FOOD
STANDARDS
Australia New Zealand

AGENTS OF
FOODBORNE ILLNESS

2nd Edition

A technical series
summarising key
information
on microorganisms
associated with
foodborne illness

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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Acknowledgements

Preface

Foodborne disease is a significant cause of morbidity and mortality across the globe and the increased internationalisation of food production and distribution means pathogens associated with food know no borders. The management and reduction of foodborne disease is therefore a core objective of all food agencies. As one strategy to support this objective a number of resources have been developed and made available by food and scientific research agencies to inform food manufacturers, consumers and physicians about foodborne illness. The FDA Bad Bug Book for example, provides information to consumers and physicians primarily focused on the clinical characteristics of the food illness produced by each organism, to assist in their recognition and diagnosis. Advice to manufacturers on principles of safe food production to avoid contamination with pathogenic microorganisms is also available, such as the CSIRO Food and Nutrition Sciences' "Guide to Food Safety - Make it Safe". An area not comprehensively addressed by any current, readily available resource, however, is the behaviour of foodborne pathogens under varying food and environmental conditions.

Although a limited range of organisms are responsible for the majority of foodborne disease, their potential survival, growth and toxin production, and therefore their pathogenicity, is dependent to a significant extent on the food matrix in which they are present. This interplay between the characteristics of foods and potentially pathogenic microorganisms they may contain, creates a high level of complexity and challenge in the discipline of food microbiology. Prediction, prevention and management of foodborne disease is therefore dependent on an understanding of the behaviour of microorganisms under different conditions and in different food matrices. This technical series provides monographs summarising the key biological characteristics of foodborne pathogens to support microbiological risk assessment, including hazard identification.

This series is aimed at a scientific audience with general knowledge of microbiology and provides contemporary information on the characteristics of microorganisms that may be useful to technical members of the food industry, food safety consultants and food regulators.

Each chapter contains details on growth and survival characteristics of the pathogen, symptoms of disease, virulence factors, epidemiological data (including a summary of large, well-document outbreaks), occurrence of the pathogen in food, susceptible populations and the dose-response relationship. At the end of each chapter is a list of recommended reading and useful links for further information.

This Second edition contains updated information and includes an additional six chapters. The series has also been expanded to consider parasites and infectious prion particles. FSANZ welcomes and invites comments, suggestions, corrections or additional information to enhance the material presented in the series.
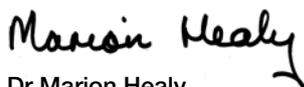
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Bacillus cereus

Bacillus cereus is a spore forming bacterium that produces toxins that cause vomiting or diarrhoea. Symptoms are generally mild and short-lived (up to 24 hours). *B. cereus* is commonly found in the environment (e.g. soil) as well as a variety of foods. Spores are able to survive harsh environments including normal cooking temperatures.

Description of the organism

B. cereus is a Gram-positive, motile (flagellated), spore-forming, rod shaped bacterium that belongs to the *Bacillus* genus. Species within this genus include *B. anthracis*, *B. cereus*, *B. mycoides*, *B. thuringiensis*, *B. pseudomycoides* and *B. weihenstephanensis* (Rajkowski and Bennett 2003; Montville and Matthews 2005). Genomic sequencing data has shown *B. anthracis*, *B. cereus* and *B. thuringiensis* to be very closely related (Rasko et al. 2004) with their 16S rRNA gene sequence sharing more than 99% similarity (Ash et al. 1991).

B. cereus is widespread in nature and readily found in soil, where it adopts a saprophytic life cycle; germinating, growing and sporulating in this environment (Vilain et al. 2006). Spores are more resistant to environmental stress than vegetative cells due to their metabolic dormancy and tough physical nature (Jenson and Moir 2003).

B. cereus produces two types of toxins – emetic (vomiting) and diarrhoeal – causing two types of illness. The emetic syndrome is caused by emetic toxin produced by the bacteria during the growth phase in the food. The diarrhoeal syndrome is caused by diarrhoeal toxins produced during growth of the bacteria in the small intestine (Ehling-Schulz et al. 2006).

Growth and survival characteristics

Strains of *B. cereus* vary widely in their growth and survival characteristics (refer to Table 1). Isolates from food and humans can be subdivided as either mesophilic or psychrotrophic strains. Mesophilic strains grow well at 37°C but do not grow below 10°C; psychrotrophic strains grow well at refrigeration temperatures but grow poorly at 37°C (Wijnands et al. 2006a). All isolates of *B. cereus* associated with emetic toxin production have been found to be mesophilic in nature (Pielaat et al. 2005; Wijnands et al. 2006b).

The maximum salt concentration tolerated by *B. cereus* for growth is reported to be 7.5% (Rajkowski and Bennett 2003). *B. cereus* growth is optimal in the presence of oxygen, but can occur under anaerobic conditions. *B. cereus* cells grown under aerobic conditions are less resistant to heat and acid than *B. cereus* cells grown anaerobically or microaerobically (Mols et al. 2009).

Mesophilic strains of *B. cereus* have been shown to have greater acid resistance than psychrotrophic strains (Wijnands et al. 2006b). The observed average decimal reduction value or D-value (the time required to reduce the initial concentration of bacterial cells or spores by 1 $\log_{10}$ unit) was 7.5 min for mesophilic strain stationary phase cells (pH 3.5, 37°C). In comparison the D-value for psychrotrophic strains under the same conditions was 3.8 min (Wijnands et al. 2009; Augustin 2011).

There is considerable strain variability in the heat resistance of *B. cereus* spores. The D-values of some strains is up to 15 to 20 times greater than the more heat sensitive strains. The D-value at 85°C is 33.8–106 min in phosphate buffer, and at 95 °C is 1.5–36.2 min and 1.8–19.1 min in distilled water and milk, respectively (ICMSF 1996). Heat resistance is increased in high fat and oily foods, for example in soybean oil the D-value at 121°C is

30 min. Spores are more resistant to dry heat than moist heat, with heat resistance usually greater in foods with lower water activity. Spores are also more resistant to radiation than vegetative cells (Jenson and Moir 2003).

Nisin is a preservative that is used to inhibit the germination and outgrowth of spores. Antimicrobials which inhibit the growth of *B. cereus* include benzoate, sorbates and ethylenediaminetetraacetic acid (Jenson and Moir 2003).

Table 1: Limits for growth of *B. cereus* and toxin production when other conditions are near optimum (Kramer and Gilbert 1989; Sutherland and Limond 1993; ICMSF 1996; Fermanian et al. 1997; Finlay et al. 2000)

	Bacterial Growth		Emetic Toxin Production		Diarrhoeal Toxin Production	
	Optimum	Range	Optimum	Range	Optimum	Range
Temperature (°C)	30–40	4–55	12–15	12–37	32	10–43
pH	6.0–7.0	4.9–10.0	-	-	8.0	5.5–10
Water activity	-	0.93–0.99	-	-	-	-

Symptoms of disease

B. cereus causes two types of foodborne illness – emetic (vomiting) and diarrhoeal syndromes. The emetic syndrome is an intoxication that is caused by ingestion of a cyclic peptide toxin called cereulide that is pre-formed in the food during growth by *B. cereus*. This syndrome has a short incubation period and recovery time. The symptoms of nausea, vomiting and abdominal cramping occur within 1–5 hours of ingestion, with recovery usually within 6–24 hours (Schoeni and Wong 2005; Senesi and Ghelardi 2010).

The diarrhoeal syndrome is caused by enterotoxins produced by *B. cereus* inside the host. The incubation period before onset of disease is 8–16 hours and the illness usually lasts for 12–14 hours, although it can continue for several days. Symptoms are usually mild with abdominal cramps, watery diarrhoea and nausea (Granum 2007).

In a small number of cases both types of toxin are produced, and emetic and diarrhoeal symptoms occur (Montville and Matthews 2005). Neither form of illness is considered life-threatening to normal healthy individuals, with few fatal cases reported (Jenson and Moir 2003). *B. cereus* has been associated with non-food related illness, although this occurs rarely. The bacterium has been found in postsurgical and traumatic wounds and can cause opportunistic infections, especially in immunocompromised individuals, such as septicaemia, meningitis and pneumonia. *B. cereus* has also been known to occasionally cause localised eye infections in humans (Schoeni and Wong 2005).

Virulence and infectivity

The pathogenic mechanism for the *B. cereus* emetic illness has been well characterised. The emetic toxin (cereulide) causes vacuole formation in HEp-2 cells in the laboratory (Agata et al. 1994; Schoeni and Wong 2005). Using an animal model, Agata et al. (1995) showed that cereulide causes vomiting, potentially by binding to the 5-HT$_3$ receptors in the stomach/small intestine to stimulate the vagus nerve and brain.

Cereulide is produced by a non-ribosomal peptide synthetase (NRPS) complex (Horwood et al. 2004; Toh et al. 2004). The entire NRPS cluster has been characterised (Ehling-Schulz et al. 2006) resulting in a highly specific method for detection of cereulide producing *B. cereus* strains (Fricker et al. 2007).

Production of the emetic toxin has been shown to occur in skim milk within the temperature range of 12–37°C, with more toxin produced at 12 and 15°C compared to higher temperatures (Finlay et al. 2000). The emetic toxin is highly resistant to environmental factors, showing stability from pH 2–11 and during heating to 100°C for 150 minutes (pH 8.7–10.6) (Jenson and Moir 2003; ESR 2010).

Three types of enterotoxins are associated with the diarrhoeal form of disease. These are: the three component enterotoxin haemolysin BL (HBL), the three component non-haemolytic enterotoxin (NHE) and the single component enterotoxin cytotoxin K. After consumption of food containing *B. cereus*, the enterotoxins are released into the small intestine during vegetative growth following spore germination, and by any surviving vegetative cells (Wijnands et al. 2009).

The diarrhoeal enterotoxins can be produced in the temperature range of 10–43°C, with an optimum of 32°C (Kramer and Gilbert 1989; Fermanian et al. 1997). Production occurs between pH 5.5–10, with an optimum of pH 8 (Sutherland and Limond 1993). The diarrhoeal enterotoxins are stable at pH 4–11 and inactivated by heating to 56°C for 5 minutes (Jenson and Moir 2003). Maltodextrin is known to stimulate growth of *B. cereus* and to aid diarrheal enterotoxin production in reconstituted and stored infant milk formulae (Rowan and Anderson 1997). It has also been shown that *B. cereus* produces more HBL and NHE under conditions of oxygen tension (low oxygen reduction potential) that simulate the anaerobic, highly reducing fermentative conditions encountered in the small intestine (Zigha et al. 2006).

Up to 26% of *B. cereus* vegetative cells can survive conditions that simulate passage through the stomach. The survival rate of the vegetative cells is dependent on the strain type, phase of vegetative cell growth and the gastric pH (Wijnands et al. 2009). As diarrhoeal enterotoxins are unstable at low pH and are degraded by digestive enzymes, any enterotoxins pre-formed in food would be destroyed during passage through the stomach and so not cause illness if ingested (Jenson and Moir 2003).

In contrast, spores of *B. cereus* are able to pass unaffected through the gastric barrier. The spores contain receptors that need triggering by certain low molecular weight substances to commence germination. These inducers may be present in the food as well as the intestinal epithelial cells. In the small intestine the spores germinate, grow and produce enterotoxins (Wijnands 2008).

A crucial virulence factor required for causing the diarrhoeal symptoms is the ability of the vegetative cells and spores of *B. cereus* to adhere to the epithelial cell wall of the small intestine. The adhesion efficiency of spores and cells has been shown to be low, approximately 1% (Wijnands 2008).

The ability of the enterotoxins to act as tissue-destructive proteins and damage the plasma membrane of the epithelial cells of the small intestine suggests a role for these enterotoxins in causing diarrhoea (Senesi and Ghelardi 2010). Beecher et al. (1995) showed HBL causes fluid accumulation in ligated rabbit ileal loops, implicating a role in diarrhoea. However, direct involvement of NHE and cytotoxin K in causing diarrhoea is yet to be demonstrated (Senesi and Ghelardi 2010).

Efficient horizontal DNA transfer systems are present within the *B. cereus* group, enabling plasmids to be transferred among strains of different species of this group (*B. cereus*, *B. anthracis* and *B. thuringiensis*). The plasmids are known to be important determinants of virulence properties of *B. cereus* strains, since they contain genes responsible for virulence such as the *ces* gene cluster required for cereulide formation and emetic disease (Arnesen et al. 2008). Furthermore, chromosomal DNA contains genes associated with the diarrhoeal disease,

and is therefore present in all strains. In view of the homogeneity of the *B. cereus* group, an online tool has been developed for ascertaining the foodborne virulence potential of strains (Guinebretière et al. 2010).

