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REVIEW ARTICLE

Control of enteric pathogens in ready-to-eat vegetable crops in organic and 'low input' production systems: a HACCP-based approach

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Summary

Risks from pathogens such as *Salmonella*, *Yersinia*, *Campylobacter* and *Escherichia coli* O157 have been identified as a particular concern for organic and 'low input' food production systems that rely on livestock manure as a nutrient source. Current data do not allow any solid conclusions to be drawn about the level of this risk, relative to conventional production systems. This review describes six Risk Reduction Points (RRPs) where risks from enteric pathogens can be reduced in ready-to-eat vegetables. Changes can be made to animal husbandry practices (RRP1) to reduce inoculum levels in manure. Outdoor livestock management (RRP2) can be optimized to eliminate the risk of faecal material entering irrigation water. Manure storage and processing (RRP3), soil management practices (RRP4) and timing of manure application (RRP5), can be adjusted to reduce the survival of pathogens originating from manure. During irrigation (RRP6), pathogen risks can be reduced by choosing a clean water source and minimizing the chances of faecal material splashing on to the crop. Although preventive measures at these RRPs can minimize enteric pathogen risk, zero risk can never be obtained for raw ready-to-eat vegetables. Good food hygiene practices at home are essential to reduce the incidence of food-borne illnesses.

Introduction

Organic farming standards prohibit the use of chemosynthetic fertilizers and pesticides, but recommend regular organic matter-based fertility inputs into soils. An integrated/holistic approach to fertilization and crop protection is used to ensure food quality and safety (Cooper *et al.* 2007 for a recent review and Fig. 1 for the logical framework for organic crop production). Organic standards also prescribe that livestock is reared outdoors for extended periods (Cooper *et al.* 2007).

The lower risk of pesticide residues in organic produce and consumer perceptions about animal welfare benefits of outdoor livestock rearing systems have significantly contributed to the increase in demand for organic foods (Benbrook 2007). In addition, the benefits of regular organic matter inputs to the structural stability, biological activity, organic matter content and inherent fertility of soils are widely acknowledged (Reganold *et al.* 1987, 1993; Mäder *et al.* 2002).

However, the use of animal manure-based fertility inputs in organic fruit, vegetable and salad crops has resulted in concerns about an increased risk of enteric pathogens entering the food supply chain (Trewavas 2001, 2004). In addition, a recent report into an *E. coli* O157:H7 outbreak associated with spinach in the USA, highlighted the potential pathogen-transfer risks associated with outdoor/free-range livestock production (US Food and Drug Administration 2006; California Department of Health Services 2007).

Enteric bacterial pathogens such as *Salmonella, Yersinia, Campylobacter* and *E. coli* O157 currently pose the greatest

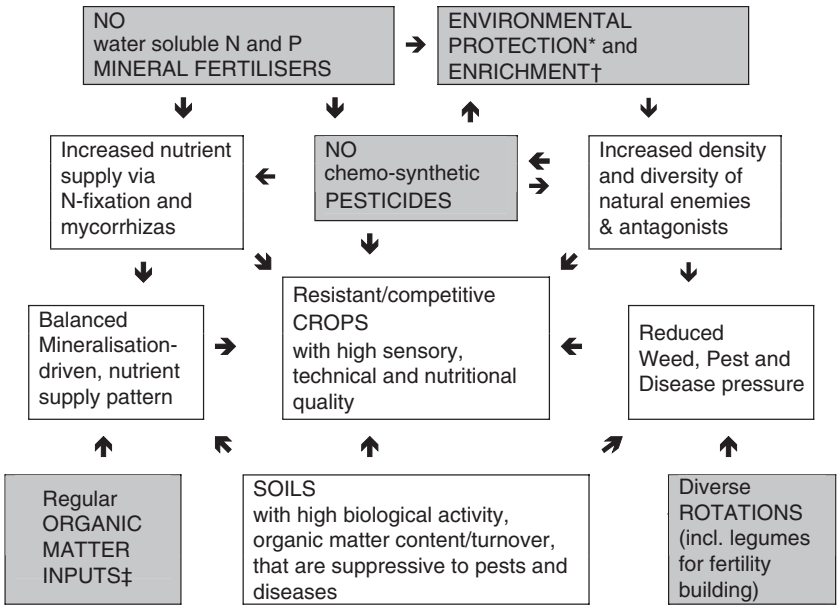

Figure 1 Logical Framework for organic (and other 'low input') crop production systems (redrawn with permission from Cooper *et al.* 2007). Agronomic practices prescribed or recommended under organic and other low-input farming standards. Positive impacts on soils, crops and the environment associated with agronomic practices in organic and other low-input food production systems. ↓↑ ← → = positive effects or impacts. *Measures to minimize pollution (e.g. N-leaching and P-run-off) and soil erosion events associated with agricultural activities (e.g. noncropped field margins, fertility catch crops). †Measures taken to increase biodiversity on farms (introduction of noncrop vegetation e.g. woodlands, hedges, noncropped field margins, beetle-banks). ‡Animal and green manures, manure and waste-based composts, but no sewage, sewage sludge or sewage based composts.

food-borne risk of serious illness on an international scale (Fernandez-Alvarez *et al.* 1991; Beuchat 1996). The main focus of this review will therefore be to (i) carry out a Hazard analysis on the risk for enteric bacterial pathogen transfer at different steps/points in organic and low-input primary production systems and (ii) identify approaches/methods that can be used to minimize risks of pathogen transfer onto ready-to-eat crops. Risks associated with viral protozoal and mycotoxin-producing fungi are discussed in detail elsewhere (see Cooper *et al.* 2007 for recent reviews). We will use the term 'Risk Reduction Point' or 'RRP' (rather than Critical Control Point or CCP) to describe the different primary production steps/points associated with risk, to reflect the fact that complete control is unlikely to be possible at any primary production steps/points (=RRPs) associated with risk (see Table 3 for definitions of RRPs and CCPs).

Several approaches can be taken to assess the potential risks of pathogen transfer into the supply chains for organic and low input-foods. These include:

1. Statistics/literature reviews of reported cases of food-borne diseases where the source of infection has been confirmed (Nguyen-the and Carlin 1994; O'Brien *et al.* 2000; Food Standards Agency 2001a, 2007; Adak *et al.* 2005; Kopke *et al.* 2007).

2. Microbiological assessments/surveys of contamination levels in specific foods (O'Brien *et al.* 2000; Food Standards Agency 2001a,b; Sagoo *et al.* 2001b).

3. Hazard analysis-based risk assessments which evaluate/compare risks of pathogen contamination at specific 'Risk Reduction Points' during primary production, and 'Critical Control Points' during processing and all other stages of the food supply chain (e.g. transport, storage and display in supermarkets and food storage or preparation at home).

According to recent statistics of food-borne diseases in the UK *Campylobacter* spp. causes the highest number of infections (340 000) and the highest number of hospital days (60 000), while nontyphoidal salmonellae and *Clostridium perfringens* cause the highest number of deaths (209 and 177, respectively). *E. coli* O157:H7 infections, while being relatively rare (approx. 1000 cases) result in the highest death rate (2%) (Adak *et al.* 2005).

Adak *et al.* (2005) report that most infections are caused by foods of animal origin (meat, fish, eggs and dairy products; 65% of cases and 68% of deaths) and most of the remaining cases/deaths are associated with consumption of complex foods (e.g. ready meals; 26% of cases and deaths) (Table 1). Similar trends are also

Table 1 Annual cases and deaths because of food-borne diseases in England and Wales, by food group and type (1996–2000) (data from Adak *et al.* 2005)

	Reported cases (per annum)		Deaths (per annum)	
Food group/type	No.	%	No.	%
Animal product-based foods	1 118 505	65	468	68
Poultry/eggs	605 374	35	237	35
Red meat	287 485	17	164	24
Beef	115 929	7	67	10
Pork	46 539	3	24	4
Bacon/ham	17 450	1	9	1
Lamb	46 239	3	27	4
Mixed/unspecified	61 329	4	36	5
Milk/dairy products	116 837	7	42	5
Seafood	116 603	7	30	4
Shellfish	77 019	4	16	2
Fish	22 311	1	10	2
Mixed/unspecified	17 273	1	4	1
Plant product-based foods	64 477	4	19	3
Salad vegetables (ready-to-eat)	37 496	2	11	2
Cooked vegetables	6870	0	2	0
Fruit	5275	0	1	0
Rice	26 981	2	5	1
Complex foods*	453 237	26	181	26
Infected food handler	67 157	4	14	2
Total	1 724 315		687	

*Multicomponent/processed foods (e.g. ready meals).

reported in more recently published statistics for the UK covering the period between 1992 and 2003 (Hughes *et al.* 2007) and for the USA (Sharp and Reilly 1994; Mead *et al.* 1999).

