REDUCING FOOD SAFETY RISKS FROM PRE-HARVEST WATER

Pre-harvest water

Vegetables can't grow without water. In Australia's often dry and unpredictable climate, irrigation is essential in order to supplement, or even replace, natural rainfall. Water is also used to apply fertilisers, spray pesticides, provide cooling and stabilise soils. This water may be sourced from dams, rivers, underground bores or town water supplies.

Water is also a potential source of microbial contamination. Human pathogenic bacteria such as *Escherichia coli* (*E. coli*), *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Yersinia enterocolitica* and others can readily contaminate water. Certain strains of these bacteria – particularly *Salmonella* spp. and *E. coli* – can cause severe illness or death. Human pathogens can potentially survive for extended periods in contaminated water, reaching populations of millions in every litre.

Faeces, dead animals and birds are key sources of these microbes. Surface water in dams and waterways is particularly vulnerable, with contamination due to wild birds, runoff from manure storage or animals entering water to drink. Bores usually pose less risk but may still be contaminated by seepage from septic systems or intensive livestock production. Even rainwater tanks can be contaminated by birds or animals entering them, or by faeces from birds or rodents washed into tanks after rain.

Assessing risk from water sources

Assessing the microbial risks from different water sources can help determine the potential for pre-harvest contamination of vegetables. The assessment should include consideration of how water quality can change seasonally and over time, particularly as this relates to rainfall and temperature. For example, water sources can become anaerobic, saline or be affected by toxic algal blooms during drought. If drought is followed by heavy rains, accumulated organic materials, including faeces, are likely to wash into water sources.

Risk is increased if there is a lot of bird or animal activity in and around the water source, or it is close to sources of contamination such as intensive animal feeding operations (feedlots, saleyards, poultry farms, dairy farms, piggeries, etc.), garbage dumps, manure storage and septic overflows.

Testing for *E. coli* is the best indicator of whether water has been contaminated, and is the standard method used to assess water quality around the world. Coliforms and/or thermotolerant coliforms are very common in the environment, so tests for these bacteria are less specific.

Water should be tested at least annually, and more often if the water source is variable. Testing needs to be conducted at the time of greatest risk, or whenever water quality changes. For example, both heavy rainfall events and drought can increase the risk that a water source is contaminated. Regular testing is most important if the water contacts the harvestable part of crops that are eaten uncooked, particularly those with surface characteristics that can provide refuges for bacteria (e.g. leafy greens, herbs).

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Reducing risk

The best way to reduce risk is to prevent water becoming contaminated. Livestock should be kept away from dams and watercourses. Runoff from potentially contaminated areas (e.g. manure stockpiles) must be diverted away from dams and cropping areas. Water pipes and tanks should be constructed so as to prevent pest entry (e.g. enclosing water tanks) and kept well maintained. Installing backflow devices can help prevent contaminated water entering the main system.

Figure 1. Keeping cattle away from watercourses will reduce the risk of contaminating irrigation water. Photo: Keven Law

Despite these precautions, it can be difficult to ensure water is always free of human pathogens. In addition to chemical sanitisers, new systems are available which can treat irrigation water, potentially in-line. Electrolysis produces low levels of chlorine and other compounds using natural salts present in the water. So long as the water is clean (free of organic matter), these systems can significantly reduce levels of bacteria and fungi in the water supply.

If water cannot be confirmed free of human pathogens, then the best way to ensure vegetables are safe is to avoid contact with the harvestable part. Sub-surface irrigation, drippers and hydroponic systems all avoid irrigation water touching the upper parts of the plant. However, it is important that water used in recirculating hydroponic systems does not splash the leaves during harvest and packing.

Figure 2. Hydroponic systems avoid water contacting the crop, but it is important to avoid water splashing the leaves during harvest.

If potentially contaminated water contacts the harvestable part of the vegetables, either during irrigation or spray application, then withholding periods apply.

Figure 3. Withholding periods apply between application of water of unknown quality and harvest if the water contacts the harvestable part of the crop and the product may be eaten uncooked.

Human pathogens, such as *E. coli* and *Salmonella* spp., evolved to live in the warm, wet environment of the gut. They can survive wide ranges of pH and the actions of digestive enzymes. However, they are poorly adapted to the exposed, dry surfaces of plant leaves and fruit. Populations of these bacteria, therefore, die off over time.

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Apart from time, factors that have been shown to reduce survival of *E. coli* on plants include:

- High and/or fluctuating temperatures
- Strong UV light
- Rapid leaf drying due to low relative humidity and/or an open canopy
- Hydrophobic or waxy surfaces
- Low availability of nutrients
- Presence of diverse microflora, including microbial predators.

Figure 4. Human pathogens are less able to survive on plants which have hydrophobic or waxy surfaces.