Mode of transmission

B. cereus food poisoning can be caused by either ingesting large numbers of bacterial cells and/or spores in contaminated food (diarrhoeal type) or by ingesting food contaminated with pre-formed toxin (emetic type). Transmission of this disease results from consumption of contaminated foods, improper food handling/storage and improper cooling of cooked foodstuffs (Schneider et al. 2004).

Incidence of illness and outbreak data

B. cereus related food poisoning is not a notifiable disease in most countries, including Australia and New Zealand, and therefore incidence data is extremely limited. It is recognised that there may be significant under reporting of *B. cereus* illness due to the generally mild, short duration and self-limiting symptoms, in addition to it being infrequently tested for in routine laboratory analyses of stool samples.

There was one reported outbreak of *B. cereus* foodborne illness in Australia in 2011 and one outbreak reported in 2010 (OzFoodNet 2012a; OzFoodNet 2012b). It has been estimated that *B. cereus* accounts for 0.5% of foodborne illness caused by known pathogens in Australia (Hall et al. 2005). In New Zealand there was one foodborne *B. cereus* outbreak reported in 2011, there were no outbreaks reported in 2010 (Lim et al. 2012).

In the European Union there were 0.04 reported cases of *B. cereus* foodborne illness per 100,000 population in 2011 (ranging from <0.01–0.24 per 100,000 population between countries). This was an increase from the 2010 case rate of 0.02 cases per 100,000 population (EFSA 2012; EFSA 2013).

B. cereus was reported as a major causative agent of foodborne illness in the Netherlands in 2006 (causing 5.4% of the foodborne outbreaks) and in Norway in 2000 (causing 32% of foodborne outbreaks) (Wijnands 2008). Scallen et al. (2011) estimated that in the United States (US), *B. cereus* caused 0.7% of foodborne illness caused by 31 major pathogens.

Meat, milk, vegetables and fish have been the predominant food types associated with the diarrhoeal syndrome. In contrast, rice products, potato, pasta and cheese products have been the predominant foods associated with the emetic syndrome (FDA 2012) (refer to Table 2).

Table 2: Selected major outbreaks associated with *B. cereus* (>50 cases and/or ≥1 fatality)

Year	No. of cases (fatalities)	Food	Syndrome type	Country	Comments	References
2008	1 (1)	Spaghetti with tomato sauce	Emetic	Belgium	Food stored at room temperature for 5 days after preparation. *B. cereus* and cereulide isolated from pasta	(Naranjo et al. 2011)
2007	2 (1)	Asparagus sauce	Emetic	Australia	Prior to serving, the sauce was stored for 2 hours in a hot kitchen (up to 37°C), permitting *B. cereus* growth	(NSW Food Authority 2013)
2003	4 (1)	Pasta salad	Emetic	Belgium	Food stored for 3 days in fridge at 14°C, permitting *B. cereus* growth. *B. cereus* isolated from food	(Dierick et al. 2005)
2000	173	Cake	Diarrhoeal	Italy	*B. cereus* isolated from food and rolling board. Rolling board likely source of contamination	(Ghelardi et al. 2002)
1998	44 (3)	Vegetable puree	Diarrhoeal	France	Cytotoxin K produced by *B. cereus* involved	(Jenson and Moir 2003)
1991	139	Barbequed pork	Diarrhoeal	US	*B. cereus* spores from dried foods, slaughtered animals or worker hands likely source of contamination. Unrefrigerated storage of cooked pork for >18 hours permitted *B. cereus* growth	(Luby et al. 1993)
1989	55	Cornish game hens	Diarrhoeal	US	Inadequate thawing and cooking, cross-contamination from basting brush used before and after cooking, inadequate refrigeration	(Slaten et al. 1992)

Occurrence in foods

As *B. cereus* is found in soil, raw plant foods such as rice, potatoes, peas, beans and spices are common sources of *B. cereus*. The presence of *B. cereus* in processed foods results from contamination of raw materials and the subsequent resistance of spores to thermal and other manufacturing processes. During the cooling processes, spores may germinate, enabling *B. cereus* to multiply in the food and/or produce high levels of the emetic toxin cereulide, depending on the strain(s) present (Wijnands 2008).

B. cereus has been recovered from a wide range of food types. A survey carried out in Brisbane on 1,263 retail food products reported the prevalence of *B. cereus* as 1.6% on unbaked pizza bases (n=63), 4.5% on ready-to-reheat frozen cooked meat pies (n=157), 0.3% on processed meats (n=350) and 5.5% on raw diced chicken (n=55) (Eglezos et al. 2010).

In the Netherlands, an investigation was carried out on the prevalence of potentially pathogenic strains of *B. cereus* in retail food samples (Wijnands et al. 2006b). The strains containing potential toxin producing genes were classified as psychrophilic, intermediate and mesophilic in nature. It was found that 89.9% of the isolates were mesophilic, with psychrophilic and intermediate strains amounting to 4.4% and 5.7%, respectively (n=796). Of the isolates found in flavourings, 98.9% were mesophilic (n=92). Prevalence of mesophilic isolates was also high in ready-to-eat products (92.7%, n=384), vegetables and vegetable products (91.4%, n=115) and pastry (90.1%, n=81). A higher prevalence of psychrophilic strains of *B. cereus* have been reported in meat and meat products (20.8%, n=24) and in fish and fish products (40%, n=40) (Wijnands et al. 2006b). A study by Ankolekar et al. (2009) undertaken in the US reported that 93.3% of the *B. cereus* strains isolated from retail uncooked (raw) rice (n=83) were positive for NHE or HBL, representing diarrhoeal strains.

Agata et al. (2002) performed an investigation into cereulide in foods. When an emetic type strain of *B. cereus* was added to food products and the food was stored under conditions designed to simulate temperature abuse (30°C, 24h), cereulide production occurred in rice dishes and other starchy foods. Addition of ingredients such as mayonnaise, vinegar and other condiments retarded the growth of bacteria and the quantity of cereulide formed. Bacterial growth and/or cereulide production was inhibited in egg and egg products, meat and meat products, milk and soybean curd. Shaking (aeration) of milk and soymilk resulted in increased cereulide production (Agata et al. 2002).

Host factors that influence disease

All people are believed to be susceptible to *B. cereus* food poisoning. However, some individuals, especially young children, are particularly susceptible and may be more severely affected (ICMSF 1996). Individuals vary in their response to cereulide dosages; this may be associated with differences in the number of 5-HT$_3$ receptors in the stomach/small intestine of individuals (Wijnands 2008).

The risk of illness after ingestion of vegetative cells is influenced by the strain, composition of the food, the liquid nature of the food and the age of the individual. Liquid foods are transported faster to the small intestine and therefore are protected from the influence of gastric conditions, providing more opportunity for survival of the pathogen (Wijnands 2008).

Dose response

No human dose response relationship is available for either the emetic or diarrhoeal toxin produced by *B. cereus*. Epidemiological evidence suggests that the majority of outbreaks worldwide due to *B. cereus* have been associated with concentrations in excess of 10^5 cfu/g in implicated foods. Rare cases of both emetic and diarrhoeal illness have been reported involving 10^3–10^5 cfu/g of *B. cereus* in food. These cases occurred in infants or aged and infirm individuals (Kramer and Gilbert 1989; Becker et al. 1994). Laboratory studies on the formation of emetic toxin in boiled rice cultures support this finding, with >10^6 cfu/g of *B. cereus* required for toxin production to occur (Finlay et al. 2002). The use of a threshold is analogous to the No Observed Adverse Effects Level (NOAEL), commonly used in the assessment of risk from chemical substances in food. The threshold of 10^5 cfu/g is at any point after cooking, and not just the final concentration as used by McElroy et al. (1999) (described below).

A surrogate approach using vegetative cell concentrations has been used to estimate dose response for the emetic toxin. McElroy et al. (1999) developed a two-step approach that linked the probability of illness, based on *B. cereus* concentrations from outbreaks and an attack rate (assumed to be independent of dose). A weakness of this approach is that the probability of illness is based on outbreaks where toxins may have been pre-formed in the food vehicle and subsequently cooked. As a result of the cooking, the total *B. cereus* concentration in the food vehicle may be reduced and therefore not directly related to the presence or concentration of toxin. This may account for disease outbreaks being reported where *B. cereus* concentrations in the food have been as low as 10^3 cfu/g.

Epidemiological data gathered during outbreaks in the Netherlands has been used to estimate that a dose of cereulide of approximately 9.5 µg/kg of bodyweight is required to cause the onset of the emetic syndrome (Finlay et al. 1999).

Recommended reading and useful links

Arnesen SLP, Fagerlund A, Granum PE (2008) From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiology Reviews 32:579-606

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 93–96.
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Campylobacter species

Campylobacter spp. are bacteria that cause the gastrointestinal disease campylobacteriosis, with symptoms that can mimic appendicitis. Most cases of campylobacteriosis are not fatal. Infection with *Campylobacter* spp. has also been associated with Guillain-Barré syndrome, which results in progressive muscle weakness or paralysis. *Campylobacter* spp. are widespread in nature and are present in the intestine of many wild and domestic animals and birds.

Description of the organism

Campylobacter spp. are Gram-negative, non-spore forming bacteria and are members of the family *Campylobacteraceae*. The genus *Campylobacter* comprises of 17 species and 6 subspecies (Nachamkin 2007; Silva et al. 2011). The two species most commonly associated with human disease are *C. jejuni* and *C. coli*. *C. jejuni* accounts for more than 80% of *Campylobacter*-related human illness, with *C. coli* accounting for up to 18.6% of human illness. *C. fetus* has also been associated with foodborne disease in humans (Gurtler et al. 2005; FDA 2012).

Growth and survival characteristics

The growth and survival of *Campylobacter* spp. depends on a variety of factors. *Campylobacter* spp. are sensitive to environmental conditions, such as temperature, availability of water and oxygen; and have limited capacity to survive environmental stress (refer to Table 1).

Campylobacter spp. grow in the 30–45°C temperature range. At 32°C, *C. jejuni* may double its number in approximately 6 hours (Forsythe 2000). *Campylobacter* spp. do not multiply at temperatures below 30°C, such that the number of *Campylobacter* spp. will not increase in foods held at room temperature (20–25°C) (Park 2002).

Although unable to grow below 30°C, *Campylobacter* spp. survive at temperatures as low as 4°C under moist conditions (Hazeleger et al. 1998; Park 2002). Survival in food is extended at refrigeration temperatures compared with room temperature, with viable cells being found after 7 months storage at 4°C (Lazaro et al. 1999). In a study of *Campylobacter* spp. that examined survival on naturally contaminated chicken skin and minced meat at freezing temperatures (–22°C), Sampers et al. (2010) found that numbers declined by approximately 1 $\log_{10}$ over the first 24 hour period. No further significant reduction was achieved by prolonged freezing, with *Campylobacter* spp. being detected in samples by enrichment after 84 days.

Although *Campylobacter* spp. survive well at cold temperatures, they are sensitive to heat and are readily inactivated by pasteurisation treatment or domestic cooking. Heating at 55–60°C for several minutes readily destroys *Campylobacter* spp. (ICMSF 1996).

Campylobacter spp. are highly sensitive to loss of moisture and do not survive well on dry surfaces (Fernandez et al. 1985). *C. jejuni* grows best at a sodium chloride concentration of 0.5% and does not grow in the absence of sodium chloride or in the presence of 2% or higher concentrations of sodium chloride (Doyle and Roman 1982; Wallace 2003).

Campylobacter have varying degrees of oxygen tolerance (3–5%) between species (Forsythe 2000). Most strains of *Campylobacter* do not grow in the presence of air, other than a few strains that may grow under slightly oxygen

rich conditions. Optimal growth occurs at 5% oxygen and 2–10% carbon dioxide (Park 2002). *C. jejuni* is able to adapt to aerobic conditions due to an ability to produce biofilms. The level of biofilm formation is higher in motile, flagellated strains than in non-flagellate, non-motile strains. This ability enhances the survival and spread in food processing environments such as poultry processing (Reuter et al. 2010).

Several studies have shown that *C. jejuni* is sensitive to acids such as formic, acetic, ascorbic and lactic acids (Murphy et al. 2006).

