E/S/R Science for Communities

New Zealand Food Safety
Ministry for Primary Industries
Manatū Ahu Matua

SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*

THE ORGANISM/TOXIN

- *Escherichia coli* (*E. coli*) are facultatively anaerobic, Gram-negative rods that are members of the family Enterobacteriaceae (1, 2).
- Shiga toxin-producing *E. coli* (STEC) are a group of *E. coli* that produce one or more extracellular Shiga toxins (Stx). The toxins are classified into two major groups comprising Stx1 and Stx2 and their subtypes. STEC can also be called verocytotoxin-producing *E. coli* (VTEC) (2, 3).
- There are several hundred STEC serotypes with only a small number correlating to illness in humans (1, 3).
- Serotypes O157:H7, O103, O26, O111, O45, O121 and O145 are consistently associated with severe human disease. More severe health outcomes have been associated with STEC producing Stx2, compared with those producing Stx1 (3, 4, 5, 6).
- STEC infections cause a range of diseases varying in severity from asymptomatic carriage to haemorrhagic colitis and haemolytic uraemic syndrome (HUS) (1, 3).
- STEC does not produce toxins in food (7).

GROWTH AND ITS CONTROL

Unless otherwise stated, the information below was derived from the following references (2, 8).

Growth:

	Minimum	Optimum	Maximum
Temperature	7-8°C	35-40°C	46°C
Water activity	0.95	0.995	
pH*	4.4	6-7	10
Atmosphere	• Although STEC do not require oxygen for growth, they grow better in aerobic conditions. • Growth of STEC can occur in vacuum-packed meat at 8 and 9°C, but not when the meat is packed under 100% CO_2.		

*Minimum pH is influenced by other factors including temperature of incubation, acidulant used, a_w and the presence of inhibitory substances such as nitrate.

Survival:

Temperature	• Heat resistance of STEC is dependent on the composition, pH and a_w of the food. • Heat resistance increases as the a_w decreases. • Low temperature (chilling and freezing) has little effect on STEC survival. For example, *E. coli* O157:H7 has been shown to survive on mangoes and papayas stored at -20°C for at least 180 days (9).
pH	• Tolerant to acidic conditions with many strains able to survive at pH 2.5–3.0 for over four hours (10). • O157:H7 has been demonstrated to survive in low pH foods (including fermented sausage, mayonnaise and apple cider vinegar) for up to several weeks depending on the storage temperature and pH (7). • O157:H7 is more tolerant to acid when it is in stationary growth phase or starved during its log-phase of growth (11). It is therefore possible that STEC may be able to survive in food products previously considered too acidic. • Induction of acid-resistance in O157:H7 can increase tolerance to other environmental stresses including heating, radiation and antimicrobials (7).
Atmosphere	• An atmosphere of 100% CO_2 enhanced survival of uninjured STEC at both 4 and 10°C. Survival on fermented meat was equivalent when packed under air or under vacuum.
Biofilm production	• STEC serotypes can form biofilms on different food or food contact surfaces, contributing to persistence in food processing environments (12).
VBNC	• O157:H7 can transition to the Viable but Non-Culturable (VBNC) state after exposure to low temperatures (8ºC) in nutrient-limiting microcosms on the surface of lettuce. These metabolically active VBNC cells can produce small amounts of Shiga toxin (13). • No reports of successful resuscitation of STEC from the VBNC state on plant surfaces, or evidence to show a link between VBNC cells and human illness.

Inactivation:

Thermal inactivation:	
Temperature	• *E. coli* is sensitive to heat however, sensitivity depends on the food composition (fatty foods for example, ground beef, increase resistance), pH, a_w and the growth phase of the cells (stationary phase cells are more resistant than log phase cells, for example after freezing). • Thermal treatments, including hot water rinses, steam pasteurisation and steam vacuuming, are widely used for decontaminating hides and carcasses. None of these treatments are however 100% effective (13).
***D* values**	• **A literature review suggests the following time/temperature (ºC/minutes) in "beef/pork D_{60} 6.1; D_{65} 1.1; D_{70} 0.2 and "all meats": D_{60} 6.9; D_{65} 1.3; D_{70} 0.3** (15).
pH	• Inactivation is dependent on the pH, acidulant and temperature. Studies on inactivation of O157:H7 with lactic, citric or acetic organic acid sprays, on beef carcasses at concentrations up to 1.5% have shown that pathogen concentrations were not affected by any of the treatments (16).
Drying	• Drying is not an effective intervention to kill STEC as 35 strains survived 24 hours of drying at 35°C (14).