Among the foods of animal origin, poultry and eggs are associated with the highest risk of food-borne disease (35%), hospitalization (48%) and deaths (35%) (Adak *et al.* 2005). Most infections associated with poultry are due to *Salmonella* and *Campylobacter* species. While improved quality assurance in farm to retail processing introduced in the mid-1990s has successfully reduced salmonellosis in the UK and other developed countries, these measures have had little effect on *Campylobacter* infection rates, which have increased over the last 20 years (Engvall and Andersson 1999; Adak *et al.* 2002; Wegener *et al.* 2003; Food Standards Agency 2004; Patrick *et al.* 2004; Hughes *et al.* 2007).

In contrast, vegetables and fruits accounted for only 3% of cases and 2% of deaths in the Adak *et al.* (2005) study, with the majority of cases/deaths associated with salad vegetables (Table 1). Overall, the authors concluded that '*vegetables and fruits had the lowest disease and hospitalization risks*' and that '*disease linked to plant-based food had a minor impact on the population*' (Adak *et al.* 2005).

However, surveys have indicated that a wide range of raw fruits and vegetables can be contaminated with potential human pathogens, and outbreaks of gastrointestinal illness caused by bacteria, viruses and parasites have been reported for different intact or processed vegetables and, to a lesser extent, fruits (Adak *et al.* 2005). Products associated with the highest risk of enteric pathogen contamination are sprouted seeds and unpasteurized fruit juices (Griffin 1998; Hilborn *et al.* 1999; Doyle 2000b). For these products, the EU has introduced specific microbiological standards (European Commission 2005).

In the majority of reports that identified fruits and vegetables as the source of food-borne disease, these foods came from conventional systems, although some reports identifying organically produced plant foods as sources of gastrointestinal diseases are available (Haward and Leifert 1999; Kopke *et al.* 2007). The organic plant foods most frequently associated with food-borne diseases such as salmonellosis and *E. coli* O157:H7 are 'ready-to-eat' bean sprouts and lettuce products (Doyle 2000b). Because of the minor contribution of vegetables and fruits to food-borne disease in the developed world and the low level of organic food consumption (<4% in most European countries; Soil Association 2006), it is unclear whether organic or conventional foods in the diet were responsible for the reported food-borne disease incidences. As a result it is currently impossible to make comparative quantitative assessments of the proportion of food-borne disease caused by fruits and vegetables from organic, low-input and conventional production systems (Haward and Leifert 1999; Adak *et al.* 2005).

There is, to our knowledge, only one large-scale UK survey in which the microbiological status of uncooked ready-to-eat organic vegetables collected from retail establishments was examined (Table 2) (O'Brien *et al.* 2000; Sagoo *et al.* 2001a). This study showed that of the 3200 samples examined, 99·5% were of satisfactory or acceptable quality, while 0·5% were of unsatisfactory quality (because of the detection of *E. coli* or *Listeria* spp. at levels in excess of 10^2 CFU g^{-1}). None of the samples were of unacceptable microbiological quality and the authors concluded that '*overall agricultural, hygiene, harvesting and production practices were good*' in organic systems. A less extensive survey (examining 85 vegetable samples from retail outlets in the UK) carried out during the same time period (McMahon and Wilson 2001) did not detect *Salmonella, Campylobacter* and *Listeria* spp. or *E. coli* and *E. coli* O157 in any sample, thus confirming results from the Sagoo *et al.* (2001a) study.

In contrast to these findings, for certain 'high-risk' ready-to-eat crops (in particular, lettuce and bean

Table 2 Microbiological quality of ready-to-eat organic vegetables based on the 2000 PHLS Microbiological Guidelines (Sagoo *et al.* 2001b)

Parameter	Microbiological quality classification			
	Satisfactory	Acceptable	Un-satisfactory	Un-acceptable*
No. of samples	3146	39	15	0
Percentage of samples	98·5	1·0	0·5	0
Key to classification of microbiological quality (CFU g^{-1} unless stated)				
Escherichia coli	<20	20 to $<10^2$	$\geq 10^2$	NA†
Listeria spp.	<20	20 to $<10^2$	$\geq 10^2$	NA
Listeria monocytogenes	<20	20 to $<10^2$	NA	$\geq 10^2$
Campylobacter spp.	Not detected‡	Not detected‡	Not detected‡	Detected‡
Salmonella spp.	Not detected‡	Not detected‡	Not detected‡	Detected‡
E. coli O157	Not detected‡	Not detected‡	Not detected‡	Detected‡

Seventy-four per cent of vegetables examined were grown in close proximity or in contact with the soil (e.g. broccoli, cabbage, carrot, cauliflower, celeriac, celery, cress, lettuce, mushrooms, radish, spring onions, watercress), the remainder were nonsoil salad vegetables (e.g. cucumber, pepper, tomato).

*Potentially hazardous.

†NA, not applicable.

‡In 25 g of vegetable tissues.

sprouts) some small-scale microbiological surveys in the US have indicated that a higher frequency of contamination and/or higher concentration of enteric pathogens may be present in organically produced crops compared with those produced conventionally (Griffin 1998; Hilborn *et al.* 1999; Doyle 2000a). For example, *Salmonella* was detected in 3 of 39 samples of organic bean sprouts, but in none of the 39 samples of conventional sprouts. In addition, generic *E. coli* was present in 8 of 48 samples of organic 'spring mix' lettuce (average count of 10^6 CFU g^{-1}), but only in 4 of 48 samples of conventionally produced lettuce (average count of 10^4 CFU g^{-1}). The authors suggested that these differences may be due to the use of organic fertilizers and/or the inability to use hypochlorite as a disinfectant in the processing of lettuce and bean sprout products under organic production standards. The sample numbers in these studies were low and too few producers were included in the surveys to draw solid conclusions about the relative risks associated with organic and conventional products. Nevertheless, these studies indicate the areas of potential risk and should trigger more extensive surveys of such 'high risk' products.

As the currently available survey-based data do not allow relative risks associated with organic, 'low input' and conventional produce to be compared, this review will focus on describing HACCP (Hazard Analysis Critical Control Point)-based quality assurance systems used in organic and other 'low input' food production systems (Haward and Leifert 1999; Kopke *et al.* 2007). This is based on a Hazard Analysis and the identification of 'Risk Reduction Points' (Table 3) at which risks can be reduced in primary production. The risks associated with different RRPs and the approaches taken to minimize microbiological hazards will be described in separate sections for the different RRPs below.

Risk reduction points in organic and low-input production systems

RRP1 animal husbandry

Animals can shed significant numbers of pathogens in their faeces. Many of the enteric pathogen species causing disease in humans, are also known to cause gastrointestinal diseases in farm animals (e.g. *E. coli* and *Salmonella* strains can cause diarrhoea in pigs, poultry and calves) and faecal shedding rates increase significantly in diseased animals (Engvall and Andersson 1999; Hoyle *et al.* 2004; Bonde and Sørensen 2007; Diez-Gonzalez 2007; Hirt *et al.* 2007). The health status of livestock herds/flocks is therefore the first RRP at which the risk for pathogen transfer into the food chain can be reduced.

