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Shiga toxin-producing *Escherichia coli* (STEC) contamination of leafy vegetables and risk management

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Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are a sub-group of *Escherichia coli* (*E. coli*) bacteria that produce toxins known as Shiga toxins, which can cause severe foodborne illness in humans. STEC infections can cause a wide range of symptoms, from mild gastroenteritis to severe conditions like haemolytic uremic syndrome and life-threatening kidney disease. The severity of illness depends on factors such as the strain of STEC involved, the number of bacteria ingested, and the individual's health. Vulnerable populations, such as children, the elderly, and immunocompromised individuals, are at higher risk of severe disease outcomes.

STEC are transferred to humans via contaminated food and water, direct animal contact, environmental contamination and human-to-human transfer via the faecal-oral route. The most common foodborne sources of STEC include undercooked meat, unpasteurised milk, ready-to-eat meat products, seed sprouts and leafy vegetables.

STEC infections are characterised by Shiga toxin (Stx) production. There are 2 main types (Stx1 and Stx2), and the *stx* toxin genes (*stx1* and *stx2*) are carried by lambdoid bacteriophages integrated into the *E. coli* chromosome. Additional virulence genes are consistently associated with severe illness, most notably the *eae* gene, which encodes the intimin protein required for adherence. However, this virulence factor is not always essential for illness severity (EFSA 2020). The severity of illness caused by STEC depends on the combination of *stx* genes and additional virulence factors.

STEC serogroups

Among about 470 serogroups of STEC, the most commonly associated with serious human illness is O157. Other strains are grouped as non-O157. In Australia, the O157 has been reported to be the main STEC serogroup contributing to more than half of all STEC isolates serotyped, followed by O111 and O26 (Vally et al. 2012; Ingle et al. 2019). Several other serogroups have been reported with low prevalence.

In the USA, there are 6 main non-O157 strains including O26, O45, O103, O111, O121 and O145 (USDA 2012), while in Europe, there are 5 leading non-O157 strains including O26, O103, O91, O146 and O145 (EFSA 2020). In Europe, all STEC strains are considered pathogenic in humans, capable of causing at least diarrhoea and, based on the analysis of the *stx* sub-types and the presence/absence of the *eae* gene, all STEC sub-types can be associated with severe illness (EFSA 2020).

STEC illness notifications

STEC infection is a notifiable disease in Australia. Between 2000 and 2010, there were 822 notifications of STEC illness in Australia, making the annual rate 0.4 cases per 100,000 per year (Vally et al. 2012). From 2011 to 2024, there were 6,677 STEC notifications and the annual rate of cases (1.99 cases per 100,000 per year) increased significantly during this period (NNDSS 2025).

Although most cases of STEC infection in Australia are considered sporadic, major STEC outbreaks can occur, with the largest reported outbreak of serogroup O157 STEC in Australia occurring among 57 patients in Queensland in 2013, associated with exposure to an animal nursery at an agricultural show (Vasant et al. 2017). Further information on foodborne transmission of STEC in Australia is limited.

STEC sources

STEC are frequently isolated from the gastrointestinal tracts of various animal species including cattle, sheep, goats, pigs, horses, deer, rodents and birds (Persad and LeJeune 2014). Animals are exposed to STEC by direct or indirect contact with the faeces of a shedding animal. Most animals lack receptors for Shiga toxin, and their presence does not typically lead to disease. In contrast, in humans, the presence of additional virulence factors alongside the *stx* gene are linked to disease development (Persad and LeJeune 2014).

Healthy cattle are regarded as the primary reservoir of STEC. In an Australian study on beef cattle faeces, the prevalence of *E. coli* O157 was highest (6.7%), followed by 1.0% for O26 and 0.3% for O111. No STEC strains of the O45, O103, O121, or O145 serotypes were isolated (Mellor et al. 2016). The study also reported a higher prevalence of STEC among younger animals than adult animals. **Sheep** are also a reservoir for STEC in Australia, with the serotype of importance being O26. However, the risk of human infection is low due to lower prevalence rates (Gyles 2007; Duffy et al. 2010). **Wild birds and animals** pose a unique risk in their ability to travel long distances, increasing the dissemination of STEC in the environment.

Major sources and routes of STEC contamination

The major sources and routes of STEC contamination in leafy vegetables are:

1. **Irrigation water**: one of the primary sources of STEC is using contaminated water for irrigation. Water contaminated with faecal matter from animals (livestock and wildlife) can carry pathogens like STEC, which then contaminate the surface of leafy vegetables. Surface water sources such as rivers, creeks and canals are highly prone to contamination with STEC from animal operations. Applying surface irrigation water with high levels of *E. coli* via an overhead irrigation system (Figure 1), particularly closer to harvest, can elevate the risk of STEC contamination. Though *E. coli* could die off at the rate of 0.5 log per day, the residual population becomes the contaminant.