Non-thermal inactivation:	
Sanitisers /disinfectants	• STEC strains in foods are susceptible to disinfectants used in food processing environments, including chlorine-based sanitisers, hydrogen peroxide, ozone and acidified sodium chlorite. A variety of mechanisms can be used to apply antimicrobial chemicals to food products including washing, spraying, gassing, sonication and incorporation into ice (14). It has however been reported that STEC can be internalised in vegetables such as lettuce and sprouts where they may be protected from the disinfecting properties of these agents (17).
Radiation	• STEC is sensitive to UV and γ irradiation. Resistance to UV has been reported to vary amongst different STEC serogroups, with *E. coli* O157:H7 reported as one of the more resistant serotypes (17). • The D_{10} values for *E. coli* O157:H7 depend on temperature and are in the range of 0.2-0.3 kGy with values higher for frozen beef patties than refrigerated patties (18). • Combinations of radiation (1-2 kGy) and dry heat (50°C for 60 minutes) or chlorine (200 ppm) have successfully reduced STEC levels on alfalfa seeds and fresh cilantro respectively (14).
Preservatives and other non-thermal processing technologies	• STEC is inhibited by 8.5% NaCl at 37°C, with growth retarded above 2.5% NaCl. The amount of salt required for inhibition reduces as other factors such as temperature and pH become sub-optimal. For example 5% salt inhibited *E. coli* O157:H7 at 12°C. • High Hydrostatic Pressure (HHP), in conjunction with elevated temperatures, has proved effective in reducing STEC from some food matrices (14). • Ultrasound, UV light and pulsed-electric field interventions have proved successful in reducing STEC numbers from some food matrices (14). • Host-specific bacteriophages have been successfully used to kill STEC on the surfaces of beef samples (19). However, another study found that treatment of cattle hides with bacteriophages before processing did not produce a significant reduction of O157:H7 on hides or carcasses during processing (20).

THE ILLNESS

Incubation: 3 to 9 days (mean 4 days) (4).

Symptoms:

- Symptoms ranging from mild watery diarrhoea, abdominal cramps, fever and vomiting to bloody diarrhoea, thrombocytopaenia, uraemia and acute kidney failure. Both O157 and non-O157 STEC have similar clinical presentations but disease severity can differ depending on the serotype (7, 21).
- Most patients recover within 10 days of the initial onset of symptoms, but a proportion (up to 10%) go on to develop more serious forms of the disease (7, 22).

Conditions: Haemorrhagic Colitis (HC); Haemolytic Uraemic Syndrome (HUS); Thrombocytopaenic purpura (TPP) (1, 23).

At Risk Groups:

- Any age group, but most often causes disease in young children (< 5 years), the elderly (>65 years) and people with compromised immune systems (4, 24).
- Risk factors include consumption of contaminated food or water and direct contact with farm or domesticated animals and their manure (24, 25).

Long-term effects:

- HUS, chronic and end-stage kidney disease. Less frequently gastrointestinal complications, neurological disorders and diabetes mellitus (23).
- Up to 10% of STEC-infected individuals develop HUS (26), with a fatality rate of 3-5% (4, 23, 26).

Dose:

- Infective dose of *E. coli* O157:H7 in food is estimated to be in the range of < 50 to a few hundred cells (24, 27, 28).
- There are few data on which to base a dose-response relationship for non-O157 STEC. However, available information suggests that the minimum dose estimates for STEC serogroups O111 and O145 are comparable, or slightly higher, to minimum dose estimates for *E. coli* O157:H7 (4, 6).

