used to quantify *E. coli* in the wastewater samples. Results were reported as most probable number (MPN) per 100 mL of wastewater. A membrane filtration technique was used to detect *C. perfringens*. A total of 10 mL of wastewater was heat shocked at 75 °C for 20 min to kill the vegetative cells and stimulate vegetation of *C. perfringens* spores. Serial dilutions of the wastewater were made and these dilutions were filtered through a sterile 0.45 µm membrane filter (Millipore Inc., Bedford, MA, USA), and the membranes were transferred to mCP media (Acumedia, Acumedia Manufacturers, Inc., Baltimore, MD, USA) and incubated in an anaerobic jar for 24 h at 45 °C. Yellow colonies that turned pink to red after exposure to ammonium hydroxide vapor were considered as presumptive *C. perfringens* colonies.

Vegetable samples analysis

Each produce sample consisted of four randomly selected items (i.e., lettuce heads, carrots, peppers) from each of the experimental plots. The dimension of the lettuce heads was measured to determine the surface area of the processed sample. The four outermost layers of lettuce head were aseptically removed and weighed. The dimension of each individual carrot and bell pepper was measured and used to calculate the surface area for each sample. All produce samples were placed in a 4-L plastic beaker and phosphate buffered saline solution (0.85% NaCl in 0.02 M phosphate buffer, pH 7.4–8.0) was added at the ratio of 1:2 (weight of vegetables to volume of rinse solution). Bacteria on the produce surface were eluted by shaking the beakers on a horizontal shaker for 10 min at approximately 200 strokes per minute. The rinse solution was processed for the studied bacteria as described for wastewater analysis.

The efficiency of recovery of *E. coli* and *C. perfringens* was determined in laboratory experiments by adding known concentrations of the organisms to the different vegetables and processing as described in the previous paragraph.

Soil analysis

Two randomly selected soil samples from each plot were collected before and after the application of wastewater (total of six samples for each event). Ten grams of soil was placed inside an oven at 200 °C for 24 h to determine the dry soil weight. An additional 10 g of soil was mixed with 96 mL of 0.1% peptone water (0.1 g peptone in 100 mL distilled water) in a 250 mL centrifuge bottle. The bottle was shaken by hand for 30–60 s and then placed on a horizontal shaker and shaken for another 20 min. After shaking, the bottle was allowed to stand for approximately 30 s and serial dilutions were made by transferring 1 mL from the suspension to a 9 mL 0.1% peptone water blank. A multiple tube technique using EC medium with MUG (4-methylumbelliferyl-β-D-glucuronide) (Difco Laboratories, Detroit, MI, USA) was used for the detection of *E. coli*. Triplicate dilutions (equivalents of 1.0, 0.1, and 0.01 g) were placed in tubes with 10 mL of EC medium with MUG containing an inverted Durham tube and incubated at 44.5 °C for 18–24 h. After incubation, tubes showing gas formation and fluorescence under UV light were considered as positive for *E. coli*. *C. perfringens* was detected by the membrane filter method as previously described.

Survival experiments

Experiments were conducted to determine the persistence of the studied enteric bacteria on the produce and the soil after wastewater irrigation was terminated in the second year. Three carrot, bell pepper, and soil samples were taken from each treatment plot weekly from July 7 through August 20 during which time the plots were irrigated with well water. Samples were analyzed for the presence of *E. coli* and *C. perfringens*. Samples were processed and analyzed as previously described.

All positive Quanti-Tray™ for vegetable samples and positive tubes for soil samples were further confirmed using API 20 E biochemical test strips (bioMérieux Vitek,

Inc., Hazelwood, MO, USA). *C. perfringens* colonies on m-CP agar were verified by streaking the colonies onto blood agar plates (Difco Laboratories) and the formation of double hemolysis around colonies verified *C. perfringens*.