Campylobacter spp. have been shown to enter a viable but non-culturable state when subjected to unfavourable conditions, such as low nutrient availability, elevated temperature, freezing or stationary phase (Levin 2007). In this state, cells transform from a motile spiral form to a coccoid form (Rollins and Colwell 1986). The nature and role of this coccoid form is uncertain.

Table 1: Limits for growth of *Campylobacter* spp. when other conditions are near optimum (ICMSF 1996; Forsythe 2000)

	Minimum	Optimum	Maximum
Temperature (°C)	32	42–43	45
pH	4.9	6.5–7.5	9.5
Water activity	0.987	0.997	–

Symptoms of disease

Symptoms of campylobacteriosis include diarrhoea (sometimes bloody), nausea, abdominal pain, fever, muscle pain, headache, and vomiting. The incubation period before onset of disease is usually 2–5 days, with illness generally lasting for 2–10 days. The unique feature of the disease is the severity of abdominal pain which may become continuous and sufficiently intense to mimic acute appendicitis (Young and Mansfield 2005; FDA 2012). As a consequence of *C. jejuni* infection a small number of individuals develop a secondary condition such as reactive arthritis or Guillain-Barré syndrome, in which a harmful immune response of the body attacks part of the peripheral nervous system leading to symptoms of muscle weakness or paralysis (Havelaar et al. 2009).

Virulence and infectivity

Campylobacter spp. have four main virulence properties: motility, adherence, invasion and toxin production. The exact nature of how *Campylobacter* spp. adhere to and invade the intestinal epithelial cells is not fully understood (Levin 2007). It is thought that the combination of its spiral shape and flagella leads to rapid motility that enables the organisms to penetrate through the intestinal lining unlike conventional bacteria (Levin 2007; Bhavasar and Kapadnis 2007).

Campylobacter organisms produce two types of toxins: enterotoxin and cytotoxins. The enterotoxin of *C. jejuni* is similar to the *Vibrio cholerae* toxin and the *Escherichia coli* heat-liable toxin. This enterotoxin is produced to a lesser degree by *C. coli*. It has been suggested that enterotoxin produced by *Campylobacter* spp. results in watery diarrhoea, as opposed to bloody diarrhoea due to cytotoxin production. However, in some studies enterotoxigenic strains have been isolated from asymptomatic carriers (Wassenaar 1997). There have been at least six types of cytotoxins identified in *Campylobacter* spp. This includes a 70 kDa cytotoxin, a Vero/HeLa cell cytotoxin, a cytolethal distending toxin (CDT), a shiga-like toxin, a haemolytic cytotoxin and a hepatotoxin.

The CDT toxin has been shown to cause cell distension and cell disintegration of human tumour epithelial cells (Pickett et al. 1996). Active CDT toxin has been found in roughly 40% of over 70 *Campylobacter* strains tested (Johnson and Lior 1988). However, the role of enterotoxin and cytotoxins in *Campylobacter* pathogenesis has not been fully characterised.

Mode of transmission

Campylobacter spp. are transmitted to humans via the faecal-oral route, predominantly through the consumption of contaminated food or water or direct contact with infected animals (CDC 2010a). They are often present in the intestines of domestic and wild animals, such as cattle, sheep, poultry, dogs, wild birds and rodents, and are shed in the faeces of these animals (Hu and Kopecko 2003; Ellis-Iversen et al. 2012).

Campylobacter spp. present on raw meats may contaminate work areas and the hands of kitchen staff before being transferred to ready-to-eat foods or causing self-infection (Coats et al. 1987). External packaging material of raw meat (raw chicken, game-fowl, lamb and beef) has been reported to be a vehicle of cross-contamination of *Campylobacter* spp. in retail premises and consumer homes (Burgess et al. 2008).

Incidence of illness and outbreak data

Campylobacter infection is notifiable in all Australian states and territories except in New South Wales. In 2012 *Campylobacter* was the most frequently notified foodborne infection in Australia, with a rate of 102.3 cases per 100,000 population (15,664 cases). This was a decrease from the previous 5 year mean of 112.8 cases per 100,000 population (ranging from 107.4–119.9 cases per 100,000 population per year) (NNDSS 2013).

In New Zealand the notification rate in 2011 was 151.9 cases per 100,000 population (6,692 cases). This was a decrease from the 2010 rate of 168.2 cases per 100,000 population (Lim et al. 2012).

While not a notifiable disease in the United States (US), surveillance through FoodNet (representing 15% of the population) reported a rate of *Campylobacter* infection of 13.6 cases per 100,000 population in 2010. This was similar to the 2009 rate of 13.0 cases per 100,000 population (CDC 2010b; CDC 2011). The number of confirmed human campylobacteriosis cases in the European Union (EU) was 50.3 per 100,000 population in 2011 (ranging from 0.3–178 cases per 100,000 population between countries). This was a 2.2% increase in the number of cases from 2010 (EFSA 2013).

The incidence of *Campylobacter* infections is known to be associated with seasonal changes in many countries. *Campylobacter* infection is most prevalent during spring in Australia (Unicomb et al. 2009). A main peak of *C. jejuni* during summer and a peak of *C. coli* during winter has been observed in Germany (Gurtler et al. 2005). *C. jejuni* is one of the most commonly reported agents associated with foodborne illness in many developed countries, including New Zealand, the United Kingdom (UK) and the US (Mead et al. 1999; Park 2002).

Foods associated with *Campylobacter* spp. outbreaks include poultry meat, raw (unpasteurised) milk and milk products, beef, pork and shellfish (IFT 2004) (refer to Table 2). Outbreaks of campylobacteriosis linked to consumption of raw (unpasteurised) milk have been increasingly reported in the US (FDA 2010). *Campylobacter* infections generally occur sporadically, rather than being associated with outbreaks.

Table 2: Selected major foodborne outbreaks associated with *Campylobacter* spp. (>50 cases and/or ≥1 fatality)

Year	No. of cases	Food	Country	Comments	References
2008	98	Raw peas	US	Peas were contaminated in the field with bird faeces. Same strain of *C. jejuni* isolated from peas and bird faeces	(Gardner et al. 2011)
2007	68	Cheese	US	Cheese from raw milk was prepared and consumed as part of community celebration activities	(KDHE 2007)
2005	79	Chicken salad	Denmark	Cross-contamination from raw chicken to the chicken salad during preparation/storage. *C. jejuni* implicated	(Mazick et al. 2006)
2005	86	Chicken liver pate	Scotland	Pate preparation involved using undercooked chicken livers by flash frying, followed by mechanical homogenization. More than one strain of *C. jejuni* implicated	(Forbes et al. 2009)
2003	81	Custard prepared from UHT milk	Spain	Cross-contamination from raw chicken to custard	(Jiménez et al. 2005)
1998	79	Tuna salad	US	Precise route into tuna salad unknown. Rare strains of *C. jejuni* implicated. Several deficiencies identified in the camp kitchen operation	(Roels et al. 1998)
1995	78	Cucumber	South Australia	Cucumber served at self service salad bar. Suspected cross-contamination from raw meat	(Kirk et al. 1997)

Occurrence in foods

Poultry meat is generally recognised as a primary source of *Campylobacter* infection in humans (Sahin et al. 2002). The reported incidence of *Campylobacter* spp. on raw meat products from other food animal species tends to be lower than those reported for poultry. Using population genetics approaches, Wilson et al. (2009) confirmed that the vast majority (97%) of sporadic *Campylobacter* infections in the UK could be attributed to animals farmed for meat and poultry. Chicken and cattle were the principal sources of *C. jejuni* pathogenic to humans, with wild animal and environmental sources responsible for the remaining 3% of human disease.

In an Australian baseline survey carried out during 2007–2008 on the incidence and concentration of *Campylobacter* and *Salmonella* in raw chicken, 84.3% of post-processing carcass rinse samples (n=1,104) were positive for *Campylobacter* spp. These results were similar to those from a retail baseline microbiological survey carried out in 2005–2006 in South Australia and New South Wales, which found that 90.0% of retail poultry samples (n=859) were contaminated with *Campylobacter* spp. (FSANZ 2010).

A retail survey conducted in New Zealand between 2005–2008 found 72.7% of poultry carcasses were contaminated with *C. jejuni* (n=500). Several internationally rare serovars as well as common human clinical serovars were isolated, both ubiquitous and supplier-associated (Mullner et al. 2010).

A baseline survey carried out in the EU in 2008 revealed that 75.8% of broiler carcasses sampled (n=9,213) were contaminated with *Campylobacter* spp. The prevalence of *C. jejuni* and *C. coli* were 51.0% and 35.5%, respectively. *Campylobacter* spp. were also commonly detected in live poultry, pigs and cattle (EFSA 2010).

In the UK, a survey of poultry sold at retail carried out during 2007–2008 indicated that 65.2% of samples tested (n=3,274) were contaminated with *Campylobacter* spp. *C. jejuni* was present in 52.9 % of the samples while 47.1% contained *C. coli* (FSA 2009).

In a survey of retail food stuffs in Ireland between 2001–2002, *Campylobacter* spp. were found in 49.9% of raw chicken (n=890), 37.5% of raw turkey (n=88), 45.8% of raw duck (n=24), 3.2% of raw beef (n=221), 5.1% of pork (n=197), 11.8% of lamb (n=262), 0.8% of pork pate (n=120), 2.3% of raw oysters (n=129), and 0.9% of fresh mushrooms (n=217) tested. Of the positive samples, 83.4% were contaminated with *C. jejuni* and 16.6% were contaminated with *C. coli* (Whyte et al. 2004).

Host factors that influence disease

It is now known that individuals and populations express acquired immunity against *Campylobacter* infections. This immunity may be achieved via non-specific host-defence mechanisms (innate immunity) as well as via a pathogen specific immune response (adaptive immunity). The bacterial factors that induce the innate response in humans are known to be variable among strains of *Campylobacter* spp. and therefore influence the extent of the innate immune response (Havelaar et al. 2009). Following infection by *C. jejuni*, immunoglobulin (Ig) A and IgM antibodies appear one week after infection and IgG antibodies peak a few weeks later. IgA and IgM antibodies disappear within two to three months, while IgG antibodies remain for much longer (Havelaar et al. 2009).

IgA antibodies directed against *Campylobacter* spp. are present in breast milk (Ruiz-Palacios et al. 1990; Nachamkin et al. 1994). Therefore, susceptibility in early infancy may be reduced by passive immunity acquired from milk and/or placentally transferred immunity from immune mothers (Havelaar et al. 2009). Available data suggests that young children under the age of four (with the exception of early infants) and young adults in the age range of 20 to 30 years old are most susceptible to *Campylobacter* spp. infection (WHO/FAO 2009).

The bacterium-specific immune response limits the disease and leads to the development of protective immunity. Phagocytes and *Campylobacter*-specific secreted IgA antibodies play a part in this immune response. Repeated exposure is known to increase levels of protective immunity, however, this immunity is often strain specific (Havelaar et al. 2009). In some cases, acquired immunity could lead to resistance to colonisation by *Campylobacter* spp. (Tribble et al. 2010).

The incidence of *Campylobacter* infection in patients with acquired immune deficiency syndrome (AIDS) has been calculated to be 40-fold higher than that in the general population (Sorvillo et al. 1991). People with AIDS, immunosuppressive therapy, and liver disease are predisposed towards *Campylobacter* infections (Pigrau et al. 1997).

Dose Response

Volunteer studies have shown that 800 cells are able to cause campylobacteriosis in healthy adults (Black et al. 1988). The dose-response relationship and the illness-to-infection ratio appeared to differ between different *C. jejuni* isolates (Medema et al. 1996). Due to the sensitivity of *C. jejuni* to acids, it has been suggested that ingesting *Campylobacter* spp. with buffers such as milk or water which aid rapid wash through the gastric acid of the stomach, may reduce the oral infective dose (Blaser et al. 1980). Recent data confirm that doses of less than 100 cells have been associated with human illness (Teunis et al. 2005; Tribble et al. 2010).

Recommended reading and useful links

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Cyclospora cayetanensis

Cyclospora cayetanensis is a protozoan parasite that causes the gastrointestinal illness cyclosporiasis. Infected individuals shed a non-infectious form of the parasite in their faeces which requires an extended period of environmental exposure to develop into the infectious form. In developed countries cyclosporiasis is typically associated with international travel or consumption of imported produce.