Incidence:

- New Zealand incidence rate of 8.9/100,000 (2016) (25). Notifications for STEC have been increasing since 1997. This is partly due to changes in laboratory testing practices with increasingly sensitive assays and algorithms being used.
- New Zealand incidence rates are higher than the EU (1.59/100,000 (2013)) (29), the USA (2.2/100,000 (2015)) (30) Australia (0.5/100,000 (2014)) (31).

Treatment:

- Treatment rarely required.
- In more severe cases, fluid replacement therapy may be given, with platelet and blood transfusions together with dialysis for cases of HUS.
- The use of antibiotics to treat STEC infections has become contentious as certain antimicrobials can stimulate Shiga toxin production (4).

SOURCES

Human:

- Infected person (stools) (1).

Animal:

- Exposure to ruminant (cattle, sheep and goats) faecal matter is the main source of STEC (O157 and non-O157 serotypes) infections for humans. (4, 24, 25, 32).
- A study in Scotland showed that cattle faeces can contain from 100 to over 10^6 CFU/g of *E. coli* O157:H7 (33).
- Exposure by direct contact with domesticated animals or birds from farms or petting zoos (7).

Food:

- Foods of particular concern include ground and

fermented meat products, consumption of undercooked hamburgers, unpasteurised apple cider and juice, raw dairy products and fresh produce (fruit and vegetables) (1, 4, 7, 24).
- Consumption of undercooked ground beef has been implicated in up to 40% of all major outbreaks (4, 9).

Environment/Water:
- STEC shed in faeces can contaminate pasture, soil and water (24)
- Contamination in the environment can act as a source of infection for humans and/or other animals (4).
- STEC can survive for 150 days in soil (34, 35) and 90 days in cattle faeces (36).
- Water from ponds, streams, swimming pools, wells and water troughs have been implicated in a number of large outbreaks overseas (4, 7).

Transmission Routes:
- Consumption of contaminated food and water, person-to-person transmission (faecal-oral route), environmental exposure, direct contact with animals (4, 6, 7, 37).

OUTBREAKS AND INCIDENTS

Outbreaks:

New Zealand
- The number of foodborne outbreaks, and associated cases, has increased gradually over the past 10 years (38). This trend can be partly attributed to changes in laboratory testing practices. The majority of outbreaks in recent years have been from person-to-person contact.

New Zealand

Notable foodborne outbreaks in recent years are included below. Updates can be found on the ESR website* https://surv.esr.cri.nz/surveillance/annual_outbreak.php

Year	Foodborne outbreaks (cases)	Suspected foods
2010	1 (3)	O157:H7 No identifiable source
2013	2 (4)	1 outbreak associated with dairy products
2014	4 (15)	2 outbreaks associated with dairy products

*Some outbreaks have no identifiable source

Worldwide

Notable foodborne outbreaks in recent years are included below.

Year	Cases (deaths)	Suspected foods	Country	Strain	Control measure failure
2011	3950 (53), 800 cases developed HUS	Fenugreek seeds	EU member States, North America. All cases had been in Germany or France shortly before becoming ill.	O104:H4	Contaminated, imported Egyptian seeds (Beutin *et al*., 2012) (39).
2016	111	Beef, veal and bison products	USA (multistate)	O157:H7	Meat products contaminated at the slaughterhouse (40).
2016	63	Flour	USA (multistate)	O121 and O26	Contamination of flour at mill (41).
2017	32	SoyNut butter products	USA (multistate)	O157:H7	Contamination of product at manufacture (42).
2017	25	Leafy greens	USA (multistate)	O157:H7	Contaminated leafy greens (43).