Many food safety standards stipulate a withholding period between application of potentially contaminated water and harvest. In Australia, **vegetables must not be harvested for at least 48 hours** if irrigation water or sprays have contacted the harvestable part. This interval is intended to allow human pathogens on plant surfaces to return to normal environmental levels.

Exceptions to this apply:

- The water has been tested and found to contain <100 CFU (colony forming units i.e. individual bacteria) of *E. coli* per 100ml
- The product is always eaten cooked e.g. rhubarb
- Water containing more than 1,000 CFU/100ml *E. coli* is not recommended to be used in ways that contact the harvestable part of crops.

Is 48 hours enough?

Some research in the scientific literature suggests that human pathogens can survive on leaf surfaces for several days, or even longer. If this is the case, it suggests that the withholding period between water contact and harvest may need to be increased.

However, many studies have been conducted in relatively cool environments with lower light intensity than Australia. Also, they have often used very high initial populations of pathogens. For example, surveys of irrigation water have indicated that although *E. coli* populations in water occasionally exceed 1,000 CFU/100ml, they are more often 100 CFU/100ml or less. In contrast, research studies commonly use water containing as many as 1,000,000 CFU *E. coli*/100ml.

Testing the time limit

As part of the project "Pathogen persistence from paddock to plate" (VG16042), the project team conducted a series of trials examining die-off rates of pathogens on the surfaces of vegetables after irrigation with contaminated water.

Water containing approximately 3,000 CFU/ml *E. coli* and 300 CFU/ml *Salmonella* spp. was used to irrigate vegetables, including cos lettuce, parsley, kale, silverbeet and baby spinach. Initial trials were in pots inside and outside a glasshouse, with later trials in the field. Some plants were lightly damaged by compressing or clipping the leaves; others were left intact.

Figure 5. Some products were lightly damaged by compressing or clipping leaves before irrigation with contaminated water.

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Whole plants were sampled immediately after irrigation, then each day for up to a week. Testing conducted by Symbio laboratory determined the populations of *E. coli* (CFU/g) and presence/absence of *Salmonella* spp. on the leaf material.

Figure 6. Water containing *E. coli* and *Salmonella* spp. was used to irrigate a range of vegetables, including baby spinach.

The difference damage makes

In nearly every case, *E. coli* on undamaged leaves fell below the level of detection (10 CFU/g *or* log 1 CFU/g) within 48 hours of irrigation. This occurred in both the field and the greenhouse, even though ultraviolet radiation was blocked in the latter. Results were similar across all of the vegetables tested.

However, it soon became clear that damage – even slight damage not obvious to the eye – increased survival of human pathogens. Cos lettuce was particularly susceptible; simply creasing the leaves resulted in detections of *E. coli* continuing for up to a week after the leaves had been contaminated.

Even heavy rain the day after irrigation, hot temperatures (>35°C) and high UV radiation did not eliminate *E. coli* from damaged leaves. Moreover, some researchers have found that *E. coli* is able to both survive and multiply inside damaged leaves. Our results support this, with increased populations occasionally recorded three days after the plants were contaminated.

In total, 58 tests were conducted for presence of *Salmonella* spp. on intact plants. Apart from a single positive result for spinach, there were no detections of *Salmonella* spp. two days after irrigation with contaminated water. In contrast, 36% of damaged plants were positive for *Salmonella* spp. two days after irrigation; while 14% remained positive six days later.

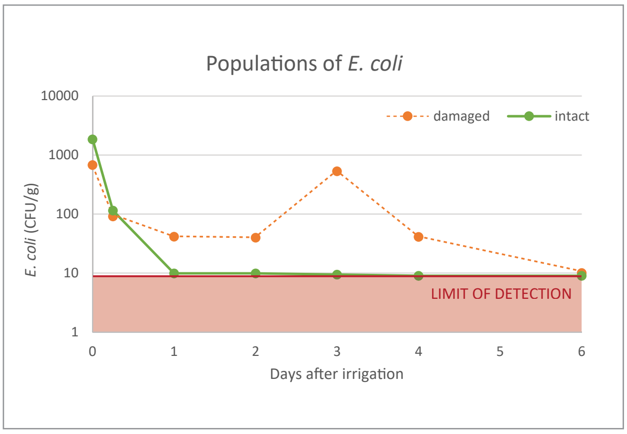

Figure 7. Average populations of *E. coli* on damaged and undamaged vegetables for up to six days after irrigation with contaminated water. Results compiled from three greenhouse and two glasshouse trials.