A range of enteric bacteria (*Campylobacter* spp., *Salmonella*, *E. coli* O157) that can cause serious diseases in humans, do not cause disease, but are part of the commensal gut flora and are present at relatively high numbers (10^2–10^7 CFU g^{-1}) in the faeces of healthy animals (Himathongkham *et al.* 1999; Sahin *et al.* 2002; Newell and Fearnley 2003). This is of particular concern for *E. coli* O157, which can infect humans when present in very low levels (<100 CFU g^{-1}) in food, and is associated with the highest proportion of hospitalization and death rates (Adak *et al.* 2005).

Table 3 Risk reduction points (RRPs) for pathogen transfer from manure-based fertilizers onto crops (revised from Haward and Leifert 1999)

No.	Description	Strategies prescribed or recommended (e.g. by organic farming standards/principles) to reduce pathogen transfer risks at the RRP
RRP1	Livestock husbandry	Use of appropriate: feeding regimes (e.g. high dietary forage intake), rearing systems (e.g. housing, bedding, stocking density), breed choice (e.g. robust and locally adapted breeds), health management (e.g. minimum antibiotic use, integration of preventative, management and intervention-based approaches)
RRP2	Outdoor livestock management	Remove and keep livestock out of fields used for ready-to-eat vegetable crops for at least 9 months prior to planting of such crops. Do not put livestock into fields neighbouring ready-to-eat vegetable crops. Omit livestock from fields where run-off may potentially reach (i) ready-to-eat vegetable crops or (ii) surface water used for irrigation
RRP3	Manure storage and processing	Manure from intensive (e.g. cage) based conventional rearing units not permitted. Composting for 6 months prescribed for manure from acceptable conventional systems. Controlled (e.g. windrow or in-vessel) composting systems recommended for high-risk vegetable/salad crops. Controlled composting systems prescribed for mushroom crops. Production and use of slurry is discouraged. Use of human manure/slurry not permitted
RRP4	Soil management	Long rotations, regular organic matter inputs and green manures are prescribed to achieve high soil biological activity and structural stability. Inversion-ploughing to burry manure
RRP4·1	Soil moisture/matric potential	
RRP4·2	Soil type & pH	
RRP4·3	Soil temperature	
RRP4·4	Soil biological activity and organic matter content	
RRP5	Manure addition to soil	Application of manure to a growing crop are not permitted. Manure has to be applied 6 months prior to planting of ready-to-eat vegetable/salad crops. Inversion-ploughing to bury manure recommended. Livestock has to be removed from field 9 months prior to planting of crops
RRP6	Irrigation	Where there is a risk of contamination from livestock faeces, manure or sewage, surface water should not be used for irrigation of ready-to-eat vegetables. Irrigation water from deep wells recommended. The microbiological quality of water used for irrigation should be monitored regularly. Where possible minimum or tape irrigation should be used
RRP6·1	Source/type of irrigation water	
RRP6·2	Application of irrigation water	
RRP7	Handling of crops and harvest	Ensure that machinery used for mechanical weeding and harvest is not contaminated with faecal materials. Operator hygiene should be ensured according to established guidelines (Stanley and Knight 2000)

Primary production – RRPs: RRPs are points, stages or practices in the primary production system where measures can be applied to reduce the likelihood of enteric pathogens being transferred onto produce preharvest.
Postharvest/processing – critical control points (CCPs): CCPs are raw materials, locations, practices, formulations or processes where measures can be applied to prevent or minimize the likelihood of enteric pathogens being present at unacceptable levels.
Postharvest safety assurance is not the subject of this review; for details see Knight and Stanley (2000) and ILSI (2004).

Risks associated with cattle

Most disease outbreaks of *E. coli* O157 have been associated with beef, beef products and, to a lesser extent, dairy products, and so most studies of faecal shedding in cattle have focussed on this organism. Several studies have indicated that increasing the proportion of forage (e.g. fresh grass or hay) can reduce the population density of *E. coli* in the colon and rumen of cattle and the proportion of cows with detectable levels of *E. coli* O157 in their faeces (see Diez-Gonzalez 2007 for a recent review). High-concentrate diets are known to increase the acidity in the gastrointestinal system, which is thought to increase the relative competitiveness of *E. coli*, one of the most acid-tolerant enteric bacterial species (Diez-Gonzalez *et al.* 1998; Diez-Gonzalez 2007). The level of forage in the ruminant diets tends to be higher in organic than conventional systems, which may indicate a lower level of risk of *E. coli* being present in the faeces. However, some studies reported no effect of dietary forage levels on pathogen shedding, while others indicated that the type of concentrate also affects the levels of *E. coli* and/or human pathogens (Diez-Gonzalez 2007). A range of feed additives or treatments (including sodium chlorate, antibiotics, bacteriophages and probiotics) have also been investigated as control methods for *E. coli* O157 in cattle,

Table 4 Effect of organic and conventional outdoor and indoor production systems on the proportion of *Salmonella enterica* antibody-positive pigs and pigs shedding *Salmonella* spp. on farm and at slaughter (QLIF WP2·2·3; modified from Bonde and Sorensen 2007)

Characteristic assessed	Organic outdoor	Conventional		ANOVA (*P*-value)
		Outdoor	Indoor	
Herd faecal samples (on farm)				
No. of samples*	593	615	610	<0·0001
Salmonella-positive samples (%)	0·2	0·2	2·6	
Duration of transport between farm and abbatoir (min)	175	129	97	<0·0001
Abattoir faecal samples				
No. of samples*	537	555	474	<0·01
Salmonella-positive samples (%)	1·9	1·1	4·0	
Meat juice test (ELISA *S. enterica* O-antigens)				
No. of samples*	559	558	484	NS
Antibody-positive pigs (%)	9·3	7·2	6·8	

*Up to ten animals per herd were sampled.

but only one probiotic feed additive based on *Lactobacillus acidophilus* has shown satisfactory activity (see LeJeune and Wetzel 2007 for a recent review).

A recent survey of ampicillin resistance in faecal *E. coli* in Scotland reported a significantly lower prevalence of ampicillin resistance in organic compared with conventionally reared calves, which was correlated to the lower levels of antibiotic use in organic farms (Hoyle *et al.* 2004) The risk of antibiotic-resistant pathogen strains being present in cattle faeces may therefore be lower in organic production.

Risks associated with pigs

In pigs, most studies have focussed on *Salmonella* shedding, as pork and pork products are recognized as major sources of human salmonellosis (Wegener and Baggesen 1996; Lo Fo Wong *et al.* 2002). The percentage of seropositive pigs and levels of *Salmonella* antibodies were found to be higher in outdoor-compared with indoor-reared pigs (Jensen *et al.* 2004) and it has been assumed that the risk of faecal *Salmonella* shedding is also higher in organic and free-range/outdoor production systems.

However, recently, a large-scale Danish survey (Bonde and Sorensen 2007) showed that (i) faecal *Salmonella* shedding in organic and conventional outdoor-raised pigs is significantly lower than that in indoor-raised pigs, both on farm and at slaughter, (ii) transport stress increased the proportion of pigs shedding *Salmonella* spp., and (iii) the duration of transport between the farm and abattoir was significantly different between organic and indoor-raised conventional pigs (Table 4), although (iv) duration of transport had no significant effect on shedding at slaughter (Bonde and Sorensen 2007). The authors concluded that '*pigs from low-input* (i.e. organic and conventional) production systems *may be more resistant to the pathogen, or may have encountered the infection earlier in life, so they have cleaned themselves from infection at time of slaughter*'.

Risks associated with poultry

In poultry, most reported studies have focussed on *Campylobacter* (and to a lesser extent, *Salmonella*) prevalence, as poultry meat is the most important source of *Campylobacter* infections in humans (Corry and Atabay 2001; Sahin *et al.* 2002; Newell and Fearnley 2003; Table 5).