LIST OF NZ RISK PROFILES ON STEC IN FOODS

Matrix	Date	Link
Shiga Toxin-producing *Escherichia coli* in red meat and meat products	2002	http://www.mpi.govt.nz/dmsdocument/26042-risk-profile-shiga-toxin-producing-escherichia-coli-in-red-meat-and-meat-products
Shiga-like Toxin-producing *Escherichia coli* in uncooked comminuted fermented meat (UCFM) products	2003	http://www.mpi.govt.nz/dmsdocument/25988-risk-profile-shiga-like-toxin-producing-escherichia-coli-in-uncooked-comminuted-fermented-meat-products
Shiga Toxin-producing *Escherichia coli* in leafy vegetables	2006	http://www.mpi.govt.nz/dmsdocument/26045-risk-profile-shiga-toxin-producing-escherichia-coli-in-leafy-vegetables
Shiga Toxin-producing *Escherichia coli* in uncooked comminuted fermented meat products	2007	http://www.mpi.govt.nz/dmsdocument/26000-risk-profile-shiga-toxin-producing-escherichia-coli-in-uncooked-comminuted-fermented-meat-products
(Update) Shiga Toxin-producing *Escherichia coli* in red meat	2014	http://www.mpi.govt.nz/dmsdocument/7272-risk-profile-update-shiga-toxin-producing-escherichia-coli-in-red-meat-and-meat-products
(Update) Shiga Toxin-producing *Escherichia coli* in raw milk	2014	http://www.mpi.govt.nz/dmsdocument/1121-risk-profile-shiga-toxin-producing-escherichia-coli-in-raw-milk

REFERENCES

1- Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M. and Finlay, B.B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical Microbiology Reviews. **26(4):** 822-880.

2- Desmarchelier, P.M. and Fegan, N. (2003). Enteropathogenic *Escherichia coli.* Ch 9 In: Hocking A.D. (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (AIFST) (NSW Branch), Sydney, p. 267-310.

3- Rivas, L., Mellor, G.E., Gobius, K. and Fegan, N. (2015). Detection and typing strategies for pathogenic *Escherichia coli*. Springer brief in food, health and nutrition. Published by Springer, New York. ISBN 978-1-4939-2345-8.

4- Bryan, A., Youngster, I. and McAdam, A.J. (2015). Shiga toxin-producing *Escherichia coli.* Clinical Laboratory Medicine. **35:** 247-272.

5- Mingle, L.A., Garcia, D.L., Root, T.P., Halse, T.A., Quinlan, T.M., Armstrong, L.R., Chiefari, A.K., Schoonmaker- Bopp, D.J., Dumas, N.B., Limberger, R.J. and Musser, K.A. 2012. Enhanced identification and characterization of non-O157 Shiga toxin-producing *Escherichia coli*: a six-year study. Foodborne Pathogens and Disease **9(11):**1028–1036.

6- Smith, J.L., Fratamico, P.M. and Gunther, N.W. (2014). Shiga toxin-producing *Escherichia coli.* Advances in Applied Microbiology. **86:** 145-197.

7- Meng, J., Doyle, M.P., Zhao, T. and Zhao, S. (2007). Enterohemorrhagic *Escherichia coli.* Ch 12 in: Doyle, M.P, Beuchat, L.R. (eds). Food Microbiology: Fundamentals and Frontiers. 3rd ed, ASM Press, Washington D.C., p. 249-269.

8- The International Commission on Microbiological Specifications for Foods (ICMSF) (1996). Intestinally pathogenic *Escherichia coli*. In Micro-organisms in Foods 5 Microbiological Specifications of Food Pathogens, pp 126-140. Blackie Academic and Professional, London.

9- Strawn L.K. and Danyluk M.D. (2010). Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. on fresh and frozen cut mangoes and papayas. International Journal of Food Microbiology. **138**: 78-84.

10- Molina, P.M., Parma, A.E. and Sanz, M.E. (2003). Survival in acidic medium of Shiga toxin-producing *Escherichia coli* O157:H7 and non-O157:H7 isolated in Argentina. BMC Microbiology. **3:** 17.

11- Arnold, K.W. and Kaspar, C.W. (1995). Starvation and stationary-phase-induced acid tolerance in *Escherichia coli* O157:H7. Applied and Environmental Microbiology. **61(5):** 2037-2039.

12- Wang, R., Bono, J.L., Kalchavanand, N., Shackelford, S. and Harhay, D.M. (2012). Biofilm formation by shiga toxin-producing *Escherichia coli* O157:H7 strains and their tolerance to sanitizers commonly used in the food processing environment. Journal of Food Protection. **75(8):** 1418-1428.