Figure 1. Applying contaminated surface irrigation water with an overhead irrigation system elevates the risk of leafy vegetables contamination.

2. **Organic soil amendments**: raw or improperly composted manure contaminated with STEC can transfer these pathogens to leafy vegetables when used as fertilisers (Ongeng et al. 2015). STEC can be transferred to the leaves during production through direct contact with soil or indirect splashing with rain and overhead irrigation.
3. **Growing site**: the coexistence of fresh produce and livestock industries poses elevated risks of pathogen transfer from animal operations to the production field via contaminated irrigation water, dust storms and run-off. Compost yards and piles can be a significant risk for contaminating leafy vegetables in the field. They should be located as far away as possible and be screened or protected to avoid contamination via wind and run-off. Wildlife incursions in the production field and water sources are another major risk factor.
4. **Harvesting**: if workers or harvesting equipment come into contact with contaminated water, soil, or surfaces, they can spread the bacteria onto the leafy vegetables. Inadequate personal and equipment hygiene can also contribute to cross-contamination.
5. **Cool chain management**: pre-cooling leafy vegetables immediately after harvest reduces microbial risk. Maintaining a cool chain is critical to minimise microbial proliferation, particularly for businesses transporting produce long distances between harvest and postharvest processing.
6. **Postharvest processing**: after harvest, leafy vegetables are washed in water to remove dirt and residues, but this water can be contaminated if sanitisers are not maintained at appropriate concentrations to compensate for organic and microbial loads on harvested produce (Figure 2).
7. **Extreme weather conditions**: heavy rainfall that results in run-off or flooding (Figure 3), dust storms, bushfires and heatwaves affect fresh produce safety (Singh 2023). Natural disasters often exacerbate the transfer of microbial pathogens from the environment into production fields, water sources and postharvest processing facilities.

Figure 2. The wash water must have appropriate sanitiser concentrations.

Figure 3. Extreme weather conditions such as heavy rainfall can transfer microbial pathogens from the environment into production fields, water sources and postharvest processing facilities.

STEC risk management strategies

Global Food Safety Initiative (GFSI) benchmarked quality assurance schemes provide guidance for microbial risk management. Having these schemes in place can help with managing microbial risk, including STEC. Comprehensive food safety strategies must be implemented at various points along the leafy vegetable production and supply chain (Singh 2025). Essential risk management practices include:

Preharvest water quality management: irrigation water from surface sources such as rivers and canals should be pumped and stored in a dam/pond to allow the suspended solids to settle. The presence of sediment in irrigation water indicates a higher risk of faecal contamination. Using coarse filtration and frequent backflushing minimises the sediment and reduces the microbial population in irrigation water (Figure 4).

Irrigation water quality should be monitored regularly (Figure 5), at least once a month. Surface water quality deteriorates in summer and in response to extreme weather conditions such as heavy rainfall.

Generic *E. coli* is a reliable indicator of water quality, and higher levels of *E. coli* are linked to the presence of STEC and other pathogens in water.

The *E. coli* population in irrigation water for leafy vegetables should be no more than 100 CFU/100 mL (ANZG 2023). If overhead sprinklers are used to irrigate leafy vegetables, especially within 2 days of harvest, the water quality should be monitored closely, aiming for the lowest possible levels of *E. coli*.

Figure 4. Using coarse filtration and frequent backflushing minimises the sediment and reduces the microbial population in irrigation water.

Figure 5. Collecting samples to test irrigation water.

Organic soil amendments: composts containing animal manure should be free from harmful pathogens (Figure 6). Commercial products must be treated in accordance with the Australian Standard AS4454-2012 Composts, soil conditioners and mulches. Traceability of composts containing animal products (e.g. source, batch, certification) sourced and applied in the production system is important to take corrective actions if required.

Figure 6. Microbiological sampling of compost samples.

Coexistence with animal operations: animal operations located near land used for growing leafy vegetables are recognised as a significant risk for the transmission of STEC. Appropriate buffer zones between animals and leafy vegetable operations are required, along with transmission barriers such as perennial windbreaks. Cattle, in particular, are considered one of the highest-risk animals for STEC contamination. Extreme weather conditions (e.g. dust storms, heavy rainfall, cyclones) influence the transmission and spread of contamination within the broader environment surrounding cattle operations. Proper drainage systems should be installed to curtail run-off risk from neighbouring animal operations.