Description of the organism

C. cayetanensis is a protozoan parasite which belongs to the phylum *Apicomplexa*, subclass *Coccidiaina* and family *Eimeriidae*. Many species of *Cyclospora* have been identified in animals. However, *C. cayetanensis* is the only species identified in humans, and appears to be restricted to this host (Arrowood 2003; Ortega and Sanchez 2010). Once sporulated, organisms of the genus *Cyclospora* have an oocyst that contains two sporocysts, and each sporocyst contains two sporozoites. *C. cayetanensis* oocysts are spherical, measuring 8–10μm in diameter, and as such are smaller than many other species of *Cyclospora* (Ortega et al. 1994; Lainson 2005; Smith 2007).

Growth and survival characteristics

C. cayetanensis can only multiply within the host. Factors that influence the survival of unsporulated and sporulated oocysts in the environment are poorly understood. Available data suggests that the viability of unsporulated oocysts is maintained for up to two months when stored at 4°C (as evidenced by sporulation occurring after storage for one week at 30°C) (Smith et al. 1997). Sathyanarayanan and Ortega (2004) and Ortega et al. (2008) have demonstrated that unsporulated *C. cayetanensis* oocysts are resistant to pesticides commonly used on farms and to sanitizers used by the food industry.

Sporulation of *Cyclospora* oocysts occurs optimally in the temperature range of 22–32°C, with 20–60% of purified oocysts sporulating within 2 weeks. The rate of sporulation slows at temperatures outside of this range (Ortega et al. 1993; Smith et al. 1997; Sathyanarayanan and Ortega 2006). A study by Sathyanarayanan and Ortega (2006) demonstrated that sporulation was prevented after 2 days at -20°C or exposure to extreme temperatures of -70 or 70°C for 15 minutes. Ortega and Liao (2006) showed that microwave heating of *C. cayetanensis* at 96°C for 45 seconds dramatically decreased the level of sporulation but did not completely inhibit sporulation.

Symptoms of disease

Cyclospora infection has a range of outcomes from no clinical symptoms of disease (asymptomatic infection) to severe diarrhoea resulting in dehydration and weight loss. Other symptoms can include anorexia, nausea, vomiting, abdominal bloating, cramping, fatigue, body aches and low-grade fever. The onset of illness is 2–14 days (average of 7 days). In untreated individuals a cycle of remitting and relapsing symptoms can occur that lasts for weeks to months. Shedding of oocysts occurs during the illness and can continue for several weeks after symptoms have abated (Arrowood 2003; Smith 2007; Hall et al. 2012). Infection with *C. cayetanensis* can lead to longer term sequelae including malabsorption, biliary disease, Reiter's syndrome (reactive arthritis) and Guillain-Barré syndrome (a peripheral nervous system disorder that causes paralysis) (Herwaldt 2000; Ortega and Sanchez 2010).

Virulence and infectivity

There are currently no animal models or *in vitro* cultivation methods to determine the infectivity of *Cyclospora* oocysts and consequently knowledge of factors that influence the virulence of *C. cayetanensis* is limited (Ortega and Sanchez 2010).

C. cayetanensis oocysts are unsporulated and non-infective when shed in the faeces, and it is thought that a prolonged period outside the host is required for the oocysts to sporulate and become infectious. Factors that affect the sporulation process of *C. cayetanensis* are not well characterised, however, ambient temperature and the presence of higher concentrations of atmospheric oxygen appear to be involved (Smith 2007).

Cyclospora spp. appear to have a higher binding affinity for particular types of fresh produce. For example the fine hair-like projections on the surface of raspberries are thought to facilitate the attachment of "sticky" *Cyclospora* oocysts. The adhesins responsible for this strong attachment are unknown (Ortega and Sanchez 2010).

Mode of transmission

C. cayetanensis is transmitted via the faecal-oral route by consumption of contaminated food or water. Direct person-to-person transmission is unlikely as the oocysts shed from individuals are not infectious and require extended periods of time outside the host to sporulate (Ortega and Sanchez 2010; Hall et al. 2012).

Figure 1: Life cycle of *C. cayetanensis* (CDC–DPDx 2009)
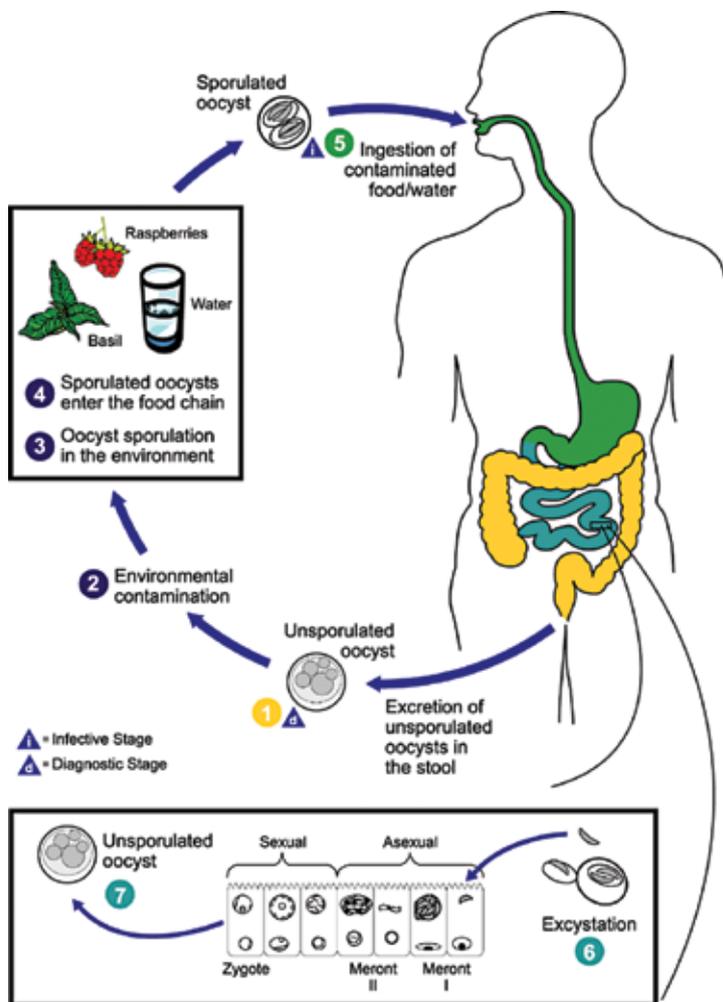
(1) When shed in the faeces, *C. cayetanensis* oocysts are unsporulated and non-infective.

(2) Oocysts contaminate the environment.

(3) Oocysts sporulate in the environment and develop sporocysts and sporozoites. The sporulated oocysts are the infectious form of *C. cayetanensis*.

(4) Fresh produce and water can serve as vehicles for transmission.

(5) The sporulated oocysts are ingested (in contaminated food or water).

(6) The oocysts excyst, releasing sporozoites in the gastrointestinal tract. The sporozoites infect the epithelial cells lining the small intestine, in particular the jejunum. Once inside the intestinal cell, sporozoites multiply asexually and produce type I meronts which in turn form type II meronts. The type II meronts infect other intestinal cells and initiate sexual multiplication by producing either microgametocytes or macrogametocytes. The microgametocytes fertilize the macrogametocytes, leading to the production of a zygote.

(7) The zygotes differentiate into unsporulated oocysts, which are released into the lumen of the intestine and shed in the faeces.

(Smith 2007; CDC–DPDx 2009; Ortega and Sanchez 2010)

Incidence of illness and outbreak data

C. cayetanensis has been reported worldwide, however, it is more common in tropical and sub-tropical environments and in many developing countries, particularly in parts of South America, the Caribbean and Asia. The incidence of *C. cayetanensis* is seasonal, with cases most commonly occurring in spring and summer (Ortega and Sanchez 2010; Chacin-Bonilla 2010; Hall et al. 2012).

Infection with *C. cayetanensis* is not a notifiable disease in Australia or New Zealand and hence very little data is available. In the United States (US) the notification rate for cyclosporiasis in 2010 was 0.07 cases per 100,000 population, which was an increase on the 2009 rate of 0.05 cases per 100,000 population (CDC 2012).

Outbreaks of *C. cayetanensis* have largely been associated with fresh produce (refer to Table 1).

Table 1: Selected major foodborne outbreaks associated with *C. cayetanensis* (>50 cases and/or ≥1 fatality)

Year	No. cases	Food	Country	Comments	Reference
2010	314	Fresh produce (cantaloupe, chives & lettuce)	Cruise ship (multiple countries)	Food taken on board the cruise ship from south east Asian destinations	(Gibbs et al. 2013)
2005	142	Fresh basil	Canada	Fresh basil imported from Mexico, contamination possibly from human handling or irrigation water	(Milord et al. 2012)
2004	96	Fresh snow peas	US	Snow peas imported from Guatemala, all from the same batch	(CDC 2004)
2000	54	Raspberry filling of a wedding cake	US	Frozen unwashed raspberries were thawed and mixed into the cream filling of the cake. Raspberries were imported from Guatemala	(Ho et al. 2002)
1997	93	Mesclun lettuce	US	Lettuce thought to be imported from Peru	(Herwaldt 2000)
1996	1465	Raspberries	US and Canada	Raspberries imported from Guatemala. Berries were picked and sorted by hand, well water was used for washing	(Herwaldt et al. 1997)

Occurrence in food

C. cayetanensis has been identified in international surveys of fresh produce. Dixon et al. (2013) detected *Cyclospora* spp. in 1.7 % of packaged leafy greens purchased from grocery stores in Canada (n=544). In a study of herbs in Vietnam, 10.4% of basil (n=96), 11.6% of coriander (n=86) and 7.7% of marjoram samples (n=26) were positive for *Cyclospora* spp. oocysts (Tram et al. 2010). Ortega et al. (1997) detected *C. cayetanensis* oocysts in 1.7% of vegetables sampled from markets in Peru (n=172). In Nepal *Cyclospora* spp. have been detected in drinking water and on cabbage and lettuce (Sherchand et al. 1999).

As *C. cayetanensis* infections have only been observed in humans (Ortega and Sanchez 2010), there does not appear to be an animal reservoir. It is likely that the most significant transmission occurs where sewage, or water contaminated by human faeces, has been applied to horticultural crops (Dawson 2005). This could occur via the use of contaminated water for the application of pesticides, sprinkling contaminated water on horticultural produce to maintain freshness, or washing the produce in contaminated water drawn from ponds, lakes or

rivers. Contaminated hands of food handlers, baskets and containers in markets could also lead to contaminated produce (Sherchand et al. 1999; Ortega and Sanchez 2010; Tram et al. 2010).

Host factors that influence disease

People of all ages are susceptible to *C. cayetanensis* infection. Young children, the elderly and immunocompromised individuals develop more severe clinical symptoms. In endemic areas, the severity of symptoms and duration of infection tends to decrease after repeated infections, suggesting possible immunity in older children and adults (Ortega and Sanchez 2010).

Dose response

The number of *C. cayetanensis* oocysts required to cause infection is not known, however, it is presumed to be low (possibly as low as 10 oocysts) on the basis of data from outbreak investigations (Smith 2007; RTI International 2009).

Recommended reading and useful links

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 127–129.
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Milord F, Lampron-Goulet E, St-Amour M, Levac E, Ramsay D (2012) *Cyclospora cayetanensis*: A description of clinical aspects of an outbreak in Quebec, Canada. Epidemiology and Infection 140:626–632

Ortega YR, Liao J (2006) Microwave inactivation of *Cyclospora cayetanensis* sporulation and viability of *Cryptosporidium parvum* oocysts. Journal of Food Protection 69(8):1957–1960

Ortega YR, Sanchez R (2010) Update on *Cyclospora cayetanensis*, a food-borne and waterborne parasite. Clinical Microbiology Reviews 23(1):218–234

Ortega YR, Gilman RH, Sterling CR (1994) A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. Journal of Parasitology 80(4):625–629

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Hepatitis A virus

Hepatitis A virus (HAV) infects the liver, with disease characterised by liver inflammation and the development of jaundice. HAV infection can be asymptomatic (no clinical symptoms) in mild cases, or lead to severe liver damage in chronic cases. Hepatitis A is endemic in many developing countries, while in developed countries sporadic cases occur.

Description of the organism

HAV belongs to the *Picornaviridae* family of viruses and the genus *Hepatovirus*. The *Picornaviridae* family consists of small (25–28 nm) non-enveloped viruses which are generally more robust and survive better in the environment compared to enveloped viruses, such as herpes simplex virus. HAV particles consist of a single strand of RNA contained within an icosahedral shaped protein shell (Schoub 2003; Cook and Rzezutka 2006; Rozenberg et al. 2011).