13- Dinu, L. and Bach, S. (2011). Induction of a viable but nonculturable *Escherichia coli* O157:H7 in the phyllosphere of lettuce: a food safety risk factor. Applied and Environmental Microbiology. **77**: 8295-8302.

14- Erickson, M.C. and doyle, M.P. (2007). Food as a vehicle for transmission of Shiga Toxin-Producing *Escherichia coli.* Journal of Food Protection **70(10):** 2426-2449.

15- Horn, B. (2015). *D* and *z* values for the heat inactivation of pathogens in raw meat. Prepared for the Ministry for Primary Industries under project MFS/14/4. Client report number: FW 15001.

16- Brackett, R., Hao, Y. and Doyle, M. (1994). Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. Journal of Food Protection. **57:**198-203.

17- Smith, J.L., Fratamico, P.M. and Gunther, N.W. (2014). Shiga toxin-producing *Escherichia coli.* Advances in Applied Microbiology. **86:** 145-197.

18- Clavero, M., Monk, J., Beuchat, L., Doyle, M. and Brackett, R. (1994). Inactivation of *Escherichia coli* O157:H7, salmonellae and *Campylobacter jejuni* in raw ground beef by gamma irradiation. Applied and Environmental Microbiology. **60:** 2069-2075.

19- O'Flynn, G., Ross, P., Fitzgerald, F. and Coffey, A. (2004). Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. Applied Environmental Microbiology **70:** 3417-3424.

20- Arthur, T.M., Kalchayanand, N., Agga, G.E., Wheeler, T.L. and Koohmaraie, M. (2017). Evaluation of bacteriophage application to cattle in lairage at beef processing plants to reduce *Escherichia coli* O157:H7 prevalence on hides and carcasses. Foodborne Pathogens and Disease **14(1):** 17-22.