RESULTS

Efficiency of methods

The efficiency of recovery of the studied organisms is shown in Table 1. *E. coli* exhibited a greater range of recoveries than did *C. perfringens*.

Dairy wastewater, diluted wastewater, and well water

Samples were collected weekly for each type of water (dairy wastewater, diluted dairy wastewater, and well water). A total of 24 samples was collected during the first year (May through July), and 33 samples during the second year (April through June) for each type of water (Table 2). In the diluted wastewater, *E. coli* concentrations averaged 8.2×10^7 MPN/100 mL and *C. perfringens* concentrations averaged 5.0×10^4 CFU per 100 mL. Out of 57 well water samples examined, only eight were positive for *E. coli* at very low average concentrations (2.94 MPN/100 mL).

Produce

Lettuce, carrots, and bell peppers were positive for *E. coli* during both growing seasons. Arithmetic and geometric averages for each produce type over the two growing seasons, with two harvests per season, are shown in Table 3. Overall comparison of average *E. coli* concentrations on three vegetable crops indicates that the greatest numbers were detected on carrots, followed by lettuce and bell peppers. The percentage of positive samples for *E. coli*

Table 1 | Percentage recovery of *E. coli* and *C. perfringens* from vegetables

Vegetable	<i>E. coli</i> (%)	<i>C. perfringens</i> (%)
Lettuce	74.2 ± 9.6	46.1 ± 7.8
Carrot	66.6 ± 11	41.7 ± 10.3
Bell pepper	28.6 ± 1.6	63.1 ± 5.4

Table 2 | Concentration of *E. coli* and *C. perfringens* in dairy wastewater (WW), diluted dairy wastewater (DWW), and well water

	<i>E. coli</i> ^a			<i>C. perfringens</i> ^b		
	WW	DWW	Well	WW	DWW	Well
Arithmetic average	1.2×10^8	8.2×10^7	2.94	8.1×10^4	5.0×10^4	<1
Standard deviation	1.5×10^8	9.0×10^7	9.7	7.5×10^4	4.6×10^4	<1
Geometric average	1.4×10^7	1.5×10^7	1.3	4.8×10^4	2.6×10^4	<1
Standard deviation	85.5	23.7		3.5	4.5	

^aMPN/100 mL.^bCFU/100 mL.**Table 3** | *E. coli* and *C. perfringens* concentrations on lettuce, bell pepper, and carrot

Crop		<i>E. coli</i>		<i>C. perfringens</i>	
		MPN/g	MPN/cm ²	CFU/g	CFU/cm ²
Lettuce	Arithmetic average	332.9	46.8	2.0	0.1
	Standard deviation	1,078.4	105.4	4.5	0.3
	Geometric average	2.9	1.1	0.4	0.02
	Standard deviation	40.4	28.8	7.3	5.3
	Range	<0.02–4,800	<0.01–373.5	<0.02–29.2	<0.002–1.5
	% positive	81.2		87.5	
Bell pepper	Arithmetic average	3.5	2.5	0.21	0.2
	Standard deviation	12.6	8.8	0.5	0.4
	Geometric average	0.1	0.1	0.07	0.04
	Standard deviation	23.5	25.4	3.0	2.5
	Range	<0.02–70	<0.01–49	<0.02–3.6	<0.01–2.9
	% positive	60.4		75	
Carrot	Arithmetic average	12,700	11,571	14.7	11
	Standard deviation	37,639	34,147	9.7	7.2
	Geometric average	133	107.5	10.3	8.2
	Standard deviation	10.5	11.6	3.9	4.9
	Range	<0.02–140,000	<0.01–130,000	0.1–39.6	0.2–34
	% positive	98		100	

demonstrates the same trend, with most positive samples on carrots (98%), followed by lettuce (81%) and bell peppers (60%). The large standard deviations reflect the high viability of contamination of the produce, likely because of diurnal and weekly changes in temperature, relative humidity, and other environmental factors that affect survival and the nature of the random distribution of microbes in the environment. *C. perfringens* spores were detected on carrots, lettuce and bell peppers in both years. The highest percentage positive samples were observed in carrots (100%), followed by lettuce (87%), and bell peppers (75%).