In Denmark, *Campylobacter* was significantly more prevalent in outdoor organic flocks than in both intensive and extensive conventional indoor flocks (Heuer *et al.* 2001). However, while *Campylobacter* prevalence decreased, the proportion of *Campylobacter* resistant to at least one antibiotic increased with production intensity (Table 5). In the USA, the prevalence of *Campylobacter* in organic and conventional retail chicken was similar (around 75%), while *Salmonella enterica* could be detected in a larger proportion of organic (61%) than conventional (44%) chickens sampled (Cui *et al.* 2005). Levels of resistance to antibiotics were similar in organic and conventional chicken, but a greater prevalence (especially of multiple) antibiotic resistance was detected in *S. enterica* strains isolated from conventional chicken systems (Table 5). Most importantly, all 12 *S. enterica* serovar *typhimurium* strains isolated from conventional chicken were resistant to five or more antibiotics, while most (79%) of the 19 strains isolated from organic chicken were susceptible to all 17 antimicrobials tested (Cui *et al.* 2005).

Resistance to antimicrobials in *Campylobacter* and *Salmonella* strains isolated from both food and clinical sources appears to be increasing in many countries (Threlfall *et al.* 1996, 2000; Besser *et al.* 1997; Glynn *et al.* 1998; White *et al.* 2001; Nachamkin *et al.* 2002; Ge *et al.* 2003).

These and other studies (Corry and Atabay 2001; Wilson 2002; Newell and Fearnley 2003) indicate that contamination of chicken with *Campylobacter* and *S. enterica* can currently not be prevented in both organic and conventional primary production systems.

Table 5 Effect of organic and conventional outdoor and indoor production systems on the prevalence of *Campylobacter* and/or *Salmonella* in retail chicken or broiler flocks (data from Heuer *et al.* 2001; Cui *et al.* 2005)

Characteristic assessed	Organic free-range	Conventional indoor	
		Extensive	Intensive
Denmark (Heuer *et al.* 2001)			
Prevalence of *Campylobacter* in chicken flocks			
No. of flocks sampled*	22	12	59
Campylobacter positive (%)	100 a	49 b	37 b
Antibiotic-resistant strains			
No. of strains tested	21	29	12
No. showing resistance*	1†	4‡	2§
Strains showing resistance* (%)	5	14	17
USA (Cui *et al.* 2005)			
Prevalence of *Campylobacter* in retail chickens			
No of chickens sampled	198		61
Campylobacter positive (%)	76		74
Salmonella positive (%)	61		44
% of *Campylobacter* strains showing resistance to			
Chloramphenicol	0		0
Ciprofloxacin	20		3
Erythromycin	35		44
Tetracycline	81		70
No. of strains tested	150		45
% of *Salmonella enterica* strains showing resistance to			
7 ABs (AU-AM-CE-FO-ST-TE-TI)	2		9
6 ABs (AU-AM-CE-FO-TE-TI)	0		14
5 ABs (AU-AM-CE-FO-TI)	1		41
4 ABs (GE-SM-ST-TE)	7		0
3 ABs (KA-ST-TE)	1		0
2 ABs (ST-TE or KA-NA)	46		27
1 AB (TE or NA)	1		0
0 AB None of the antibiotics tested	42		9
No. of strains tested	91		22

Percentage data within rows with different letters are significantly different ($P < 0{\cdot}001$).
AB(s), antibiotic(s); AU, amoxicillin-clavulanic acid; AM, ampicillin; CE, caphalothin; TI, ceftiofur; FO, cefoxitin; GE, gentamicin; KA, kanamycin; SM, sulfamethoxazole; ST, streptomycin; TE, tetracycline; NA, nalidixic acid.
*Ten broilers per flock were sampled.
†To ampicillin and tetracycline.
‡Two to streptomycin, one to erythromycin and one to enrofloxacin and nalidixic acid.
§One to streptomycin and one to enrofloxacin and nalidixic acid.

It is therefore essential to process (dry, heat treat and/or compost) manure from both organic and conventional chicken production systems (see Table 6 and RRP3 below).

Physiological and environmental factors

It is important to note that faecal pathogen shedding is also affected by a range of physiological and environmental factors. For example, shedding may be higher in young livestock (Buret *et al.* 1990; Hoyle *et al.* 2004), during pregnancy and birth (Jones 1999), when feeding regimes are changed (Chapman *et al.* 1997), and during transport or other forms of stress (Grau *et al.* 1969; Mechie *et al.* 1997). In addition, manure is a heterogeneous substrate consisting not only of faeces and urine but also of secretions from the nose, throat, vagina, blood, mammary glands, skin and placenta, as well as carbon-rich bedding material (straw, sawdust, etc.) and/or milking parlour waste (Alice 1997). Inocula of human pathogenic bacteria present in manure may therefore not only originate from animal faeces, although there is virtually no published information on the contribution of nonfaecal livestock secretions to pathogen risks associated with manure use.

Based on the currently available published information, it is possible to draw the following general conclusions:

Table 6 Soil association standards for the storage and treatment of manure used in organic farming systems (Soil Association 2005)

Treatments you should use for organic manure and plant waste – treatment for slurry: aerated treatment for manure and plant waste, including straw
Stacked for three months
Stacked for two months and turned at least twice, or
Properly composted
You must treat your nonorganic manure and plant waste as follows – treatment for slurry: aerated treatment for pig and poultry manure from systems described in standard 4·7·8:
Stacked for 12 months
Stacked for six months and turned at least twice, or
Properly composted
Treatment for other livestock manure and plant waste, including straw and by-products from nonorganic food processing:
Stacked for six months
Stacked for three months and turned at least twice, or
Properly composted

1. Complete control of pathogens (prevention of pathogen inocula being present in manure) through livestock management practices is not possible.
2. The risk of potential human pathogens being present in manure may be reduced in cattle production for *E. coli* (by forage-based feeding regimes prescribed under organic farming standards) and in pig production for *Salmonella* (by more outdoor production).
3. In poultry production, the risk of *Campylobacter* being present in manure may be reduced by indoor rearing systems used in conventional farming.
4. The risk of antibiotic-resistant potential human pathogens being present in manure appears to be lower in organic livestock production systems.
5. The safe use of manure relies on additional control being achieved at other RRPs (see below).

RRP2 outdoor livestock management

Several outbreaks of *E. coli* O157:H7 associated with conventional prepacked spinach and lettuce products were linked to close proximity to grazing cattle. Detailed investigations of such outbreaks often did not pinpoint the exact route of transmission, but provided some data suggesting that well- or river water contaminated with bovine faecal material and/or wildlife vectors may have been the route of transmission of faecal material from grazing cattle onto vegetables (US Food and Drug Administration 2006; California Department of Health Services 2007).

Organic standards currently prescribe methods used for storage/processing of manure (see Table 6), but few standards prescribe time intervals between the presence of livestock or application of manure, and planting of crops. This is an area where the introduction of additional recommendations or even prescriptive standards could further decrease potential risks of enteric pathogen transfer onto crops. For example, intervals of 9 months between livestock being present in the field and the planting of high-risk, ready-to-eat vegetable crops could be recommended or prescribed. This would put organic standards in line with other quality assurance schemes (e.g. Nature's choice) which already prescribe similar intervals.

In addition, it is important that fields used for livestock grazing are well separated from vegetable-growing areas of farms, and that it is not possible for livestock to escape into vegetable fields. Faecal contamination via the irrigation water (resulting from livestock grazing, but also other sources such as sewage plants) must also be prevented (see also RRP6) and the risk of contamination

Table 7 Prevalence and concentrations of zoonotic pathogens observed in British livestock manure (data from Hutchison *et al.* 2004)

	Manure samples containing zoonotic agents (%)		Mean concentration in positive samples (log_{10} CFU g^{-1})	
	Fresh	Stored	Fresh	Stored
Escherichia coli O157				
Cattle	13	9	6·5	3·9
Pig	12	16	4·8	3·9
Poultry	ND	ND	ND	ND
Sheep	21	22	4·0	3·4
Salmonella				
Cattle	8	10	4·6	5·3
Pig	8	5	4·0	2·9
Poultry	18	12	3·7	3·7
Sheep	8	11	3·0	3·8
Listeria				
Cattle	30	31	4·2	4·3
Pig	20	19	4·7	4·2
Poultry	19	15	4·5	2·7
Sheep	29	44	2·7	3·3
Campylobacter				
Cattle	13	10	3·9	4·0
Pig	14	10	3·3	4·4
Poultry	19	8	3·6	2·8
Sheep	21	11	2·9	2·0
Chryptosporidium parvum				
Cattle	5	3	2·4	1·8
Pig	14	5	2·5	2·1
Poultry	ND	ND	ND	ND
Sheep	29	0	1·7	0
Giardia intestinalis				
Cattle	4	3	2·3	0·8
Pig	2	2	4·7	1·1
Poultry	ND	ND	ND	ND
Sheep	21	0	3·6	0

Number of manure samples assessed: cattle, fresh (n = 810), stored (n = 429); pig, fresh (n = 126), stored (n = 58); poultry, fresh (n = 67), stored (n = 26); sheep, fresh (n = 24), stored (n = 9).