21- Karmali, M.A., Mascarenhaus, M., Shen, S., Ziebell, K., Johnson, S., Reid-smith, R., Issac-Renton, J., Clark, C., Rahn, K. and Kaper, J.B. (2003). Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. Journal of Clinical Microbiology. **41**: 4930-4940.
22- World Health Organisation (WHO). 2011. Fact sheet No 125- Enterohaemorrhagic *Escherichia coli* (EHEC). World Health Organisation, Geneva.
23- Gould, L.H., Demma, L., Jones, T.F., Hurd, S., Vugia, D.J., Smith, K., Shiferaw, B., Segler, S., Palmer, A., Zansky, S. and Griffin, P.M. (2009). Haemolytic uremic syndrome and death in persons with *Escherichia coli* O157:H7 infection. Foodborne Diseases Active Surveillance Network Sites, 2000-2006. Clinical Infectious Diseases **49:** 1480-1485.
24- Gyles, C.L. (2007). Shiga toxin-producing *Escherichia coli*: An overview. Journal of Animal Science **85** (E suppl.): E45-E62.
25- ESR (2016) Notifiable diseases in New Zealand. Annual Report 2015. ESR, Porirua. *https://surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2016/2016AnnualNDReportFinal.pdf* (accessed February 2018).
26- European Food Safety Authority Panel on Biological Hazards (BIOHAZ). (2013). Scientific opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. EFSA Journal. **11**:3138.
27- Tilden, J., Young, W., McNamara, A.M., Custer, C., Boesel, B., Lambert-Fair, M.A., Majkowski, J., Vugia, D., Werner, S.B., Hollingsworth, J. and Morris, J.G. (1996). A new route of transmission for *Escherichia coli*: Infection from dry, fermented salami. American Journal of Public Health **86:** 1142-1145.
28- Bell, B.P., Godoft, M.,Griffin, P.M., Davis, M.A., Gordon, D. Tarr, P.I., Bartleson, C.A., Lewis, J.H., Barrett, T.J. and Wells, J.G. (1994). A multistate outbreak of Escherichia coli O157:H7-associated bloody diarrhoea and haemolytic uremic syndrome from hamburgers. The Washington Experience. JAMA **272:** 1349-1353.
29- European Centre for Diseases Prevention and Control. The European Union Summary Report on trends and sources of Zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA, Parma, Italy and ECDC, Stockholm, Sweden *http://ecdc.europa.eu/en/publications/Publications/EU-summary-report-trends-sources-zoonoses-2013.pdf* (accessed February 2018).
30- Centers for Disease Control and Prevention. Summary of Notifiable Infectious Diseases and Conditions – United States, 2015. *https://www.cdc.gov/mmwr/volumes/64/wr/mm6453a1.htm?s_cid=mm6453a1_w* (accessed February 2018).
31- Annual Report of the National Notifiable Diseases Surveillance System. Australia's Notifiable Disease Status (2014): NNDS Annual Report Working Group. *https://health.gov.au/internet/main/publishing.nsf/Content/cda-cdi4001-pdf-cnt.htm/$FILE/cdi4001e.pdf* (accessed February 2018).
32- Rivas, L., Lake, R., Cressey, P., King, N., Horn, B. and Gilpin, B. (2014). Risk profile (update): Shiga toxin-producing *Escherichia coli* in red meat and meat products. Prepared for the Ministry for Primary Industries under code MRP/11/01 by Environmental Science and Research Client report no: FW12014.
33- Chase-Topping, M.E., McKendrick, I.J., Pearce, M.C., MacDonald, P., Matthews, L., Halliday, J., Allison, L., Fenlon, D., Low, J.C., Gunn, G. and Woolhouse, M.E.J. (2007). Risk factors for the presence of high-level shedders of *Escherichia coli* O157 on Scottish farms. Journal of Clinical Microbiology. **45:**1594-1603.
34- Ogden, I.D., Hepburn, N.F., MacRae, M., Strachan, N.J.C., Fenlon, D.R., Rusbridge, S.M. and Pennington, T.H. (2002). Long-term survival of *Escherichia coli* O157 on pasture following an outbreak associated with sheep at a scout camp. Letters in Applied Microbiology. **34:** 100-104.
35- Bolton, D.J., Monaghan, A., Byrne, B., Fanning, S., Sweeny, T. and McDowell, D.A. (2011). Incidence and survival of non-O157 verocytotoxigenic *Escherichia coli* in soil. Journal of Applied Microbiology. **111:** 484-490.
36- Williams, A.P., McGregor, K.A., Killham, K. and Jones, D.L. (2008). Persistence and metabolic activity of *Escherichia coli* O157:H7 in farm animal faeces. FEMS Microbiology Letters. **287:** 168-173.
37- Hale, C.R., Scallon, E., Cronquist, A.B, Dunn, J., Smith, K., Robinson, T., Lathrop, S., Tobin-D'Angelo, M. and Clogher, P. (2012). Estimates of enteric illness attributable to contact with animals and their environments in the United States. Clinical Infectious Diseases of Clinical Microbiology. **54** (Suppl 5): S472-S479.

38- ESR (2015) Summary of outbreaks in New Zealand 2015. Annual Report (2015). ESR, Porirua. *https://surv.esr.cri.nz/surveillance/annual_outbreak.php?we_objectID=4480* (accessed February 2018).
39- Beutin, L. and Martin, A. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains. Journal of Food Protection. **75(2):** 408-418.
40- Centers for Disease Control and Prevention (CDC. (2016). Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to beef products produced by Adams Farm (final update).*https://www.cdc.gov/ecoli/2016/o157h7-09-16/index.html* (accessed February 2018).
41- U.S. Food and Drug Administration (FDA) (2017). FDA investigated multistate outbreak of Shiga toxin-producing E. coli infections linked to flour. *https://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm504192.htm* (accessed February 2018).
42- Centers for Disease Control and Prevention (CDC. (2016). Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to I.M. Healthy Brand SoyNut Butter (final update). *https://www.cdc.gov/ecoli/2017/o157h7-03-17/index.html* (accessed February 2018).
43- Centers for Disease Control and Prevention (CDC. (2016). Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked leafy greens (final update). *https://www.cdc.gov/ecoli/2017/o157h7-12-17/index.html* (accessed February 2018).