Analysis of variance tests showed that *C. perfringens* and *E. coli* concentrations on three crops were statistically

different ($p < 0.0001$). Comparison of means of each pair by Student's *t*-test suggests that these means are significantly different for the different vegetables. This statistical analysis suggests that depending on where the edible part of the crops is situated (i.e., in the soil or above the soil) has a statistically significant effect on the degree of contamination on the surface of the vegetable crops.

Soil

Two soil samples were collected from each plot before and after application of dairy wastewater to the plots. Another set of samples was taken at the second harvest of each

vegetable. The results are summarized in Table 4. Prior to application of wastewater to the plots in the first year of study, no *E. coli* or *C. perfringens* were detected. However, prior to the application of the wastewater in the second year very low numbers of *E. coli* was detected (0.3 and 0.4 MPN/g), but no *C. perfringens*.

At the end of second year, *E. coli* and *C. perfringens* concentrations of 25 MPN/g and 133 CFU/g were detected in the experimental soil plots, respectively. There were positive correlations ($p < 0.05$) between *E. coli* and *C. perfringens* density and soil moisture content.

Table 4 | *E. coli* and *C. perfringens* in treatment and control plot soil before and after application of dairy wastewater

	<i>E. coli</i> ^a		<i>C. perfringens</i> ^b	
	Year 1	Year 2	Year 1	Year 2
<i>Before application of dairy wastewater</i>				
Arithmetic average	<1	0.4	<1	<1
Geometric average	<1	0.37	<1	<1
Standard deviation	0	0.3	0	0
<i>After application of dairy wastewater</i>				
Arithmetic average	280	24.5	387	133
Geometric average	174	5	375	37
Standard deviation	236	43	102	166

^aMPN/g.

^bCFU/g.

Survival study

To assess how long the bacteria would survive on the produce and soil in the second year wastewater irrigation was terminated after the second harvest and subsequent irrigation was with well water only. A total of eight bell pepper and carrot samples were collected weekly from experimental plots after wastewater irrigation had ceased. Lettuce was not studied as it was beyond the growing season in southern Arizona. *E. coli* and *C. perfringens* were recovered from all samples of vegetables and soil 49 days after wastewater flooding of the plots had ceased (Tables 5 and 6).

E. coli concentrations ranged from 0.2 to 124 MPN/g and 1 to 172 MPN/g on bell peppers and carrots, respectively (Table 5). Concentration of *C. perfringens* on bell peppers and carrot samples ranged from 0.02 to 0.8 CFU/g, and 1 to 11 CFU/g, respectively (Table 5). Increases on occasion in detected numbers of organisms on the plants probably reflect the randomness of the contamination from plant to plant.

DISCUSSION

Determining the degree of pathogen transfer from irrigation water and survival on produce is important in the development of risk-based standards for irrigation waters (Bos et al. 2010). There have been previous studies that used water seeded with laboratory-grown microorganisms or

Table 5 | Survival of *E. coli* and *C. perfringens* on bell peppers and carrots

Days after last wastewater irrigation	Bell pepper				Carrot			
	<i>E. coli</i>		<i>C. perfringens</i>		<i>E. coli</i>		<i>C. perfringens</i>	
	MPN/g	MPN/cm ²	CFU/g	CFU/cm ²	MPN/g	MPN/cm ²	CFU/g	CFU/cm ²
1	ND	ND	ND	ND	65.7	55.5	5.0	3.6
7	124.4	119.2	0.1	0.08	171.5	166.2	7.1	7.1
14	89.2	81.2	0.03	0.03	8.5	9.7	6.6	7.6
21	0.7	0.7	0.04	0.04	1.1	1.3	2.8	3.2
28	15.4	9.0	0.1	0.07	33.3	36.5	11.3	11.9
35	0.2	0.2	0.3	0.3	1.2	1.3	2.4	2.5
42	0.4	0.3	0.02	0.02	3.9	4.9	1.0	1.2
49	3.7	3.5	0.8	0.7	28	35.5	2.1	2.5

ND: not determined.