Table 8 Recommendations for compost and compost tea/extract preparation (revised from Ontario Ministry of Agriculture Food and Rural Affairs 2005)

Composting
Use windrow turning equipment that allowed irrigation of the windrow and moves material from the surface to the core of the windrow; since often, only the core of the windrow reaches the desired temperature of 55°C, this will ensure that all material receives an appropriate heat treatment
During wet and/or cool periods, use covers to maintain high temperature and avoid too high water contents (which results in anaerobic conditions) in the windrow
Repeatedly turn the windrow during the high-temperature phase; core temperatures should be monitored and kept above 55°C for 15 days with at least five turnings
Maintain optimum moisture levels (50–60%), optimum C : N ratios (25 : 1–30 : 1)
Measure CO_2 (or O_2) concentrations in the windrow and turn windrow regularly to maintain aerobic conditions
Records should be kept for temperature CO_2/O_2, C : N ratio, moisture and date/time of turnings
Clean compost turning equipment between use on different compost batches to avoid re-contamination with enteric pathogens
Loaders and wagons used to handle fresh manure should not be used for compost (or be cleaned before being used for compost)
Compost extracts
Prepare compost tea (CT) only from fully mature compost from controlled windrow or in-vessel composting systems
Do not add readily available carbohydrate sources (e.g. sugars or molasses) to the compost tea, since this may increase the risk for re-growth by enteric pathogens
Aerate the compost tea during the extraction process
Do not apply compost tea to edible parts of crops or apply near harvest
Extracts made from noncomposted or improperly composted manure (manure tea) may contain high levels of pathogens and should not be applied to crops; for soil applications, the same regulations/recommendations as for fresh manure should be applied

from wildlife (e.g. wild pigs, small animals, birds) minimized (e.g. by appropriate fencing).

RRP3 manure storage and processing

There are several reports that link the use of animal manure by amateur gardeners as well as commercial conventional and organic vegetable and fruit producers, with outbreaks of bacterial gastroenteritis (e.g. Morgan *et al.* 1988; Cieslak *et al.* 1993; Nelson 1997; Guan and Holley 2003). It is therefore important to use manure management and processing practices that minimize the risk of pathogen transfer from manure.

Pathogen survival in different types of manure

Zoonotic agents can be detected in a significant proportion of manure from both livestock and wild animals (Kopke *et al.* 2007). A recent extensive UK study (Hutchison *et al.* 2004; Table 7) showed that for some zoonotic pathogens (including *E. coli* O157, *Cryptosporidium parvum* and *Giardia*) standard manure storage reduced pathogen levels by between 0·6 and 3·6 log units, but that concentrations of other bacterial pathogens remained unchanged during storage.

The type of manure produced (slurry or solid manure from straw and woodchip bedding) and the method and duration of manure storage (e.g. as aerated or nonaerated slurry tanks, solid manure pits or as deep litter in barn) have a significant effect on the length of time enteric pathogens can persist in manure (Table 8; Guan and Holley 2003; Hutchison *et al.* 2004; Nicholson *et al.* 2005; Ontario Ministry of Agriculture Food and Rural Affairs 2005). Most importantly, several studies have shown that concentrations of potential pathogens such as *E. coli* decrease slowly in nonaerated manure slurry, faster in aerated manure slurry, but most rapidly in solid manures mixed with bedding materials (van Renterghem *et al.* 1991; Maule 1997; Nicholson *et al.* 2005). Several studies concluded that solid manure storage for 1 month was sufficient to eliminate pathogens (Kudva *et al.* 1998; Himathongkham *et al.* 1999), provided that elevated temperatures of at least 55°C were reached. The rapid elimination of pathogens in solid manure is thought to be due to more rapid aerobic decomposition of fresh manure and removal of readily available nutrients required by enteric pathogens, resulting in more fastidious microbial communities out-competing and replacing the enteric bacteria (Kopke *et al.* 2007).

Effect of environmental conditions on pathogen survival in manure

The survival of enteric pathogens in manure is also affected by a range of environmental parameters, such as temperature, pH, aeration, solids content and oxidation–reduction potential (Jones 1976; Kearney *et al.* 1993; Zhao *et al.* 1995; Wang *et al.* 1996); Kudva *et al.* 1998; Stanley *et al.* 1998b; Davis *et al.* 1999; Himathongkham *et al.* 1999). For example, Himathongkham *et al.* (1999) reported exponential linear reductions in *E. coli* O157:H7 and *Salmonella typhimurium* CFU in cow manure and manure slurry, and significant reductions in the popula-

tion density and length of time that the two pathogens could be detected in manure, at increasing storage temperatures (4, 20 or 37°C).

Effect of composting on pathogen survival

Aerobic composting based on regular turning of solid manure piles/windrows can further accelerate the decomposition process (Kudva *et al.* 1998). The turning of manure can be carried out manually with the tractor forklift (as in the study by Nicholson *et al.* 2005), compost spreaders, or in a more controlled fashion by windrow turners. Guidelines for the composting of manure have been produced (Ontario Ministry of Agriculture Food and Rural Affairs 2005) and the main recommendations are listed in Table 8.

In-vessel composting systems are also available, which aerate manure either by regular mechanical turning or by forcing air through the manure pile. Composting (especially more controlled windrow and in-vessel composting systems) also results in efficient pasteurization (thermal kill of nonspore forming enteric pathogens), as temperatures can be maintained at around 55 to 60°C. For example, Kudva *et al.* (1998) reported that the persistence of *E. coli* O157 : H7, in ovine and bovine manure can be reduced from 1 year (in stockpiled undisturbed manure) to between 1 and 4 months by turning/mixing and aeration.

The efficacy of thermal inactivation depends on the length of time that high-enough temperatures can be maintained, and on uniform exposure to elevated temperatures throughout the compost heap/windrow. It has also been reported that as long as the temperature stays above a certain level (45–50°C depending on the pathogen), longer exposure to relatively lower temperatures may be as effective as shorter exposure to higher temperatures (Haug 1993). Anaerobic fermentation/composting systems are also available but are considered less efficient in removing enteric pathogens from manure than aerobic systems (Russ and Yanko 1981).

As a result, a requirement for composting of certain types of manure has been included in some organic standards (Table 6). Based on data on the relative persistence of pathogens under different storage/processing regimes, the USDA's National Organic Standard demands that during manure composting, a minimum temperature of 55°C should be reached and maintained for at least 3 days (when an in-vessel compost turning or statically aerated pile system is used) or 15 days (when a windrow composting system is used). As there is the potential for pathogen survival at lower temperatures in the outer layers of the windrows, repeated turnings are considered as essential to allow appropriate thermal disinfection of all materials in the windrow (Table 8).

Older, 'cured' compost is often free of or contains very low levels of potential enteric pathogens. In addition, re-growth of bacterial pathogens, especially *Salmonella*, is rarely observed, and this is thought to be due to 'cured' compost being a nutritionally more 'fastidious' environment in which enteric pathogens cannot persist and/or are out-competed by other micro-organisms (Kopke *et al.* 2007). However, mixing of old 'cured' compost with 'fresh', noncomposted, organic materials may encourage re-growth/re-colonization of compost with enteric pathogens (Hay 1996; Kopke *et al.* 2007).