HAV has one known serotype and six genotypes (I–VI). Genotypes I–III have been associated with human illness, while genotypes IV–VI are found in Old World monkeys. Genotypes I–III are further divided into A and B. The majority of human strains of HAV belong to genotype I or III (Hollinger and Emerson 2007; FDA 2012). Isolates from a particular HAV outbreak are usually of the same genotype (Normann et al. 2008).

Growth and survival characteristics

HAV requires specific living cells (host cells) in order to replicate. This means that the level of HAV in contaminated food will not increase during processing, transport or storage (Koopmans and Duizer 2004). While not able to replicate outside the host, HAV has been shown to survive in the environment for extended periods of time (Schoub 2003; Cook and Rzezutka 2006). The survival of HAV is influenced by environmental factors such as temperature, pH, chemicals and food composition.

It has been demonstrated that under conditions simulating typical environmental exposure, HAV remains infectious after being dried and stored for 30 days (McCaustland et al. 1982). HAV has also been shown to survive on various non-porous surfaces such as aluminium, china and latex for 60 days, however, it does not survive as well on porous materials (Abad et al. 1994). A study by Mbithi et al. (1992) demonstrated that HAV survives and remains infectious on human hands after 4 hours and can be transferred between hands and inanimate surfaces.

HAV has been shown to survive in fresh river water, seawater, groundwater and untreated tap water (Enriquez et al. 1995; Rzezutka and Cook 2004; Cook and Rzezutka 2006). However, without a standard protocol to determine virus survival it is difficult to compare the survival time of the virus in different environments. An investigation by Arnal et al. (1998) using artificial sterile seawater contaminated with HAV demonstrated that the genetic material of HAV was stable and remained in the water for 232 days, although no infectious HAV particles were detected by 35 days. In general, survival of HAV in water is enhanced at low temperatures (<4°C) (Rzezutka and Cook 2004).

Croci et al. (2002) demonstrated that when fresh produce was stored at 4°C, HAV survived and remained infective on: carrots for 4 days, fennel for 7 days and on lettuce for the study duration of 9 days. The differing survival rates observed on fresh produce may be due to the difference in surface texture of the produce and the presence of anti-viral substances. Shieh et al. (2009) showed that when spinach was stored at 5.4°C a 1 $\log_{10}$ reduction in the

level of HAV occurred over a 28.6 day period. These studies imply that HAV can persist under normal domestic storage conditions for extended periods of time.

Chemical and physical factors can affect the heat resistance of HAV. Deboose et al. (2004) investigated the inactivation of HAV in strawberry puree and found that increasing the sucrose concentration resulted in increased heat resistance of HAV. Conversely, lowering the pH was found to decrease the heat resistance of HAV. Changing the calcium concentration had no effect. Bidawid et al. (2000b) demonstrated that increases in fat content also increased the heat resistance of HAV. Dairy products with higher fat content required longer times of exposure to heat than lower fat products to achieve the same level of HAV reduction.

HAV has been found to be resistant to temperatures up to 60°C. The temperature at which 50% of HAV particles disintegrate and release their viral RNA is 61°C (10 minutes). When stabilised by 1 mol/L $MgCl_2$, 50% disintegration of HAV occurs at 81°C (Hollinger and Emerson 2007). In food, complete inactivation of HAV has been observed in shellfish when heated to 85°C for 3 minutes or 95°C for 2 minutes (Millard et al. 1987). These conditions are known to inactivate HAV in shellfish while maintaining a commercially acceptable product (Appleton 2000). For milk and cream, heating to 85°C for 30 seconds is sufficient to cause a 5 $\log_{10}$ reduction in HAV titre (Bidawid et al. 2000b).

Low temperature has little effect on HAV survival. Butot et al. (2008) showed that frozen storage of HAV contaminated berries and herbs had little effect on HAV survival over the study period of 3 months.

HAV is highly resistant to acidic conditions and solvents. Scholz et al. (1989) demonstrated that at pH 1 (24°C) HAV retained high infectivity after 2 hours and was still infectious after 5 hours. Under conditions that simulate the acidity of the human stomach (38°C, pH 1) HAV remained infectious for 90 minutes. Also, being a non-enveloped virus, HAV is resistant to solvents such as 20% ether and chloroform (ether destroys the envelop of some viruses) (Hollinger and Emerson 2007).

Symptoms of disease

HAV infection often causes mild illness in humans, or results in no clinical disease at all. In children this is particularly common, with most children under 6 years of age showing no symptoms (asymptomatic infection) (FDA 2012). For those individuals in which clinical disease occurs, initial symptoms include sudden onset of fever, nausea, anorexia, malaise, vomiting, diarrhoea, abdominal pain, myalgia (muscular pain) and headache. The initial symptoms tend to abate with the onset of jaundice (yellowing of the skin and eyes and a browning of urine due to stimulation of bile pigment production) and pale clay coloured stools. Children with symptomatic infection usually develop flu-like symptoms without jaundice (Brundage and Fitzpatrick 2006; Hollinger and Emerson 2007; FDA 2012).

Most patients show complete recovery from symptoms within 3–6 months of the onset of illness. The fatality rate for HAV is approximately 2.4%, with death more likely to occur in the elderly. Acute liver failure due to severe HAV infection has been reported in children; however, it is more frequent in middle-aged and older people and those with underlying chronic liver disease. Acute liver failure is also a rare complication of HAV infection during pregnancy (Koff 1998; FDA 2012).

The incubation period before onset of disease is 15–50 days (mean time of 30 days) (FDA 2012). HAV is shed in the faeces of infected individuals for up to 2 weeks before the onset of illness. HAV is present in the blood at the same time as viral shedding starts occurring. The virus disappears from the blood shortly after symptoms

of disease start, while faecal shedding of the virus continues for another 2 weeks (Cook and Rzezutka 2006; Hollinger and Emerson 2007).

In 3–20% of cases relapses occur, generally with milder symptoms and HAV being shed in the faeces. Multiple relapses can occur (Hollinger and Emerson 2007).

Virulence and infectivity

The target organ of HAV is the liver. HAV is initially ingested, infects the intestinal tract and is then transported to the liver via the bloodstream. In the liver, HAV attaches to receptors on the surface of the hepatocytes, enters these cells and replicates. Replication of HAV within the hepatocytes is not believed to result in immediate cell damage; this is thought to occur subsequent to replication and release of the virus. The host's immune response is responsible for destroying the HAV infected cells. As a consequence of this pathological damage the liver becomes inflamed (WHO 2000; Schoub 2003; Cook and Rzezutka 2006). Released viral particles enter the bile duct and pass into the gastrointestinal tract to be shed in the faeces (Cook and Rzezutka 2006). The resistance of HAV to inactivation by bile and intestinal proteolytic enzymes allows the virus to be shed in the faeces and facilitates faecal-oral transmission (Koff 1998).

Mode of transmission

HAV is transmitted via the faecal-oral route by either person-to-person contact or consumption of contaminated food or water (Guillois-Becel et al. 2009). Person-to-person transmission can involve young children with unrecognised HAV infection (asymptomatic infection) (Brundage and Fitzpatrick 2006).

In contrast to person-to-person transmission, outbreaks of HAV infections usually result from faecal contamination of a single source of food or water. Foods may become contaminated in their growing areas (e.g. shellfish), or during irrigation (e.g. crops), usually by coming into contact with sewage polluted water. Food can also be contaminated by infected food handlers. Infected food handlers may contaminate foods directly or contaminate surfaces on which foods are prepared. A major issue with infected food handlers is that they are often unaware they constitute a hazard, as most of the faecal shedding of HAV occurs prior to the onset of clinical symptoms (Cook and Rzezutka 2006; Hollinger and Emerson 2007). Food establishments with poor sanitary conditions and inadequate treatment and/or disposal of human waste (sewage), along with unsatisfactory manufacturing practices may also contribute to food contamination (Sattar et al. 2000).

Travel to areas in which HAV is endemic from low prevalence areas is known to be a risk factor for HAV infection. The likelihood of becoming infected with HAV depends on local hygienic and sanitary conditions, which vary from country to country (Koff 1998). In 2010, 55.1% of HAV cases reported in Australia were acquired overseas (OzFoodNet 2012).

HAV transmission through blood and blood products is rare. While HAV is present in the blood of infected individuals, this is only for approximately a 2 week period. However, post-transfusion HAV infection has occurred, as have outbreaks of HAV in haemophiliacs who received contaminated blood plasma-derived factor VIII concentrate (Mannucci et al. 1994; Hollinger and Emerson 2007).

Incidence of illness and outbreak data

HAV has a worldwide distribution; however, the prevalence of infection is related to the quality of the water supply, level of sanitation and the age of the individual when infected. In most developing countries, where HAV infection is endemic, the majority of people are infected in early childhood and virtually all adults are immune. In developed countries, HAV infections are less common due to improved sanitation. As a result very few people are infected in early childhood and the majority of adults remain susceptible to infection. Hence in developed countries the risk of epidemics and the occurrence of severe disease may increase as the majority of people infected during an outbreak would be adults (children are often asymptomatic) (Conaty et al. 2000; Issa and Mourad 2001; Koopmans and Duizer 2004).

Hepatitis A is a notifiable disease in all Australian states and territories. The incidence of HAV infection notified in Australia in 2012 was 0.7 cases per 100,000 population (164 cases). This was a decrease from the previous 5 year mean of 1.3 cases per 100,000 population per year (ranging from 0.6–2.6 cases per 100,000 population per year) (NNDSS 2013).

In north Queensland in 1996–1999 the average annual HAV notification rates in Indigenous and non-Indigenous people were 110 and 25 cases per 100,000 population, respectively. In 1999 a HAV vaccination program for Indigenous children in north Queensland was introduced. Consequently, in 2000–2003 the average annual HAV notification rates for Indigenous and non-Indigenous people were 4 and 2.5 cases per 100,000 population, respectively (Hanna et al. 2004). HAV is now included as part of the National Immunisation Program Schedule for Aboriginal and Torres Strait Islander children younger than 5 years of age living in Queensland, the Northern Territory, Western Australia and South Australia (DOHA 2011). HAV vaccination is also recommended for travellers to endemic areas and those at increased risk because of lifestyle or occupation (DOHA 2008).

The notification rate for HAV in New Zealand in 2011 was 0.6 cases per 100,000 population (26 cases), which was a decrease from the 2010 rate of 1.1 cases per 100,000 population (Lim et al. 2012). The incidence of HAV in the United States (US) has declined from 12 cases per 100,000 population in 1995 to 0.54 cases per 100,000 population in 2010. This reduction has followed the 1999 recommendation for routine vaccination of children in areas of the US with consistently elevated rates of HAV (CDC 2009; CDC 2012). In the European Union there was one strong evidence foodborne HAV outbreak in 2011 and also one in 2010 (EFSA 2012; EFSA 2013).

Foodborne outbreaks of HAV have been recognised for over 40 years, but are infrequently reported. This is because the 2–6 week incubation period for HAV makes it more difficult to associate the source of infection with a particular food (Appleton 2000).

Cold cuts and sandwiches, fruits and fruit juices, milk and milk products, vegetables, salads, shellfish and iced drinks have been implicated in HAV outbreaks (FDA 2012) (refer to Table 1).

Table 1: Selected major foodborne outbreaks associated with HAV (>50 cases and/or ≥1 fatality)

Year	No. cases (fatalities)	Food	Country	Comments	Reference
2009-2010	392 (1)	Semi-dried tomatoes	Australia	Imported product from endemic HAV countries likely source of contamination. Semi-dried tomatoes contaminated with the identical HAV strain associated with illness in the Netherlands	(OzFoodNet 2010; Donnan et al. 2012)
2004	351	Orange juice	Egypt	Significant hygiene problems at the production plant; no heat treatment of the finished product	(Frank et al. 2007)
2004	269	Raw beef	Belgium	Infected food handler identified at the meat distribution plant	(Robesyn et al. 2009)
2003	601 (3)	Green onions	US	Green onions contaminated before or during packing on the farm in Mexico	(Wheeler et al. 2005)
1997	444	Oysters	Australia	The lake from which the oysters were harvested was polluted by sewage from a local town and boats	(Conaty et al. 2000)
1997	254	Frozen strawberries	US	Consumption of items from school cafeterias containing frozen strawberries was associated with HAV infection	(Hutin et al. 1999)
1996	5620	Raw seafood	Italy		(Lopalco et al. 1997)
1988	300,000+ (47)	Raw clams	China		(Cooksley 2000)

Occurrence in food

The types of food most often implicated in HAV outbreaks are those that are either eaten raw or only slightly cooked (e.g. shellfish), or handled extensively prior to consumption (e.g. the picking and packing of raw produce in the field and the preparation of sandwiches and salads) (Koopmans and Duizer 2004; Cook and Rzezutka 2006).