Table 6 | Survival of *E. coli* and *C. perfringens* in soil of bell peppers and carrots plots

Days after last wastewater irrigation	Bell pepper plots		Carrot plots	
	<i>E. coli</i> MPN/g	<i>C. perfringens</i> CFU/g	<i>E. coli</i> MPN/g	<i>C. perfringens</i> CFU/g
1	ND	ND	44.8	96.7
7	3.5	56.6	37.6	30.3
14	17.1	117.7	0.9	32.2
21	0.3	128.8	0.4	126.6
28	110	138.9	0.7	135.5
35	38.2	185.5	38.6	324.4
42	1.9	54.4	37.1	187.7
49	18.9	68.9	37.3	61.1

ND: not done.

partially treated wastewater as irrigation water to assess the degree of vegetable contamination (Tierney *et al.* 1977; Sadowski *et al.* 1978; Rosas *et al.* 1984; Armon *et al.* 1995; Bastos & Mara 1995; Oron *et al.* 2001; Solomon *et al.* 2002; Wachtel *et al.* 2002). The problem with laboratory-grown bacteria is that they were grown under nutrient-rich conditions and they need to adapt to low-nutrient conditions in the new environment which may cause them to die off faster than expected or their behavior may be different from indigenous microorganisms.

As might be expected, the more contact the crop had with the soil, the greater the contamination of the crop. Washing dirt off crops or in the case of lettuce removing the outermost leaves of the lettuce heads will reduce the microbial load but not necessarily eliminated it (Maxcy 1978; Fascioio *et al.* 2002). We have previously tested laboratory-grown *E. coli* seeded into irrigation water (non-wastewater) used for furrow irrigation of lettuce and bell pepper (Stine *et al.* 2005). In these studies, the transfer of *E. coli* to lettuce was found to be very low (0.00007%), and no transfer could be detected to the bell peppers. Only one irrigation event was conducted in those studies. Our current study also suggests low transfer, even through the produce was irrigated multiple times with wastewater during the growing season. However, *E. coli* were routinely detected at low levels on the bell pepper, which did not occur in our previous study with laboratory-grown *E. coli*.

Similar results were obtained by Rosas *et al.* (1984) when vegetables irrigated with untreated domestic wastewater

were tested for fecal coliforms. The fecal coliform values in wastewater ranged from 500 to 3,000 CFU/100 mL. The crops selected in that study were radishes, spinach, lettuce, parsley, and celery. The leaves, stems, and roots of each crop were examined separately. The results indicated that the crop roots had the highest concentration of fecal coliforms in comparison with leaf and stem due to their direct contact with the soil. Fecal coliforms were detected on the roots and the leaves 48–91% and 6–47% of the time, respectively. Among the leaves, the highest bacterial counts were encountered on leafy vegetables, i.e., spinach and lettuce with fecal coliform concentrations of 24 and 36 CFU/g, respectively. It was concluded that these high concentrations could be attributed to their surface area, which allow for greater capture of the bacteria.

There have been numerous studies on the occurrence of *C. perfringens* in meat, but little information is available on its occurrence on produce. A survey of marketed products conducted by Strong *et al.* (1963) reported that *C. perfringens* was detected on 3.8% of the fruits and vegetables versus 16.4% of meat products. *C. perfringens* has been used as an alternative indicator of fecal contamination of surface waters and has been suggested as a model for the environmental behavior of *Cryptosporidium* spores and other persistent environmental pathogens found in wastewater (Bitton 2001). There are several advantages over other fecal indicators: it is capable of surviving for long periods of time in the environment, does not grow in the environment, and it is present in both human and

animal feces. In this study, *C. perfringens* was the indicator organism most commonly isolated (compared with *E. coli*), probably due to the greater resistance of their spores.