Risks associated with compost tea/extracts

Compost is also used in organic and low-input production systems to produce so-called compost 'extracts' or 'teas'. This is based on suspending a certain amount of compost in a perforated bag or plastic containers in water to extract minerals and other 'bioactive' compounds (e.g. antimicrobial compounds). Compost extracts may be applied directly to the soil or used as foliar sprays, and are purported to have crop protection or 'plant strengthening' activity and/or to act as micronutrient fertilizers (Diver 2002). Recommendations for the use of compost teas have been proposed (e.g. Ontario Ministry of Agriculture Food and Rural Affairs 2005; see Table 8), but their application onto edible parts of crops (especially ready-to-eat vegetables) is not recommended, because a complete absence of pathogens cannot be guaranteed.

RRP4 soil management

A range of environmental factors affects the survival of enteric pathogens entering the soil via manure applications (see Jamieson *et al.* 2002 for a recent review). Many soil management practices prescribed/recommended by organic farming standards and principles have at least partially been designed to minimize enteric pathogen survival by changing soil environmental parameters. Environmental factors that can be augmented by soil management practices include (i) soil moisture/matric potential, (ii) soil structure-related physical characteristics (particle, aggregate and pore size distribution), (iii) soil temperature and pH, (iv) soil biological activity and (v) soil nutrient availability/organic matter content. These environmental factors can be defined as sub-RRPs and are described in separate sections below.

RRP4.1 soil moisture/matric potential

Matric potential (soil moisture levels) is determined by soil type and water inputs through precipitation and/or irrigation (see also RRP6) and has been shown to be one of the most important factors influencing the survival of micro-organisms introduced into soil (Hagedorn *et al.*

1978; Tate 1978; Faust 1982; Meickle *et al.* 1995; Entry *et al.* 2000; Mubiru *et al.* 2000). High soil moisture when coupled with low temperatures was found in most studies to favour persistence of enteric pathogens (e.g. *E. coli* O157 and *Streptococcus faecalis*) in soil (Hagedorn *et al.* 1978; Kibbey *et al.* 1978; Tate 1978; Entry *et al.* 2000; Mubiru *et al.* 2000). At higher temperatures, enteric pathogen levels were shown to decrease more rapidly with increasing soil moisture levels, and this is thought to be at least partially because of the higher soil microbial activity at lower soil matric potentials (Killham 1995). It is important to note that there are also significant interactions between moisture content and other soil environmental factors, with respect to the persistence of micro-organisms added to soil (Dowe *et al.* 1997; Schmidt *et al.* 2004a, b,c). For example, a recent study on *L. monocytogenes* suggested soil moisture content, season, presence of plant root systems and decaying plant material, may interact to influence bacterial growth in soil (Dowe *et al.* 1997).

RRP4.2 soil type, pH and physical characteristics

Soil type has a significant effect on enteric pathogen survival (Tate 1978; Chandler and Craven 1980) and this may be due to differences in organic matter content, water release characteristics and particle size distribution. For example, concentrations of *E. coli* following manure application were three times higher in an organic soil than a sandy soil (Tate 1978). In addition, *E. coli* O157:H7 survival was reduced in light soils with higher matric potential (Mubiru *et al.* 2000 see also below). There is also indirect evidence for a link between higher organic matter levels and enteric pathogen persistence. For example, studies which compared the persistence of enteric pathogens in top and subsoil have all reported higher survival rates in topsoil (which has a higher organic matter level) (Chandler *et al.* 1981; Zhai *et al.* 1995). The impact of changes in soil organic matter levels, biological activity and particle size distribution associated with organic management practices, is discussed under RRP4·4 (see below).

Most studies report that pH values of between 6 and 7 are optimal for the survival of enteric pathogens and that survival decreases at either lower or higher pH values (Gerba *et al.* 1975; Ellis and McCalla 1976; Sjogren 1994). For example, when the survival of *E. coli* was compared in soils with similar texture and organic matter content, longer persistence was recorded at neutral-to-slightly alkaline pH (Sjogren 1994). Soil management practices that affect pH, for example the application of compost, which has been shown to increase soil pH in the long term (Fließbach *et al.* 2007), may therefore indirectly affect pathogen survival in soils.

RRP4.3 soil temperature

Similar to the effect of temperature on survival of pathogens during manure storage, most studies suggest that (as long as temperatures remain above freezing) enteric pathogen survival decreases with increasing temperatures (Van Donsel *et al.* 1967; Reddy *et al.* 1981). For example, in a review by Reddy *et al.* (1981), it was estimated that with every 10°C increase in soil temperature die-off rates of coliform bacteria doubled. In addition, the time required for a 90% reduction in coliform numbers after application to soil was shown to be 3·3 days in summer, but 13·4 days in winter (Van Donsel *et al.* 1967). However, freezing and thawing of soils was shown to reduce bacterial populations (Kibbey *et al.* 1978). In contrast, some studies (e.g. the laboratory study by Howell *et al.* 1996) reported increased survival and sometimes re-growth of faecal coliforms under warmer conditions.

An interaction between soil temperature and moisture was reported by Zibilske and Weaver (1978) who reported that the *Salmonella* persistence in soil was lowest at a combination of high soil temperature and low soil moisture.

RRP4.4 soil biological activity and organic matter content

Another important factor influencing the survival of newly introduced pathogenic bacteria into the soil environment is competition from the existing soil microflora (Reddy *et al.* 1981; Killham 1995; Meickle *et al.* 1995). In biologically active soils, introduced bacterial inocula were found to be reduced rapidly because of competition by the indigenous soil biota. In contrast, in sterile soils and soils with relatively low microbial activity, pathogen inocula may persist for much longer (Gerba *et al.* 1975; Reddy *et al.* 1981). The long-term (5–10 years) use of organic soil management practices which prescribe regular organic matter inputs were shown to result in increased soil biological activity (Mäder *et al.* 2002) and would therefore be expected to result in reduced persistence of enteric pathogens applied as manure. However, while regular organic fertility inputs were clearly demonstrated to increase the suppressiveness of soils against plant pathogenic fungi (e.g. Giotis *et al.* 2006), similar studies are, to our knowledge, not available for enteric human pathogens.

A high organic matter content in the soil was shown in several studies to increase the persistence of enteric pathogens (e.g. Tate 1978; see also RRP4·2 above). Soil organic matter is thought to provide organic nutrients/carbon compounds and to increase the retention of mineral and organic nutrients (Gerba *et al.* 1975). As soils with higher organic matter contents often also have a higher water-holding capacity, this may also contribute to the greater persistence of enteric pathogens observed.

Both soil biological activity and soil organic matter content were shown to increase significantly after conversion to organic farming practices (Mäder *et al.* 2002). However, based on evidence from studies carried out within the context of conventional farming practice (see above), these two changes have opposing effects on enteric pathogen persistence. This, and the lack of studies into the relative survival of enteric pathogens in long-term organic and conventionally managed soils with different inherent soil organic matter levels, make it currently impossible to predict whether organic soil management will result in an overall increase or decrease in the persistence of enteric pathogens added with manure to the soil environment. This is clearly an area that should be addressed in future research programmes.

RRP5 manure addition to soil

Application of contaminated solid manure or manure slurry poses a potential risk when applied to crops in an inappropriate fashion, especially if fresh, untreated manures are added immediately prior to or after planting of ready-to-eat vegetable crops.

Table 9 Effect of fertility input type and level on bulb yield, and pest and disease incidence in onion crops

	Parameters assessed		
Treatments	Bulb fresh weight (t ha^{-1})	Bean seed fly (% plants lost)	Onion neck rot (% plants with symptoms)
Nonfertilized†	23	10	10
Fertilized input type‡			
Chicken pellets (CP)	12	50	21
Cattle manure (FYM)	24	15	6
Compost (COM)	26	16	8
CP + FYM§	16	48	22
CP + COM§	14	48	16
FYM + COM§	27	14	8
Input level			
85 kg N ha^{-1}	23	22	12
170 kg N ha^{-1}	20	32	13
250 kg N ha^{-1}	17	41	15
ANOVA results main effects			
Input type (IT)	***	***	*
Input level (IL)	**	***	NS
Interaction (ITxIL)	NS	*	NS

†Data from nonfertilized control plots were not included in two-way anova.