Bivalve molluscs (e.g. oysters, mussels, clams and cockles) live in shallow, coastal and estuarine waters which can be polluted with human sewage. As filter feeders they collect nutrients by filtering particulate matter from the water. If molluscs are grown in water contaminated with human faeces, the molluscs can collect and concentrate HAV from the water (Appleton 2000; Moore 2001; Cook and Rzezutka 2006). HAV has been shown to be concentrated within mussels to 100-fold higher concentrations than the surrounding water and can persist for about 7 days in the mussels (Enriquez et al. 1992). HAV has been detected in oyster samples more than 2 months after the presumed contamination event; this is thought to be due to recontamination of the oysters from sediment in the water (Conaty et al. 2000).

The prevalence of HAV reported in shellfish ranges between 6–27%, depending on the location and analytical technique used. For mussels sampled from markets of major cities in south Italy, 15.6% were found to be contaminated with infectious HAV (n=180) (Croci et al. 2003). For shellfish (clams, mussels, scallops and oysters) collected from the north Adriatic sea located between the Italian and Balkan peninsulas, HAV was detected in 6% of samples (n=235) (Croci et al. 2007). For shellfish collected off the coast of Spain (cultured and wild mussels, wild clams and cockles), HAV was detected in 27.4% of samples (n=164) (Romalde et al. 2002). The methods

utilised in these studies detect the genetic material of HAV and some methods are more sensitive than others under different conditions. This suggests that the level of HAV contamination could be higher than reported.

Hernandez et al. (1997) demonstrated that 20% of pooled samples of lettuce wash water collected in Costa Rica were contaminated with HAV (n=10 pools, 5 lettuces per pool), suggesting that lettuces from this region could be a vehicle for HAV transmission.

Host factors that influence disease

People of all ages are susceptible to HAV infection (unless they have had a previous infection or vaccination). The disease is milder in young children under 6 years, with the risk of fatality increasing with age. Thus the risks are higher for unexposed older people (ESR 2001; FDA 2012).

A single HAV infection or administration of the HAV vaccine provides lifelong immunity for the individual against the virus (Leon and Moe 2006). When an outbreak of HAV occurs, if exposure can be recognised before cases begin to occur, treatment with intramuscular immunoglobulin (passive immunisation) within 2 weeks of exposure is >85% effective at preventing HAV infection. However, passive immunisation is only effective for a short time (3–6 months) and people will be susceptible to infection from another exposure (Issa and Mourad 2001; Hollinger and Emerson 2007).

Dose response

The number of HAV particles required to cause infection is not known, however, it is presumed to be 10–100 viral particles (FDA 2012). In fact it has been suggested that a single ingested viral particle may cause infection, however, the probability of this occurring is very low (Cliver 1985). It has been estimated that up to 13,000 infectious HAV particles may be present in 1 mg of faeces (Bidawid et al. 2000a).

Recommended reading and useful links

Cook N, Rzezutka A (2006) Hepatitis viruses. Ch 11 In: Motarjemi Y, Adams M (eds) Emerging Foodborne Pathogens. Woodhead Publishing, Cambridge, p. 282-308

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxinshandbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 154–158.
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Listeria monocytogenes

Listeria monocytogenes is a bacterium that causes listeriosis, a disease that can have severe consequences for particular groups of the population. It can cause miscarriages in pregnant women and be fatal in immunocompromised individuals and the elderly. In healthy people, listeriosis generally only causes a mild form of illness. *L. monocytogenes* can be found throughout the environment. It has been isolated from domestic and wild animals, birds, soil, vegetation, fodder, water and from floors, drains and wet areas of food processing factories.

Description of the organism

L. monocytogenes is a Gram-positive, non-spore forming rod-shaped bacterium. It belongs to the genus *Listeria* along with *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. selligeri* and *L. grayi* (Rocourt and Buchrieser 2007). Of these species, only two are considered pathogens: *L. monocytogenes* which infects humans and animals, and *L. ivanovii* which infects ruminants (although there have been rare reports of *L. ivanovii* being isolated from infected humans) (Guillet et al. 2010). There are thirteen known serotypes of *L. monocytogenes*: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. The serotypes most often associated with human illness are 1/2a, 1/2b and 4b (FDA 2012).

Growth and survival characteristics

The growth and survival of *L. monocytogenes* is influenced by a variety of factors. In food these include temperature, pH, water activity, salt and the presence of preservatives (refer to Table 1).

The temperature range for growth of *L. monocytogenes* is between -1.5 and 45°C, with the optimal temperature being 30–37°C. Temperatures above 50°C are lethal to *L. monocytogenes*. Freezing can also lead to a reduction in *L. monocytogenes* numbers (Lado and Yousef 2007). As *L. monocytogenes* can grow at temperatures as low as 0°C, it has the potential to grow, albeit slowly, in food during refrigerated storage.

L. monocytogenes will grow in a broad pH range of 4.0–9.6 (Lado and Yousef 2007). Although growth at pH <4.0 has not been documented, *L. monocytogenes* appears to be relatively tolerant to acidic conditions. *L. monocytogenes* becomes more sensitive to acidic conditions at higher temperatures (Lado and Yousef 2007).

Like most bacterial species, *L. monocytogenes* grows optimally at a water activity (a_w) of 0.97. However, *L. monocytogenes* also has the ability to grow at a a_w of 0.90 (Lado and Yousef 2007). Johnson et al. (1988) demonstrated that *L. monocytogenes* can survive for extended periods of time at a a_w value of 0.81. *L. monocytogenes* is reasonably tolerant to salt and has been reported to grow in 13–14% sodium chloride (Farber et al. 1992). Survival in the presence of salt is influenced by the storage temperature. Studies have indicated that in concentrated salt solutions, the survival rate of *L. monocytogenes* is higher when the temperature is lower (Lado and Yousef 2007).

L. monocytogenes can grow under both aerobic and anaerobic conditions, although it grows better in an anaerobic environment (Sutherland et al. 2003; Lado and Yousef 2007).

The effect of preservatives on the growth of *L. monocytogenes* is influenced by the combined effects of temperature, pH, salt content and water activity. For example, sorbates and parabens are more effective at preventing growth of *L. monocytogenes* at lower storage temperatures and pH. Also, adding sodium chloride

or lowering the temperature enhances the ability of lactate to prevent *L. monocytogenes* growth. At decreased temperatures (such as refrigeration storage) sodium diacetate, sodium propionate and sodium benzoate are more effective at preventing growth of *L. monocytogenes* (Lado and Yousef 2007).

Table 1: Limits for growth of *L. monocytogenes* when other conditions are near optimum (Lado and Yousef 2007)

	Minimum	Optimum	Maximum
Temperature (°C)	-1.5	30–37	45
pH	4.0	6.0–8.0	9.6
Water activity	0.90	0.97	–

Symptoms of disease

There are two main forms of illness associated with *L. monocytogenes* infection. Non-invasive listeriosis is the mild form of disease, while invasive listeriosis is the severe form of disease and can be fatal (FDA 2012). The likelihood that invasive listeriosis will develop depends upon a number of factors, including host susceptibility, the number of organisms consumed and the virulence of the particular strain (WHO/FAO 2004).

Symptoms of non-invasive listeriosis can include fever, diarrhoea, muscle aches, nausea, vomiting, drowsiness and fatigue. The incubation period is usually 1 day (range 6 hours to 10 days) (Painter and Slutsker 2007; FDA 2012). Non-invasive listeriosis is also known as listerial gastroenteritis or febrile listeriosis.

Invasive listeriosis is characterised by the presence of *L. monocytogenes* in the blood, in the fluid of the central nervous system (leading to bacterial meningitis) or infection of the uterus of pregnant women. The latter may result in spontaneous abortion or stillbirth (20% of cases) or neonatal infection (63% of cases). Influenza-like symptoms, fever and gastrointestinal symptoms often occur in pregnant women with invasive listeriosis. In non-pregnant adults, invasive listeriosis presents in the form of bacterial meningitis with a fatality rate of 30%. Symptoms including fever, malaise, ataxia, seizures and altered mental status (Painter and Slutsker 2007). The incubation period before onset of invasive listeriosis ranges from 3 days to 3 months (FDA 2012).

Virulence and infectivity

When *L. monocytogenes* is ingested, it may survive the stomach environment and enter the intestine where it penetrates the intestinal epithelial cells. The organism is then taken up by macrophages and non-phagocytic cells. The *L. monocytogenes* surface protein internalin is required for this uptake by non-phagocytic cells, as it binds to the receptors on the host cells to instigate adhesion and internalization. The bacterium is initially located in a vacuole after uptake by a macrophage or non-phagocytic cell. *L. monocytogenes* secrete listeriolysin O protein, which breaks down the vacuole wall and enables the bacteria to escape into the cytoplasm. Any bacteria remaining in the vacuole are destroyed by the host cell. Once located in the cytoplasm of the host cell, *L. monocytogenes* is able to replicate. *L. monocytogenes* is transported around the body by the blood, with most *L. monocytogenes* being inactivated when it reaches the spleen or liver. *L. monocytogenes* is able to utilise the actin molecules of the host to propel the bacteria into neighbouring host cells. In the case of invasive listeriosis, this ability to spread between host cells enables *L. monocytogenes* to cross the blood-brain and placental barriers (Montville and Matthews 2005; Kuhn and Goebel 2007; Bonazzi et al. 2009).

Mode of transmission

The most common transmission route of *L. monocytogenes* to humans is via the consumption of contaminated food. However, *L. monocytogenes* can be transmitted directly from mother to child (vertical transmission), from contact with animals and through hospital acquired infections (Bell and Kyriakides 2005).

Healthy individuals can be asymptomatic carriers of *L. monocytogenes*, with 0.6–3.4% of healthy people with unknown exposure to *Listeria* being found to shed *L. monocytogenes* in their faeces. However, outbreak investigations have shown that listeriosis patients do not always shed the organism in their faeces (FDA/USDA/CDC 2003; Painter and Slutsker 2007). Therefore the role of healthy carriers in the transmission of *L. monocytogenes* is unclear.

Incidence of illness and outbreak data

Listeriosis is a notifiable disease in all Australian states and territories. The incidence of listeriosis notified in Australia in 2012 was 0.4 cases per 100,000 population (93 cases). This is a slight increase from the previous 5 year mean of 0.3 cases per 100,000 population per year (ranging from 0.2–0.4 cases per 100,000 population per year) (NNDSS 2013). In Australia the fatality rate in 2010 was 21%, which was an increase from the 14% fatality rate of the previous year (OzFoodNet 2010; OzFoodNet 2012).

The notification rate for listeriosis in New Zealand in 2011 was 0.6 cases per 100,000 population (26 cases). This was an increase from the 2010 rate of 0.5 cases per 100,000 population. The fatality rate in New Zealand in 2011 was 3.8% (Lim et al. 2012).

In the United States (US) the notification rate for listeriosis in 2010 was 0.27 cases per 100,000 population. This was similar to the 2009 rate of 0.28 cases per 100,000 population (CDC 2012). In the European Union (EU) there were 0.32 confirmed cases of listeriosis per 100,000 population in 2011 (ranging from 0.04–0.88 cases per 100,000 population between countries). This was a 7.8% decrease in the number of cases from 2010. The reported fatality rate in the EU in 2011 was 12.7% (EFSA 2013).

Invasive *L. monocytogenes* infections can be life threatening, with average fatality rates being 20–30% among hospitalized patients (WHO/FAO 2004; Swaminathan and Gerner-Smidt 2007).

Most cases of listeriosis are sporadic. Despite this, foodborne outbreaks due to *L. monocytogenes* have been associated with cheese, raw (unpasteurised) milk, deli meats, salad, fish and smoked fish, ice cream and hotdogs (Montville and Matthews 2005; Swaminathan and Gerner-Smidt 2007) (refer to Table 2).