Both indicator bacteria could be detected on both the carrots and bell peppers for 49 days after the last irrigation with wastewater. The numbers of *E. coli* on the crops declined after 3 weeks, but small numbers could still be detected after that time. The occurrence of *C. perfringens* on the crops remained largely unchanged during the study period. In contrast soil levels of *E. coli* were erratic and no trend was evident. The level of *C. perfringens* remained fairly constant showing a small decline after 5 weeks. Stine *et al.* (2005) found in control laboratory studies that *E. coli* declined at a rate of 0.79 log per day and *C. perfringens* at 0.14 log per day on bell peppers under humid conditions similar to our study; these inactivation rates were much greater than we observed. Naturally occurring bacteria exhibit a border range of genetic types, with some members of the population capable of surviving for prolonged periods of time.

Understanding the potential for persistence of enteric organisms in soil is important, because they could serve as a source to contaminate crops during rainfall events and harvesting. Our study involved furrow irrigation, which allows for greater potential contamination of the crops compared to drip or subsurface drip irrigation (Sadovski *et al.* 1978; Choi *et al.* 2004). Soil moisture, organic matter content, temperature (Zibilske & Weaver 1978; Oron *et al.* 2001), soil type, exposure to sunlight (Tannock & Smith 1972), and protozoan predation are the most important factors affecting survival of microorganisms in soil. Soil samples were taken from the close vicinity of the plants, which means the soil samples were shaded by plants and as a result remained moist between irrigation and protected from solar radiation. Another important factor is soil organic matter content which increases after application of wastewater. It has been shown that sewage application to soil generally promotes the numbers and survival of soil microorganisms by increasing soil organic matter (Tate 1978). Higher numbers of fecal coliforms (Malkawi & Mohammad 2003) and *E. coli* (Fascioio *et al.* 2002) were also found in soil samples irrigated with treated domestic non-disinfected wastewater than those irrigated with non-wastewater. In contrast to our findings

with *E. coli*, Malkawi & Mohammad (2003), who used surface drip irrigation, found a very rapid decline of fecal coliforms in the soil after irrigation of corn.

There were positive correlations ($p < 0.05$) between *E. coli* and *C. perfringens* density and soil moisture content. Soil samples were taken from the close vicinity of the plants, which means the soil samples were shaded by plants and as a result remained moist between irrigation and protected from solar radiation. Another important factor is soil organic matter content which increases after application of wastewater. It has been shown that sewage application to soil generally promotes the numbers and survival of soil microorganisms by increasing soil organic matter (Tate 1978).

The use of diluted dairy wastes is not recommended as indicated by this study, especially by flood irrigation. Such waste may contain enteropathogenic *E. coli*, *Cryptosporidium parvum* and other enteric pathogens. Our results shown that even crops whose edible portion is grown above the ground may become contaminated and that prolonged persistence of enteric bacteria can occur during the growing season even after wastewater irrigation has ceased. In risk assessment for wastewater reuse guideline development, various credits have been given for die-off pathogens on crops in the field (Bos *et al.* 2010; Mara & Bos 2010). Our results suggested that some low level persistence of enteric bacteria occurs after wastewater irrigation with little change, even in an arid climate like Arizona. Additional studies would be needed to assess low level survival of pathogens in the field. Our studies suggest some caution in using only laboratory data to judge the survival of enteric bacteria on produce in the field.

CONCLUSIONS

The potential for contamination of three types of vegetables, lettuce, carrots, and bell peppers, was assessed during surface irrigation with the diluted dairy wastewater. The findings of this study can be summarized as follows:

- The concentrations of both *E. coli* and *C. perfringens* on three crops were significantly different ($p < 0.0001$)

which reflected the distance the edible portion grew above the ground.