‡Main effect means.

§The 2 input types were applied at equal N input levels; NS, not significant.

***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$.

All organic standards prescribe methods used for storage/processing of manure (see Table 6), but only some prescribe time intervals between application of manure and planting of crops. This is an area where the introduction of additional recommendations or even prescriptive standards could further decrease potential risks of enteric pathogen transfer onto crops. For example, intervals of 6 and 3 months between application of untreated manure and composted manure, respectively, and the planting of high-risk ready-to-eat vegetable crops, could be recommended or prescribed. This would put organic standards in line with other quality assurance schemes (e.g. Nature's choice) which already prescribe similar intervals.

It is interesting to note in this context, that within an organic production system, the application of manure immediately prior to the planting of low-fertility-demanding, ready-to-eat vegetable crops (e.g. lettuce or onion) often has no significant positive effect on yield, but may increase the susceptibility to pests and diseases, thereby decreasing crop yield and quality. This is especially true when inputs with high levels of water-soluble N (e.g. chicken manure) are applied (e.g. Kopke *et al.* 2007; Table 9). In such crops, there is an additional agronomic rationale for applying manure to preceding crops which have higher nutritional requirements (e.g. wheat) and carry a lower risk of transferring enteric pathogens into the food chain.

If intervals are to be set, they should reflect times measured for significant levels of enteric pathogen survival under conditions which favour persistence of pathogens (e.g. cool and wet soils) (see RRP4 above and Table 10). Combined with appropriate manure storage and/or treatment (which is prescribed by organic standards, see Table 6 and RRP3), such intervals would further reduce the potential for enteric pathogen transfer.

As manure application practices may further minimize the risks of pathogen transfer and allow another layer of control, they therefore represent a separate RRP (RRP5). Kopke *et al.* (2007) described several potential modes of transmission, through which enteric pathogens applied to soil can get onto the surface or into tissues of developing crop plants. Pathogens may be transmitted via (i) direct contact of plant surfaces with manure, (ii) by soil/manure particles being splashed from the soil surface onto plant tissues via precipitation and/or overhead irrigation and/or (iii) by vectors such as invertebrates, small animals and soil organisms. Approaches used to achieve control under (i) and (iii) are described here, while methods to achieve control relating to (ii) are treated as a separate CCP and described under RRP6.

Burying of manure into deeper soil layers is an effective way to reduce enteric pathogen transfer risk (Kopke *et al.* 2007) and in agricultural crop production, this is usually

Table 10 Survival of enteric pathogen in natural environments. Values in normal text are from Guan and Holley 2003; Values in parentheses are from the Ontario Ministry of Agriculture Food and Rural Affairs 2005

		Survival period of enteric pathogens (days)					
Temperature (°C)	Environment	*Escherichia coli* O157:H7	*Salmonella* spp.	*Yersinia entero-colitica*	*Campy-lobacter* spp.	*Giardia*	*Crypto-spori-dium*
<0	Water	>91 (>300)	152 (>152)	448	120 (14–56)	<7	>84 (>365)
	FYM	>100 (>100)	48 (>152)	10	21 (7)	<7	>84 (>365)
	Soil	99 (100)	63 (>84)	10	20 (14–56)	<7	>85 (>365)
4–8	Water	>91 (>300)	152 (>152)	448	8–120 (12)	77	>84 (>365)
	FYM	70 (>100)	48 (84–196)	10	12–21 (7–21)	7	56 (56)
	Soil	99 (100)	63 (84–196)	10	20 (14)	49	56 (56)
20–30	Water	49–84 (84)	45–152 (>152)	10	<2 (4)	14	70 (70)
	Slurry	27–60 (10–100)	19–60 (13–75)	10	3 (112)	7	28 (>365)
	FYM	49–56 (10)	48 (28)	10	3 (7)	7	28 (28)
	Soil	56 (2)	<45 (28)	10	10 (7)	7	28 (2)
	Compost	(7)	(7–14)		(7)		(28)
	Dry surfaces	(1)	(1–7)		(1)		(1)

Table 11 Effect of different fertilizer inputs on the populations of culturable aerobic bacteria, coliform bacteria, *Enterobacteriaceae* and *Enterococcus*, *Salmonella* and *Escherichia coli* on spring and summer crops of lettuce (QLIF WP3·4; modified from Kopke *et al.* 2007)

		Contamination associated with different fertilizers (log_{10} CFU g^{-1})			
Bacterial group	Cropping season	Fresh FYM	Composted FYM	Nettle extract	Mineral fertilizer*
Total aerobic bacteria	Spring	6·8 a	6·2 a	6·3a	6·4 a
	Summer	6·4 a	6·4 a	6·4 a	5·7 b
Total coliform bacteria	Spring	5·8 a	6·1 a	5·4 a	5·9 a
	Summer	5·2 a	5·3 a	5·1 a	4·0 b
Enterobacteriaceae	Spring	6·2 a	6·1 a	5·6 a	6·0 a
	Summer	6·3 a	6·3 a	6·1 a	5·3 b
Enterococcus spp.	Spring	2·8 a	2·7 a	2·9 a	3·1 a
	Summer	1·1 b	2·4 a	1·9 a	2·4 a
Salmonella spp.	Spring	0†	0†	0†	0†
	Summer	0†	0†	0†	0†
E. coli	Spring	0†	0†	0†	0†
	Summer	3†	1†	2†	4†

Means with different letters within rows are significantly different according to Tukey's Honest Significant Difference Test ($P < 0·05$).

*Calcium-ammonium-nitrate.

†Number of positive samples with >10 < 100 CFU g^{-1} ($n = 16$).

carried out by inversion ploughing. A similar approach/mechanism (e.g. double digging) is employed by most allotment gardeners who often use very high levels of manure as fertiliser. Although allotment gardeners often apply manure in autumn (6 months before planting of crops), incorporation in spring is also not uncommon (e.g. after winter *Brassica* crops). When manure is added in spring immediately prior to planting, the burial of manure becomes the main RRP through which enteric pathogen transfer can be prevented.

There are very few sound studies in which the effect of burying manure on coliform and enteric pathogen contamination rates in 'ready-to-eat' vegetables has been determined. An extensive recent study by Kopke *et al.* (2007) compared the effect of adding fresh FYM and regularly turned/composted FYM by inversion-ploughing with a foliar application of a plant extract-based organic fertility input and a mineral fertilizer control, on enteric pathogen populations in lettuce. There were virtually no significant differences in enteric pathogen populations between the fresh and composted FYM and Nettle extract treatments (Table 11). Lower levels (by approximately 1 log unit) of total aerobic, coliform and Enterobacteriaceae CFU were detected when mineral fertilizers were

used but only in the summer-grown lettuce, while no significant difference could be detected between fertility input treatments for *Enterococcus* spp., and *Salmonella* spp. could not be detected in lettuce from any of the treatments. *E.coli* spp. were only detected in summer crops, when similar numbers of positive lettuce samples were detected for all fertilization treatments (Table 11). Although no surface manure application control treatment was included in the study, the data obtained do indicate that inversion-ploughing may reduce the risk of enteric pathogen transfer from manure onto crop tissue (Kopke *et al.* 2007).

Surface disinfection (e.g. by low temperature steaming or solarization) of soil prior to planting of seedlings may be another method to reduce pathogen transfer risk (Bennett *et al.* 2003, 2005, 2006; van Loenen *et al.* 2003). Mulch layers may be regarded as an efficient physical barrier to reduce pathogen transmittance especially when combined with the solarization effects given by plastic mulch, as direct contact with soil is then limited and splash-transmitted pathogen transfer with soil particles reduced. However, a recent study showed that paper mulch mats, which were marketed partially as a way to reduce enteric pathogen transfer from soil, significantly increase coliform counts on lettuce (Köpke *et al.* unpublished).