Table 2: Selected major foodborne outbreaks associated with *L. monocytogenes* (>50 cases and/or ≥1 fatality)

Year	Total no. cases (fatalities)	No. perinatal cases (fatalities)	Food	Country	Comments	Reference
2011	146 (31)	7 (1)	Cantaloupe	US	*Listeria* isolated from cantaloupe and equipment at packing facility, contamination probably occurred in the packing facility	(CDC 2011; FDA 2011)
2009	36 (3)	8 (3)	Chicken wrap	Australia	*Listeria* isolated from pre-packaged chicken wraps, deficiencies in the food safety program for production of chicken meat	(OzFoodNet 2010)
2008	57 (22)	0	Deli meats	Canada	*Listeria* identified on plant equipment, company tried to correct problem with sanitation program; low sodium product	(Government of Canada 2009)
1998–1999	108 (18)	13 (4)	Frankfurters	US	Contamination due to demolition of ceiling refrigeration unit in frankfurter hopper room	(Mead et al. 2006)
1997	1566*	0	Corn and tuna salad	Italy	Possible cross-contamination from other untreated foods	(Aureli et al. 2000)
1992	279 (92)	92 (29)	Jellied pork tongue	France	*Listeria* identified at manufacturing facility	(Norton and Braden 2007)
1985	142 (48)	93 (30)	Mexican-style soft cheese	US	Cheese was made from contaminated milk that was unpasteurised or inadequately pasteurised	(Linnan et al. 1988)
1981	41 (18)	34 (16)	Coleslaw	Canada	Cabbage fertilised with manure from sheep with listeriosis	(Schlech et al. 1983)

* Non-invasive listeriosis

Occurrence in food

L. monocytogenes has been isolated from various ready-to-eat products. In a study by Meldrum et al. (2010) the prevalence of *L. monocytogenes* was 4.1% in crustaceans (n=147), 6.7% in smoked fish (n=178), 2% in sushi (n=50) and 0.9% in green salad (n=335) samples in Wales. Wong et al. (2005) isolated *L. monocytogenes* from 1% of ham (n=104) and 1.7% of pate (n=60) samples in New Zealand. *L. monocytogenes* has also been isolated from dairy products. For example, *L. monocytogenes* was detected in 1.3% of fresh cheese samples in Spain (n=78), 0.2% of hard cheese samples in the United Kingdom (n=1242) and 0.3% of ice creams in Italy (n=1734) (Busani et al. 2005; Cabedo et al. 2008; Little et al. 2009). The prevalence of *L. monocytogenes* in bulk milk tank internationally is 1–60% (FSANZ 2009).

The presence of *L. monocytogenes* in ready-to-eat products is probably due to contamination occurring after the product has been processed. This contamination may occur during additional handling steps such as peeling, slicing and repackaging. Also, in the retail and food service environment, contamination may be transferred between ready-to-eat products (Lianou and Sofos 2007). The type of handling that ready-to-eat meat receives may also influence the level of *L. monocytogenes* contamination. In a survey of retail packaged meats there was a significantly higher prevalence of *L. monocytogenes* reported in products cut into cubes (61.5%) (n=13), compared with sliced products (4.6%) (n=196) (Angelidis and Koutsoumanis 2006).

Host factors that influence disease

People at risk of invasive listeriosis include pregnant women and their foetuses, newborn babies, the elderly and immunocompromised individuals (such as cancer, transplant and HIV/AIDS patients). Less frequently reported, but also at a greater risk, are patients with diabetes, asthma, cirrhosis (liver disease) and ulcerative colitis (inflammatory bowel disease) (FDA 2012).

Dose response

Investigations of foodborne outbreaks of non-invasive listeriosis have concluded that consumption of food with high levels of *L. monocytogenes* (1.9×10^5/g to 1.2×10^9/g) is required to cause illness in the general healthy population (Sim et al. 2002).

The number of *L. monocytogenes* required to cause invasive listeriosis depends on a number of factors. These include the virulence of the particular serotype of *L. monocytogenes*, the general health and immune status of the host, and attributes of the food (for example fatty foods can protect bacteria from stomach acid). Some *L. monocytogenes* serovars are more virulent than others; this may be attributed to differences in the expression of virulence factors which could influence the interactions between the bacterium and the host cells and cellular invasion (Severino et al. 2007). The FDA and WHO have developed separate models for both healthy and susceptible populations to predict the probability that an individual will develop listeriosis (FDA/USDA/CDC 2003; WHO/FAO 2004). The probability that a healthy person of intermediate age will become ill from the consumption of a single *L. monocytogenes* cell was estimated to be 2.37×10^{-14}. For more susceptible populations the probability that illness will occur was estimated to be 1.06×10^{-12}. A more recent assessment on invasive listeriosis in susceptible populations was performed which took into account the different serotypes of *L. monocytogenes* (Chen et al. 2006). This study showed that the probability of a susceptible individual developing invasive listeriosis ranged from 1.31×10^{-8} to 5.01×10^{-11}, suggesting that there are large differences in virulence between *L. monocytogenes* serotypes.

Recommended reading and useful links

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Prions (bovine spongiform encephalopathy)

Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disease of cattle. It is caused by proteinaceous infectious particles known as prions. BSE is the only transmissible spongiform encephalopathy (TSE) of animals that is known to be infectious to humans through the consumption of contaminated meat. The human form of the disease is known as variant Creutzfeldt-Jakob (vCJD) disease.

Description of the infective agent

BSE and other TSEs are caused by a mis-folded isoform of the prion protein (PrP), a widely expressed glycoprotein. PrP is a normal constituent of cell membranes in vertebrates, and is encoded by the prion protein gene *PRNP*. The mis-folded pathogenic isoform protein is often referred to as a 'prion', a term made up from the contraction of the words 'proteinaceous' and 'infectious' (Prusiner 1982). By convention, the normal cellular isoform of PrP is represented as PrP^C. The C superscript refers to the cellular form. The prion form has the same amino acid sequence as the normal form and is represented as PrP^{Sc}. The Sc superscript is a reference to scrapie, a disease of sheep that is the prototypical animal prion disease. The prions replicate themselves by binding to the normal PrP^C protein and acting as a template that coerces the PrP^C molecule to refold into the abnormal PrP^{Sc} form (Gains and LeBlanc 2007; Cobb and Surewicz 2009).

PrP^C has been identified in mammals, birds, reptiles, amphibians, fish and yeasts. In mammals, the protein is expressed in a wide variety of tissues including spleen, lymph nodes, kidney, pancreas, salivary gland, adrenal gland, liver, thymus, and bone marrow; and is highly expressed in the nervous system (Gains and LeBlanc 2007; Linden et al. 2008; Brown and Mastrianni 2010). However, the physiological function of PrP^C remains obscure and a number of strains of mice bred not to express PrP^C show only subtle, non-lethal differences in physiologic and locomotor activity when compared to wild-type mice (Cobb and Surewicz 2009; Chakrabarti et al. 2009).

Within some prion diseases, including BSE and scrapie, strains exist which exhibit distinct disease phenotypes. Differences between strains include patterns of protein deposition in the brain and lymphoid tissues, incubation times after experimental infection of animals, histopathology and clinical manifestation. For example, with scrapie some strains preferentially propagate in the central nervous system while others are characterised by substantial infectivity in lymphoid organs (Aguzzi and Calella 2009). There are three strains of BSE which have been identified. Only one strain, classical BSE, was responsible for the BSE epidemic which started in the United Kingdom (UK) and spread to other countries, and the associated epidemic of vCJD in humans (Harman and Silva 2009). The atypical strains, known as H (high) and L (low) type, are diagnosed rarely, typically in cattle of 8–20 years of age, and appear to be sporadic and arise spontaneously (Seuberlich et al. 2010; Konold et al. 2012).

Stability characteristics

Prions are notoriously resistant to inactivation with conventional sterilization procedures used for preparation of surgical instruments and materials. PrP^{Sc} is resistant to UV irradiation at 254 nm, 70% alcohol treatment, gamma irradiation, and conventional autoclaving (121°C for 20 minutes). PrP^{Sc} can be inactivated by a number of measures including severe autoclaving conditions (134°C for 8-18 minutes) in conjunction with detergents and hydrogen peroxide gas plasma sterilization (Aguzzi and Calella 2009; Sakudo et al. 2011).

A number of procedures that modify or hydrolyse proteins can reduce the infectivity of prions (Aguzzi and Calella 2009). However, while PrP^C is protease-sensitive and soluble in non-denaturing detergents, PrP^{Sc} is insoluble in detergents and contains a protease-resistant core (Gains and LeBlanc 2007).

Symptoms and clinical signs of disease

Cattle

Field data suggest that susceptibility to BSE infection peaks around 12 months of age in cattle, although there have been BSE cases in cattle that were not fed meat-and-bone meal (MBM) until they were over 2 years of age. The incubation period in cattle is estimated to be from 30 months to 8 years (mean of 4.5–5.5 years), but the course of the clinical disease is short from the onset of clinical signs, with animals generally dying or requiring euthanasia within 6 months (USDA FSIS 2005; St Rose et al. 2006; Harman and Silva 2009). Among 124,000 UK cattle for which the age of onset of clinical signs was known, 7% were 3 years old, 31% were 4 years old, 33% were 5 years old (generally thought to be the average age of onset) and 29% were 6 years old or older (Harman and Silva 2009).

Clinical signs in cattle include changes in temperament, such as nervousness or aggression, abnormal posture, incoordination and difficulty in rising, decreased milk production, or loss of body weight despite continued appetite (USDA FSIS 2005; Seuberlich et al. 2010).

Humans

Over 200 human cases of vCJD have been reported (Aguzzi and Calella 2009). Patients have ranged in age from 17–42 years (Brown and Mastrianni 2010). The great majority of patients were UK residents during the period 1985–1996 (Mackay et al. 2011). Variant CJD is invariably fatal, and generally runs a course of approximately 18 months from the onset of symptoms. Symptoms and clinical signs include both cognitive and motor dysfunction. Early in the illness, patients usually experience psychiatric or sensory symptoms. Reported psychiatric symptoms include depression, apathy, agitation, insomnia, poor concentration, paranoid delusion, recklessness, aggression, withdrawal or anxiety. Approximately one third of the patients reported unusual persistent and painful sensory symptoms. Neurological signs develop as the illness progresses, including cerebellar ataxia, muscle spasms and involuntary movements. Late onset signs include urinary incontinence, progressive immobility, and akinetic mutism. Death is often due to opportunistic infections (Imran and Mahmood 2011a).

The great majority of cases of prion disease occurring in humans are spontaneous occurrences of sporadic Creutzfeld-Jakob disease (sCJD). In comparison to vCJD, sCJD typically affects people between 55 and 70 years old (Mackay et al. 2011). Cerebellar ataxia or progressive dementia predominate in the first few months of sCJD, which contrast with vCJD (Imran and Mahmood 2011a). Also, vCJD features distinctive histopathology (Mackay et al. 2011). Besides classical BSE, the only other TSE known to be infectious to humans by the oral route is the now nearly extinct disease known as kuru, which occurred in a small group of communities of indigenous Papua New Guinean people who practised cannibalism (Aguzzi and Calella 2009).

Pathogenic mechanism

The pathogenesis of BSE in cattle has been studied extensively although there are still a number of knowledge gaps. After oral exposure of calves to infective material, PrP^{Sc} is first observed in Peyer's patches of the ileum, and also detected in gut-associated lymphoid tissue (GALT) of the ileocaecal junction and the jejunum. The infectivity is located in macrophages and follicular dendritic cells (FDC). Later, infectivity can be identified in the enteric nervous system, although it is not clear how infectivity moves from the cells of the lymphoreticular system to those of the nervous system (Hoffmann et al. 2011). It is possible that after crossing the mucosal barrier of the intestine, prions infect the nervous tissue when they come into contact with the fine nerve fibres directly under the intestinal mucosa (van Keulen et al. 2008). Once the nervous system is infected, infectivity then ascends to the brain via both the sympathetic (e.g. splanchnic nerve) and parasympathetic (e.g. vagus nerve) nervous systems (Cobb and Surewicz 2009). Involvement of GALT is less extensive in BSE than in ovine scrapie (van Keulen et al. 2008). It has been proposed that orally acquired prion diseases can also reach the brain through the bloodstream (Caughey et al. 2009; Gough and Maddison 2010), but infectivity is not detectable in the blood of BSE-affected cows (van Keulen et al. 2008). This is in contrast to experimental BSE in sheep and human vCJD, in which GALT shows high levels of infectivity and the blood also contains prions (Gough and Maddison 2010).