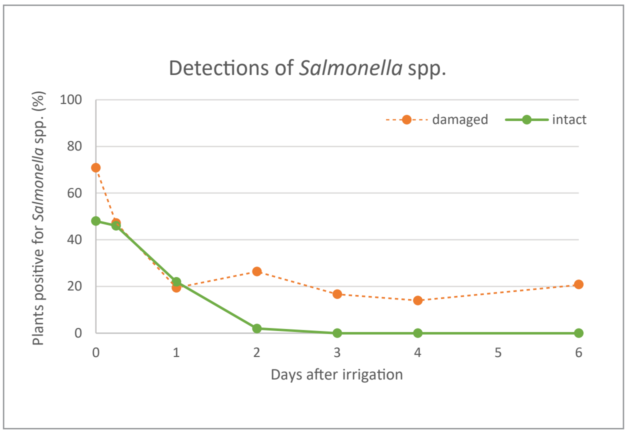

Figure 8. Percentage of plants that tested positive for *Salmonella* spp. up to six days after irrigation with contaminated water. Results compiled from three greenhouse and two glasshouse trials.

It was concluded that populations of *E. coli* declined by at least 99% after two days on the leaf surfaces. *Salmonella* spp. also rarely survived more than two days, despite being added to water at rates higher than would be expected to occur naturally in irrigation water.

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The results are, therefore, consistent with the current 48-hour withholding period between water contacting the leaves and harvest.

However, if plants are damaged, then 48 hours is not long enough to reduce risk. Even slight damage can allow bacteria on the plant leaves to survive and multiply. Easily damaged products, such as cos lettuce, appear to be particularly susceptible. Growers therefore need to take a cautious approach to water quality when irrigating these products.

Figure 9. Damage to cut spinach leaves heals over time.

My crop has been damaged – what do I do now?

Vegetable crops may be damaged by heavy rain, strong winds, farm equipment or animals moving through the paddock. If damage has occurred, irrigation with water potentially containing >100 CFU/100ml *E. coli* greatly increases the risk of it still being contaminated at harvest.

However, plants will eventually heal damaged areas. How quickly this occurs is likely to determine how soon it is safe to irrigate with water of unknown quality.

We therefore conducted a series of trials examining the effect of the time interval between damage occurring and irrigation on the likelihood that vegetables were still contaminated 48 hours after irrigation.

As lettuce and baby spinach approached commercial maturity, groups of plants were damaged by clipping the tops of a few leaves. The time interval between damage and irrigation ranged from 96 hours to one hour before irrigation. The plants were then irrigated with water containing approximately 3,000 CFU/ml *E. coli* and 300 CFU/ml *Salmonella* spp.. Half of the plants were tested immediately, as previously described, with the remainder tested two days later. The whole procedure was repeated four times.

After two days, populations of *E. coli* on undamaged lettuce and spinach plants fell from an average of 700 CFU/g to barely detectable levels.

Under the conditions in this trial, there were no significant differences in populations of *E. coli* on baby spinach damaged up to four days before contamination compared to the undamaged controls. However, *E. coli* was detected twice as frequently on

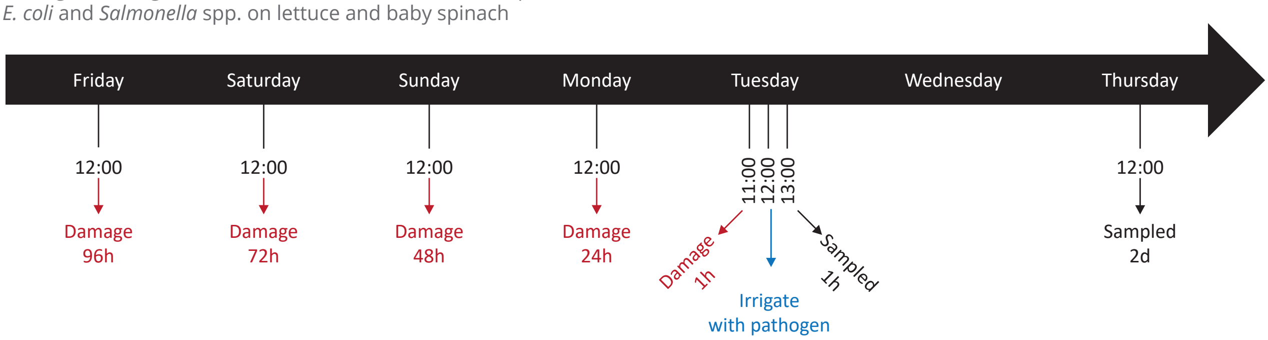

Figure 10. Timeline for examining the effect of the interval between damage and irrigation with contaminated water on the persistence of *E. coli* and *Salmonella* spp. on lettuce and baby spinach

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spinach damaged immediately before the water was applied compared to that damaged 24 hours or more beforehand. This suggests that spinach can regain resistance to contamination relatively quickly after damage.