Harvest and postharvest management

- Harvest equipment hygiene is crucial to manage cross-contamination risks.
- Rapid pre-cooling and maintaining a cool chain (<5 °C) are critical to suppress the growth and proliferation of bacterial pathogens such as STEC.
- Drinking quality water (<1 CFU/100 mL of *E. coli*) should be used for postharvest cooling, washing, and sanitising leafy vegetables.
- Pre-wash rinsing the produce helps minimise organic load in wash tanks and remove the exudate from cut leaves. The triple washing process used for baby leafy processing should have an appropriate sanitiser concentration in each washing and sanitising tank (Figure 7).
- In response to extreme weather conditions (e.g. heavy rainfall or dust storms), additional measures such as pre-wash, high-pressure rinsing of leafy vegetables; frequent wash water changes; and increasing sanitiser concentrations and washing time should be undertaken.

Traceability: establishing traceability systems allows producers and retailers to track the produce's origin and quickly identify contamination sources in the event of an outbreak or product recall. Proper documentation of each stage of the supply chain is essential for efficient trace-back procedures. Growers should maintain records of all farm inputs such as soil amendments, fertilisers and water used for leafy vegetable production and processing.

Figure 7. Appropriate sanitiser concentrations should be in each washing and sanitising tank.

Microbiological testing

To ensure production practices, processing operations, and end products are safe, microbiological testing should be conducted. In addition to the routine testing for quality assurance, growers and processors should test produce, soil and water in response to product recalls, food safety incidents and natural disasters such as floods and cyclones. **Testing is a resource-intensive process and should be based on risk levels.** Whether testing is required for produce, soil or water, should be based on the following risk factors:

Produce testing

If the crop is:

- irrigated with overhead sprinklers using water with high levels of *E. coli* (>100 CFU/100mL).
- grown in soil amended with organic products (e.g. fresh and aged animal manure).
- exposed to extreme weather conditions (e.g. heavy rainfall, dust storms).
- near livestock operations.
- grown in the field where animals have been grazing before the crop is planted.

Soil testing

If the production field is:

- exposed to extreme weather conditions (e.g. flooding).
- amended with organic products (e.g. fresh and aged animal manure) in the previous year.
- grazed by animals before the crop is planted.

Water testing

If the surface water:

- is exposed to extreme weather conditions (e.g. flooding).
- is used for overhead sprinkler irrigation, particularly in summer months.
- has historically high levels of *E. coli* (>100 CFU/100mL).
- is close to intensive livestock operations and is prone to upstream contamination.

Preharvest testing of leafy vegetables

Reyes et al. (2023) developed a leafy vegetable supply chain model and predicted that product testing is most effective when conducted early in the system before interventions (e.g. washing).

Preharvest testing of leafy vegetables is thus an effective strategy to mitigate food safety risks.

In the USA, the California Leafy Greens Handler Marketing Agreement (LGMA) developed comprehensive guidance for both routine and risk-based preharvest sampling and testing protocols for leafy vegetables. Detailed information can be obtained from California LGMA's website (https://lgma.ca.gov/).

During natural disasters and responses to recalls/outbreaks, risk-based sampling and testing protocols are appropriate.

Briefly, the following considerations for sampling lot and size should be undertaken for preharvest testing:

- Preharvest sampling: <7 days from harvest.
- Sampling lot: 1 acre (0.4 hectares) or less.
- Divide a 1-acre lot or field-level block into a grid and conduct systematic sampling within each grid, starting at a randomised location with a predetermined spacing. For example, every third bed and approximately every quartered position of the bed length within each grid.
- **Total sample size: 1,500 g** with minimum n = 60 from an acre or less.
- Target pathogens: presence/absence of STEC, *Salmonella*, *Listeria monocytogenes* and generic *E. coli* population.

Low-level and widespread contamination is more likely to be detected with a larger total composite sample mass (Quintanilla Portillo et al. 2022). Therefore, preharvest sampling is more effective when taking an increased number of smaller samples with randomisation. An example of a sampling plan adapted from LGMA is in Table 1.

Table 1. An example of a sampling plan adapted from the California Leafy Greens Handler Marketing Agreement (LGMA).

Crop examples	Total sample size per acre (g)	Sub-sample size (g)	Number of sub-samples per acre	Number of grab specimens per sample	Grab specimen size (g)
Baby leaf (e.g. spinach, rocket)	1,500	150	10	60	2.5
Romaine and iceberg lettuce, cabbage	1,500	375	4	15	25

Grab specimens should include plant tissue collected from multiple heads/plants. Sub-samples comprised of a composite of leaves should be tested as a unit. That means 4 sub-samples (375 g each) for an acre of iceberg lettuce field should be submitted to the testing laboratory.