A range of agronomic practices (e.g. mechanical and chemical weed control, harvesting) that may cause physical injuries to crops, may also increase the risk of pathogen transfer and penetration of enteric pathogens into internal plant tissues, where they are protected against environmental (and, in particular, matric) stress (Seo and Frank 1999; Takeuchi *et al.* 2000). Wounding of plant tissue may also result in a greater availability of nutrient sources and plant responses that facilitate microbial growth and colonization of plant tissues (Nguyen-the and Lund 1991). Clearly, the use of such practices should also be minimized, especially as the introduction of enteric pathogens into the plant tissues may reduce the effectiveness of disinfection treatments used in the processing of ready-to-eat vegetables.

RRP6 irrigation

There is no difference in the risk of introducing enteric pathogens via the irrigation water between organic and conventional production systems. However, as irrigation water can be an important source of enteric pathogen contamination on ready-to-eat vegetables, the main control measures that need to be introduced to achieve control at this RRP are discussed here.

The risk of introducing enteric pathogen contamination via the irrigation water can be reduced at two principal sub-critical control points: RRP6·1 Source/type of irrigation water (surface water, reservoirs, and shallow and deep wells) and RRP6·2 Irrigation method (e.g. overhead, above-ground minimum irrigation or below-ground irrigation tape systems). These sub-RRPs are described in separate sub-sections below.

RRP6·1 source/type of irrigation water

Surface water taken from lakes, streams or rivers is generally considered to be of doubtful hygienic quality. Enteric pathogens originating from livestock faeces or manure deposited on agricultural land, enter surface waters in different ways. The most important mechanisms are thought to be: (i) domestic and wild animals having direct access to surface waters, (ii) faeces or manure being transported by run-off (e.g. following heavy rain or excessive irrigation), (iii) flooding of fields, and (iv) via discharge from field drains (Jamieson *et al.* 2002; Guan and Holley 2003; Kopke *et al.* 2007). The factors affecting transport of enteric pathogens into surface water via percolation through soil into field drains have recently been reviewed (see Jamieson *et al.* 2002) and are not discussed here. However, as such studies can also be used as an indication of the relative risk of groundwater contamination, it is interesting to note that the authors described that increasing amounts of manure, light soils and high precipitation levels significantly increased enteric pathogen levels in field-drain discharge. In contrast, plough incorporation decreased faecal coliform discharge by 84% compared with disk soil treatment (Geohring *et al.* 1999).

Enteric pathogens in surface water may also originate from sewage works, which are known to discharge significant levels of enteric pathogens into river systems following breakdown or maintenance, or during periods of excess water passing through plants (e.g. following heavy rainfall) (Kopke *et al.* 2007). Faecal pathogens have also been isolated from sediments in freshwater systems (Burton *et al.* 1987; Crabill *et al.* 1998).

Thus, in several countries, agricultural use of surface water is not permitted. In areas where surface water is used extensively for irrigation of ready-to-eat vegetable crops (e.g. the USA), irrigation-related outbreaks of foodborne disease appear to be more frequently reported, although scientifically sound quantitative comparisons are currently not available.

Although generally thought to be much safer than the use of surface water, groundwater from wells may also become contaminated with faecal pathogens, especially in areas with extensive livestock production and/or manure application to soil (Howell *et al.* 1995; Jamieson *et al.* 2002). Enteric pathogens may survive in groundwater for some time and some parasites such as *Giardia* or *Cryptosporidia* may multiply once they get into the groundwater under certain environmental conditions (Gagliardi and Karns 2000). It is therefore essential to impose quality

assurance procedures on the management of irrigation wells: most importantly, this should include regular maintenance of water pipes and pumps and microbiological testing of well water (Fernandez-Alvarez *et al.* 1991; Kopke *et al.* 2007). Kopke *et al.* 2007 suggested that '*Irrigation water used for vegetable production should have tap water quality*', and that '*when no information on water quality is available, deep wells have to be considered as the best source of irrigation water*'.

RRP6·2 application of irrigation water

Kopke *et al.* (2007) suggested that '*tap or drip irrigation instead of overhead spray irrigation might help to avoid transmittance*' of enteric pathogens onto above-ground parts of ready-to-eat vegetables via water splash. Such systems are already used in the Mediterranean areas to reduce water use and irrigation costs and can be applied in most of the lighter soils used for vegetable production.

Conclusions

Clearly, as in most quality assurance schemes, there is a hierarchy of RRPs, with RRPs closest to the source of contamination being the most important ('prevention at source is better than treatment'). In the case of manure management, the order of importance would be RRP1 ≥ RRP2 ≥ RRP3 > RRP4 > RRP5. For example, RRP1 and RRP2 are clearly the most important control points, because if the animal remains healthy throughout its life and if appropriate feeding regimes minimize the risk of transfer of human pathogens that are nonpathogenic in livestock (see Diez-Gonzalez 2007), no significant pathogen loads are excreted, and pathogens do not need to be removed at a later RRP. However, as gastrointestinal diseases and shedding of human pathogens in animal faeces cannot be completely excluded, RRP3 storage/processing of manure has to be considered to be of equal if not greater importance than RRPs1 and 2. If the manure treatment kills pathogens efficiently, soil management focussed on maintaining high biological activity (RRP4) is not essential for the removal of pathogens etc.

As described above, organic farming standards already provide a very structured quality assurance scheme for the use of animal manure and sludge; however, there is a need to constantly improve standards. If new information becomes available, which shows the requirement for additional regulations and/or changes to standards (e.g. concerning the processing requirements and quantities/timing of applications to horticultural and in particular glasshouse crops), these should be introduced immediately. For example, the official reports on recent outbreaks of *E. coli* O157 associated with conventional vegetable crops in the USA suggest that control at '*RRP2 Outdoor livestock management*' and/or RRP6 broke down. The proximity of ready-to-eat vegetable crops to livestock fields and associated risks of faecal contamination of ready-to-eat vegetable crops via run-off and/or contaminated irrigation water may therefore have to be re-examined. This may also apply to the potential for enteric pathogen transfer via wildlife vectors.

The RRPs described above only relate to primary production, while HACCP-based safety assurance procedures at postharvest stages of the food supply chain are not described. This is mainly because the use of HACCP-based quality assurance systems by both organic and conventional vegetable packers, processors and retailers is now standard practice in most European countries and is described in detail elsewhere (Knight and Stanley 2000; Woteki and Kineman 2003).

However, domestic food preparation and storage (what is carried out to food after it has been purchased by consumers) has become an increasing concern. Decreasing standards of food hygiene, preparation and storage at home are thought to have contributed significantly to the rise in certain food-borne diseases (Woteki and Kineman 2003; Mitakakis *et al.* 2004). In particular, there is a concern that some consumers do not see the need to wash organic fruits and vegetables, because of a perception that recommendations to wash produce were made to ensure that pesticide residues are removed. In addition, undercooking of some foods where contamination by certain enteric pathogens cannot be prevented efficiently (e.g. *Campylobacter* in poultry) during primary production and processing, appears to be increasing (e.g. Hughes *et al.* 2007).

Clearly, awareness about food hygiene and proper food handling and preparation at home are important to prevent diseases caused by food-borne pathogens. Vegetables and fruits are living nonsterile plants and further processing must begin with washing in running water. It should, however, be pointed out that apart from eliminating the residues of soil and plant debris, washing has only a limited effect on the microflora attached to the plant surface (Nguyen-the and Carlin 1994). Washing of lettuce leaves, for example, reduced total microbial counts by 0 to 0·5 log units only (Kaferstein 1976; Bomar 1988; Jockel von and Otto 1990).

Clearly a whole food supply chain approach is needed to minimize the risk from food-borne diseases and a particular focus in the future should be on the development of strategies to improve domestic food storage, handling and preparation standards.

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