However, the same cannot be said for cos lettuce. Even when damage occurred four days before irrigation with contaminated water, survival of *E. coli* increased significantly compared to the undamaged controls. Damage immediately before irrigation had the greatest impact. Populations of *E. coli* on these lettuce had not decreased from the levels recorded immediately after the water was applied.

Salmonella spp. was detected on several undamaged spinach plants in the first two replications, and cos lettuce in the latter two replications.

- Spinach plants that were infected by downy mildew and/or had yellowing leaves were more likely to test positive for *Salmonella* spp..
- *Salmonella* spp. detections were increased on lettuce that had been severely attacked by ducks a month earlier. Although the plants appeared to recover, it seems possible that this early damage had still not fully healed.

Figure 11. Condition of 'undamaged' but poor-quality spinach that returned a number of positive tests for *Salmonella* spp. (left) and healthy spinach where all tests were negative after two days (centre). Lettuces that were severely damaged by ducks one month before commencement of the trial (right) were more likely to test positive for Salmonella spp.

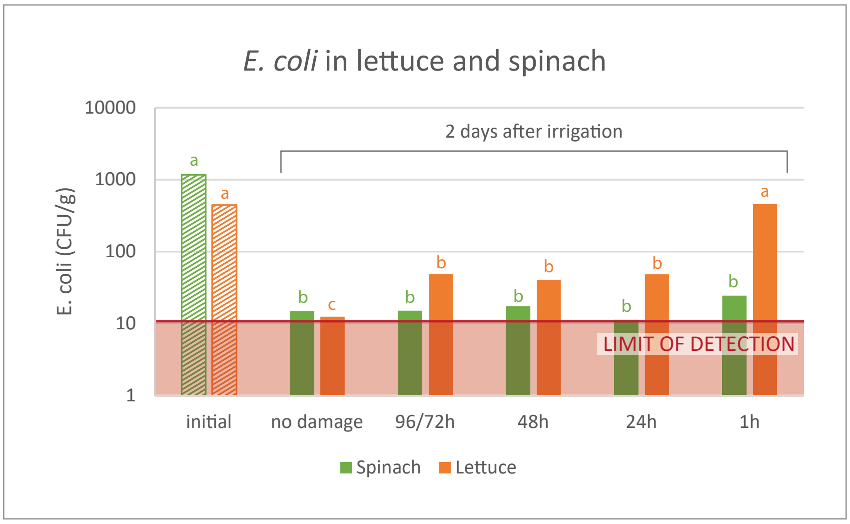

Figure 12. Average populations of *E. coli* on spinach and lettuce plants that remained undamaged or were damaged up to four days (96h) before irrigation with contaminated water. Letters indicate values that are significantly different for each vegetable type.

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What have we learned?

The quality of water that contacts the plant is the most important factor affecting the food safety of leafy vegetables. Many of the significant food safety outbreaks associated with salad greens can be traced back to contaminated water.

Human pathogens in water used for irrigation or crop sprays will persist longer on crops if they are damaged. This injury can be relatively minor, certainly below commercial thresholds. In our trials, traces of downy mildew infection and slight leaf yellowing appeared to increase detections of *Salmonella* spp. on spinach plants. It also appeared possible that damage a month earlier may have increased detections on cos lettuce.

These results are consistent with reports that human pathogens are more likely to survive if vegetables have fungal diseases, bacterial rots or light frost damage.

Once internalised, human pathogens cannot be easily removed. Even triple washing with sanitisers cannot eliminate human pathogens from vegetables if they were contaminated before harvest.

Recommendations

- Irrigation systems that avoid water contacting the harvestable part of the vegetable (e.g. sub-surface drip, run to waste hydroponics) greatly reduce risk.
- Reduce the risk that water contacting the harvestable part of plants contains *E. coli* >100 CFU /100 ml by:
  - Keeping livestock away from dams and other water sources
  - Diverting potential runoff from contaminated areas, such as neighbouring feedlots, manure storage or septic systems, so that it does not flow into dams or onto cropping areas
  - Discouraging water-birds from lingering on dams used for irrigation
  - Keeping irrigation and spray equipment clean and well maintained
  - Cleaning rainwater collection areas, keeping tanks sealed against vermin and maintaining water storage equipment
  - Verifying microbe levels through regular testing.
- Water containing *E. coli* >1,000 CFU/100ml should not be used if it contacts the harvestable part of crops that may be eaten uncooked
- If water quality is poor, investigate ways to reduce microbial load, such as filters, chemical sanitisers and electrolyzed water systems
- If water quality is poor or unknown, a 48-hour withholding period between irrigation and harvest significantly reduces the risk that vegetables will be contaminated at harvest however:
  - Longer withholding periods are needed if plants have been physically damaged
  - Contact between cos lettuce plants and contaminated water should be avoided at all times
  - Damaged baby spinach should not be irrigated for at least 24 hours if water contains *E. coli* >100 CFU/ml.