Generally, a sub-sample of 25 g is taken from a larger collection of samples. However, sampling size and methodology have evolved towards analysing larger masses of samples, especially for fresh leafy vegetables. To test the entire mass of 1,500 g, 4 sub-samples of 375 g or 10 sub-samples of 150 g should be analysed. Laboratories should have validated methods to analyse up to 375 g in a single enrichment. Based on long-established guidance, statistical assumption requires that all sample material (i.e. n = 60 totalling 1,500 g) be used for laboratory analysis.

Leafy vegetable growers and processors affected by natural disasters (e.g. cyclones, heavy rainfall) should discard the leafy vegetable crops that have been in direct contact with floodwater or heavy run-off. Replanting should be considered after at least a 60 day waiting period or after 30 days if the soil microbiological testing meets the standard of compost testing (California LGMA).

Testing of soil

Divide a 1-acre lot or field-level block into a grid and conduct systematic sampling within each grid, starting at a randomised location with a predetermined spacing. For example, every third bed and approximately every quartered position of the bed length within each grid.

Surface soil swabs: these can be collected by aseptically wearing sterile boot covers/ swabs and walking along the crop bed (Figure 8) for at least 50 meters. Fifteen pairs of boot covers (n = 30) should be collected from one acre. Alternatively, sponge swabs can be used to collect soil drags. Aggregate boot cover sampling has been shown to be a more representative and practical method for preharvest soil testing than grab sampling (Wu et al. 2023).

Figure 8. Collecting surface soil samples by aseptically wearing sterile boot covers.

Soil grab samples: collect at least 30 sub-samples of 100 g from each location to make a composite sample of 3,000 g and then mix thoroughly to draw a 300 g test unit. Collect at least 5 composite samples (~300 g each) to a depth of 50–75 mm with a soil sampling tool from an acre of field.

Testing of irrigation water

Microbiological testing of irrigation water should be undertaken to determine the population of generic *E. coli* and the presence of other target pathogens.

- For each water source, at least 3 samples should be collected from the point of application (i.e. sprinklers) and locations closest and farthest from the water source.
- Allow the irrigation system to run for a while (e.g. 3–5 minutes) before collecting samples to ensure the water sample is drawn from the irrigation source and not the residual water in the pipe line.
- Additional sampling can be conducted from primary water sources such as rivers/canals/ponds.
- From each location, collect one litre of water in sterile sampling bottles containing sodium thiosulfate.

Postharvest water testing and monitoring

Water is an important element in postharvest processing of leafy vegetables and its quality determines food safety outcomes. Growers and processors should undertake following measures:

- Microbiological testing of postharvest water used for washing produce and equipment (produce contact surfaces in particular), and workers' facilities should be tested to ensure it meets drinking quality requirements. The samples (one litre each) should be collected from various taps and outlets supplying washing tanks, cleaning hoses and workers' facilities.
- Processors should monitor the concentrations of sanitisers and contact time of leafy vegetables with sanitised water. Automated sanitiser dosing systems should be verified by manual measurement of sanitiser concentrations at regular intervals.
- Increasing the sanitiser concentration and contact time could compensate for the potentially increased microbial load on leafy vegetables exposed to extreme weather conditions. Additionally, wash water chemistry parameters such as pH, turbidity, and oxidation-reduction potential (if applicable) should be monitored regularly.
- Microbiological testing of wash water from processing tanks/flumes for generic *E. coli* and other pathogens is suggested as a measure to minimise cross-contamination risks and to verify the efficacy of sanitisers in washing process.

Testing processed products for target pathogens such as *E. coli*, STEC, *Salmonella* and *Listeria monocytogenes* is suggested; and follow the 'test and release' system. A sample size of 375 g is recommended.

All types of samples should be placed in an insulated container with sufficient freezer bricks/ice packs to keep them cool during transport. These samples should arrive in the testing laboratory within 24 hours of collection.

Conclusions

The contamination of leafy vegetables with STEC poses a significant public health risk, particularly for raw and minimally processed products. The risks can be mitigated through a combination of preventative and detective control measures, both during production and postharvest processing. A comprehensive, multi-faceted approach to risk management is essential for reducing product recalls, preventing the incidence of foodborne illnesses linked to STEC contamination in leafy vegetables, and ensuring safe, high-quality produce for consumers.

Food safety helpdesk

The food safety helpdesk service is available to leafy vegetable growers and processors to answer technical enquiries confidentially. For further information, contact Dr SP Singh on sp.singh@dpi.nsw.gov.au or 0420 593 129.

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