- The positive correlations ($p < 0.05$) between *E. coli* and *C. perfringens* density and soil moisture content indicates that the soil moisture has an important effect in occurrence and survival of these enteric microorganisms in soil.
- *E. coli* and *C. perfringens* survived on the vegetables and in soil for 49 days after wastewater application ceased.

REFERENCES

- Ackers, M. L., Mahon, B. E., Leahy, E., Goode, B., Damrow, T., Hayes, P. S., Bibb, W. F., Rice, D. H., Barrett, T. J., Hutwagner, L., Griffin, P. M. & Slutsker, L. 1998 [An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption](#). *J. Infect. Dis.* **177**, 1588–1593.
- Armon, R., Dosoretz, C. G., Azov, Y. & Shelef, G. 1995 Residual contamination of crops irrigated with effluent of different qualities: a field study. *Water Sci.* **29**, 2598–2600.
- Bastos, R. K. X. & Mara, D. D. 1995 The bacterial quality of salad crops drip and furrow irrigated with waste stabilization pond effluent: an evaluation of the WHO guidelines. *Water Sci. Technol.* **31**, 425–430.
- Beuchat, L. R. & Ryu, J. H. 1997 [Produce handling and processing practices](#). *Emerg. Infect. Dis.* **3**, 459–465.
- Bitton, G. 2011 *Wastewater Microbiology*, 4th edn. John Wiley, NY.
- Bos, R., Carr, R. & Keraita, B. 2010 Assessing and mitigating wastewater-related health risks in low-income countries: an introduction. In: *Wastewater Irrigation and Health* (P. Drechsel, C. S. Scott, K. Raschid-Sally, M. Redwood & A. Bahri, eds). Earthscan, London, pp. 29–47.
- Brandl, M. T. 2006 [Fitness of human enteric pathogens on plants and implications for food safety](#). *Ann. Rev. Phytopathol.* **44**, 367–392.
- Choi, C., Song, I., Stine, S., Pimental, J. & Gerba, C. 2004 Role of irrigation reuse: comparison of subsurface irrigation and furrow irrigation. *Water Sci. Technol.* **50**, 61–68.
- Fascioio, G. E., Meca, M. I., Gabriel, E. & Morabito, J. 2002 Effects on crops of irrigation with treated municipal wastewater. *Water Sci. Technol.* **45**, 133–138.
- Hilborn, E. D., Mermin, J. H., Mshar, P. A., Hadler, J. L., Voetsch, A., Wojtkunski, C., Swartz, M., Mshar, R., Lambert-Fair, M. A., Farrar, J. A., Glynn, M. K. & Slutsker, L. 1999 [A multistate outbreak of *Escherichia coli* O157:H7 infection associated with consumption of mesclun lettuce](#). *Arch. Intern. Med.* **159**, 1758–1764.
- Horby, P. W., O'Brien, S. J., Adak, G. K., Graham, C., Hawker, J. I., Hunter, P., Lane, C., Lawson, A. J., Mitchell, R. T., Reacher, M. H., Threlfall, E. J. & Ward, L. R. 2003 [A national outbreak of multi-resistant *Salmonella enteric* serovar Typhimurium definitive phage type \(DT\) 104 associated with consumption of lettuce](#). *Epidemiol. Infect.* **130**, 169–178.
- Jimenez, B., Drechsel, P., Krone, D., Bahri, A., Raschid, A. & Qadir, M. 2010 Wastewater sludge and excetra use in developing countries: An overview. In: *Wastewater Irrigation and Health* (P. Drechsel, C. S. Scott, K. Raschid-Sally, M. Redwood & A. Bahri, eds). Earthscan, London, pp. 3–27.
- Karpiscak, M. M., Sanchez, L. R., Freitas, R. J. & Gerba, C. P. 2001 Removal of bacterial indicators and pathogens from dairy wastewater by a multi-component treatment system. *Water Sci. Technol.* **44**, 183–190.
- Malkawi, H. I. & Mohammad, M. J. 2003 [Survival and accumulation of microorganisms in soil irrigated with secondary treated wastewater](#). *J. Basic Microbiol.* **43**, 47–55.
- Mara, M. & Bos, R. 2010 Risk analysis and epidemiology: the 2006 WHO guidelines for the safe use of wastewater in agriculture. In: *Wastewater Irrigation and Health* (P. Drechsel, C. S. Scott, K. Raschid-Sally, M. Redwood & A. Bahri, eds). Earthscan, London, pp. 51–62.
- Maxcy, R. B. 1978 Lettuce salad as a carrier of microorganisms of public health significance. *J. Food Prot.* **41**, 435–438.
- Oron, G., Armon, R., Mandelbaum, R., Manor, Y., Campos, C., Gillerman, L., Salgot, M., Gerba, C., Klein, I. & Enriquez, C. 2001 Secondary wastewater disposal for crop irrigated with minimal risks. *Water Sci. Technol.* **43**, 139–146.
- Pedrero, F., Kalavrouziotis, I., Alarcon, J. J., Koulakis, P. & Asano, T. 2010 [Use of treated municipal wastewater in irrigated agriculture-review of some practices in Spain and Greece](#). *Agric. Water Manage.* **97**, 1233–1241.
- Rosas, I., Baez, A. & Coutino, M. 1984 Bacteriological quality of crops irrigated with wastewater in the Xochimilcoplots, Mexico City, Mexico. *Appl. Environ. Microbiol.* **47**, 1074–1079.
- Sadovski, A. Y., Fattal, B., Goldberg, D., Katzenelson, E. & Shuval, H. I. 1978 High levels of microbial contamination of vegetables irrigated with wastewater by the drip method. *Appl. Environ. Microbiol.* **36**, 824–830.
- Shuval, H. I., Lampert, Y. & Fattal, B. 1997 Development of a risk assessment for evaluating wastewater reuse standards for agriculture. *Water Sci. Technol.* **35**, 15–20.
- Solomon, E. B., Potenski, C. J. & Matthews, K. R. 2002 Effect of irrigation method on transmission to and persistence of *Escherichia coli* O157:H7 on lettuce. *J. Food Prot.* **65**, 673–676.
- Strong, D. H., Canada, J. C. & Griffiths, B. 1963 Incidence of *Clostridium perfringens* in American foods. *Appl. Microbiol.* **11**, 42–44.
- Stine, S. W., Song, I., Choi, C. Y. & Gerba, C. P. 2005 The effect of relative humidity on preharvest survival of bacterial and viral pathogens on the surface of cantaloupe, lettuce, and bell peppers. *J. Food Protect.* **68**, 1352–1358.

- Tannock, G. W. & Smith, J. M. B. 1972 Studies on the survival of *Salmonella typhimurium* and *Salmonella bovis-morbificans* on soil and sheep faeces. *Res. Vet. Sci.* **13**, 150–153.
- Tate, R. L. 1978 Cultural and environmental factors affecting the longevity of *Escherichia coli* in histosols. *Appl. Environ. Microbiol.* **35**, 925–929.
- Tierney, J. T., Sullivan, R. & Larkin, E. P. 1977 Persistence of poliovirus 1 in soil and on vegetables grown in soil previously flooded with inoculated sewage sludge or effluent. *Appl. Environ. Microbiol.* **33**, 109–113.
- Wachtel, M. R., Whitehand, L. C. & Mandrell, R. E. 2002 Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. *J. Food Protect.* **65**, 18–25.
- Zibilske, L. M. & Weaver, R. W. 1978 [Effect of environmental factors on survival of *Salmonella typhimurium* in soil.](#) *J. Environ. Qual.* **7**, 593–597.

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