The role of replication of PrP^{Sc} in FDCs of the spleen in propagation of the agent is unclear, and may vary between species. Studies of scrapie have provided evidence that depletion of FDCs prevents or delays neuroinvasion, that increased nerve supply to the spleen promotes neuroinvasion and that denervation of the spleen delays or prevents neuroinvasion (Gains and LeBlanc 2007). Splenic PrP^{Sc} is found in BSE infection of mice expressing the ovine prion protein (Baron et al. 2010). However, splenic PrP^{Sc} was detected in only one of three cattle terminated in the advanced clinical stage of BSE (Murayama et al. 2010).

Once a cell is infected with PrP^{Sc}, spread of infection to adjacent cells may occur by transfer of PrP^{Sc}-containing membrane microparticles. Consistent with this hypothesis, it has been shown that PrP^{Sc} can be released from infected cells *in vitro* in association with exosomes. Exosomes are small membrane-bound vesicles that can be secreted by cells and can fuse with other cells. However, although exosome production by lymphoid cells has been demonstrated, exosomes have not been shown to be produced by neurons. Another possible route by which PrP^{Sc} could be transferred between adjacent cells is via tunnelling nanotubes, thin membranous bridges that can form between cells and allow the transfer of organelles, plasma membrane components, cytoplasmic molecules and pathogens (Caughey et al. 2009). Other proposed pathways of propagation within the nervous system include axonal transport, sequential infection of Schwann cells (cells that support and insulate peripheral nerves) and via the flow of lymph in the vicinity of neurons (Kovacs and Budka 2008).

The molecular pathways leading to cerebral damage are largely unknown, although various theories have been advanced. Depletion of PrP^{C} does not appear to be a cause, as mice that have been genetically engineered to lack PrP^{C} altogether, and those in which PrP^{C} expression is turned off in adulthood, do not develop clinical signs of TSE. In fact depletion of PrP^{C} in mice with established prion infection has been shown to reverse early spongiform degeneration and prevent progression to clinical disease. These findings suggest that the toxicity of PrP^{Sc} depends on some PrP^{C}-dependent process (Aguzzi and Calella 2009). It has been suggested that PrP^{C} is neuroprotective and its conversion to PrP^{Sc} interferes with this function and allows neurodegeneration (Caughey et al. 2009; Solomon et al. 2009). Another possibility is that binding of PrP^{Sc} to PrP^{C} triggers a signal transduction pathway leading to neuronal damage (Soto and Satani 2011).

Other theories of PrP[Sc] pathogenicity, based around *in vitro* observations, include impairment of breakdown of cellular waste by lysosomes, up-regulation of genes involved in endoplasmic reticulum function, and reduced degradation of proteins by the proteasome system (Kovacs and Budka 2008; Chakrabarti et al. 2009). These various theories are not mutually exclusive.

Infectivity

There is no robust evidence that BSE can be transmitted between cattle by routes other than consumption of feed contaminated with certain tissues from BSE-infected cattle. This is in marked contrast to the horizontal infectivity of scrapie in sheep and chronic wasting disease (CWD) in deer. CWD prions are found in saliva, urine, faeces, placenta or decomposed carcasses (Gough and Maddison 2010; Haley et al. 2011; Imran and Mahmood 2011b). PrP[Sc] from scrapie-infected sheep is found in faeces, milk, saliva, nasal secretions and placental tissues. Scrapie and CWD prions have also been shown to persist in the environment, bound to soil or other fomites, but there is no evidence that BSE has been transmitted between cattle by this route, or via exposure to excreta or secretions (Gough and Maddison 2010).

There is no evidence that vCJD has been transmitted between humans except due to medical intervention such as blood transfusion (Brown and Mastrianni 2010).

Modes of transmission

The epidemic of BSE, first recognized in 1986 in the UK, was propagated by the rendering of dead cattle infected with BSE to produce MBM which was then included in feed for cattle (Harman and Silva 2009). Ingestion of infectious material in MBM made from BSE-infected animals was the only known route of transmission of the agent between cattle.

Consumption of beef contaminated with infected bovine central nervous system tissue also led to an epidemic of vCJD in humans. Although the majority of vCJD cases have been attributed to consumption of such contaminated beef, four cases of person-to-person vCJD transmission by blood or plasma transfusion have been reported in the UK (PHE 2009; Imran and Mahmood 2011a).

Rare cases of transmission of sCJD between humans have resulted from corneal grafts, dura mater grafts and growth hormone injections (Brown and Mastrianni 2010). Similar transmission of vCJD remains a concern because retrospective analysis of tonsil and appendix specimens led to the estimation that up to 1 in 4,000 persons exposed during the UK epidemic may be a sub-clinical carrier (Harman and Silva 2009; Collinge 2012).

There is no evidence that sCJD is a TSE of animal origin because this disease develops even among lifelong vegetarians (Harman and Silva 2009).

Incidence of illness and outbreak data

Animals

More than 184,000 cases of classical BSE have been diagnosed in cattle, and at the peak of the epidemic 1,000 cases were being diagnosed each week in the UK (Imran and Mahmood 2011b; OIE 2013). The epidemic is believed to have been amplified from a single common source (Aguzzi and Calella 2009). The infection was spread elsewhere in Europe and the world by exports of infected cattle and MBM from the UK (Seuberlich et al. 2010). The feeding of MBM to cattle was banned in the UK in 1988, and in 1996 it became illegal in the UK to prepare any feed containing any mammalian protein for any farm animal. However, because of the long incubation period of BSE cases continued to occur, peaking in 1992. The incidence of new cases has steadily declined since then, and the disease is now very rare (Hueston and Bryant 2005).

A number of zoo and domestic animals developed TSEs at the same time as the BSE epidemic in cattle. All the species affected belonged to either the *Bovidae* or *Felidae* family, with the exceptions of a small number of non-human primates (Imran and Mahmood 2011b). All cases in zoo animals were attributed to ingestion of infective material derived from bovine BSE cases, as were two cases in domestic goats (Spiropoulos et al. 2011). A number of domestic cats developed TSE concurrently with the bovine BSE epidemic, and these cases were attributed to consumption of infective material in beef or beef-derived pet food (Harman and Silva 2009; Imran and Mahmood 2011b).

Epidemics of TSEs were not observed in other domestic species at the same time. Dogs and horses express PrP^C with a very stable structure that is resistant to mis-folding, and these species are resistant to infection with PrP^{Sc} (Harman and Silva 2009; Zhang 2011). Pigs are highly resistant to oral infection with the BSE prion, but may be infected by parenteral challenge (Harman and Silva 2009).

The original source of the classical BSE epidemic has been the subject of much conjecture but remains unknown. Surveillance in some countries has shown that sporadic cases of BSE occur in cattle, although to date only two strains distinct from 'classical' BSE, the L- and H-type strains, have been observed. However, it is possible that classical BSE may also occur spontaneously and that the epidemic represented amplification of infective material originating from a single sporadic case. It is also possible that the BSE epidemic originated from material from another species also rendered to produce MBM. It has been suggested that the BSE epidemic arose from rendering of scrapie-infected sheep. However, although sheep scrapie samples can cause a TSE when inoculated intracerebrally into cattle, the disease does not resemble BSE, and experimental BSE in sheep does not resemble scrapie. In fact cattle are resistant to oral infection with scrapie or CWD (Harman and Silva 2009).

Humans

The first 10 human patients with vCJD were reported in April 1996 in the UK (Mackay et al. 2011; Imran and Mahmood 2011a). As of December 2012, 227 vCJD cases have been reported in total. Current data may be found on the UK National Creutzfeldt-Jakob Disease Research & Surveillance Unit website, http://www.cjd.ed.ac.uk. The majority of cases (176) occurred in the UK (Imran and Mahmood 2011a).

There is strong evidence to show that vCJD is caused by ingestion of BSE infective material. Classical BSE prions from affected cows and vCJD prions from the brains of infected humans produce the same lesions in mice. The biochemical properties of BSE prions from cattle and vCJD prions from humans are indistinguishable.

Furthermore the great majority of vCJD cases have occurred in the UK, with a few cases in other countries including France, Ireland and Italy (Aguzzi and Calella 2009).

A two-fold difference was seen between the prevalence of vCJD in the north versus the south of the UK. Contemporaneous National Dietary Surveys showed that consumption of mechanically recovered beef products was much higher in the north of the UK (Mackay et al. 2011). Mechanically recovered beef is more likely to be contaminated with infective material in the spinal cord, and recovery of beef by this method is no longer permitted for human foodstuffs across Europe.

Occurrence in food

The incidence of classical BSE in cattle has declined markedly since the 1990s through prevention measures based on knowledge of how the disease is transmitted between cattle. There are now very few cases of BSE reported in cattle worldwide (OIE 2013).

A key component of prevention of both BSE in cattle and vCJD in humans is the prohibition on feeding ruminant derived protein to ruminants. This measure was enacted in 1994 in the UK when it became illegal to feed ruminants with mammalian proteins, with specific exceptions such as dairy proteins. The feed ban was further extended in 1996 by a ban on feeding any farmed livestock, including fish and horses, with mammalian MBM. Feeding of mammalian derived proteins was prohibited throughout the European Union (EU) in 1994 (EC No. 94/381). This was further extended in EU Regulation (EC No. 999/2001) which introduced further EU-wide controls to combat the spread of BSE, including a ban on the feeding of processed animal proteins to any animals kept, fattened or bred for the production of food.

With regards to infectivity in food, the risk of exposure of humans to BSE can essentially be removed by withholding the particular lymphoid and central nervous tissues known to harbour infectivity, termed specific risk materials (SRM), from the food supply. The list of SRM, and the ages of cattle from which they must be removed, have been modified over time with advancing knowledge, but currently include brain, eyes, tonsils, spinal cord and intestines, as well as the entire spinal column of older cattle. Bans on the use of SRM from bovine carcasses above specified ages have been implemented throughout Europe and other countries to ensure the safety of beef and beef products. Slaughter procedures of cattle are designed to prevent contamination of the carcass with SRM, and SRM are rendered and incinerated to destroy infectivity in countries with BSE risk factors. In addition, mechanical recovery of meat from bones is prohibited in order to prevent inclusion of dorsal root ganglia, which may contain infectivity. Beef and beef products from countries with these and animal feed control systems are therefore considered to be safe for human consumption. Countries assessed as being of negligible risk of BSE in the cattle population are not required to practise these precautions.

Human host factors that influence disease

A polymorphism at position 129 of the PrP^C amino acid sequence has been identified in humans, with different genotypes exhibiting different susceptibilities to TSEs. Approximately 40% of Caucasians are homozygous for methionine (Met) at this position, 10% are homozygous for valine (Val) and 50% are Met/Val heterozygotes. To date, all confirmed clinical cases of vCJD have been homozygous for Met at codon 129 (Mackay et al. 2011). A presumptive clinical case in a Met/Val heterozygote was reported in 2009, but an autopsy was not done and the MRI findings were not typical of vCJD (Kaski et al. 2009; Mackay et al. 2011). A confirmed pre-clinical infection in a Met/Val heterozygote was identified in a patient who died of a ruptured aortic aneurysm five years after receiving a blood transfusion from a person who subsequently developed vCJD. The Met/Val heterozygote had PrP^{Sc} in

the spleen but not in the central nervous system, and there was no evidence of central nervous pathology typical of vCJD (Gains and LeBlanc 2007; Mackay et al. 2011). The three known clinical cases of vCJD infection by blood transfusion were all Met/Met homozygotes (Mackay et al. 2011). Two of three PrP^{Sc}-positive samples in an anonymous postsurgical study of appendices were from Val/Val homozygotes. This indicates that lymphoid tissue, at least, of all three genotypes may become infected (Harman and Silva 2009; Will 2010; Mackay et al. 2011).

It is not yet clear whether the Met/Val and Val/Val genotypes are protective against neurological infection with vCJD, or whether onset is delayed rather than prevented (Mackay et al. 2011). Some authors have predicted a multiphasic vCJD epidemic with a late peak of cases affecting people heterozygous at codon 129 (Aguzzi and Calella 2009; Will 2010). Besides vCJD, the only other orally acquired prion disease known in humans is kuru, a historical disease of a small number of communities in Papua New Guinea who propagated the disease through the practice of funerary cannibalism. The mean incubation period of kuru is 12 years, but the incubation period has exceeded 50 years in some individuals (Imran and Mahmood 2011a). Retrospective analysis of blood samples from kuru patients shows an age stratification of codon 129 genotype. The young kuru patients were mainly Met/Met or Val/Val homozygotes, whereas the elderly patients were mostly Met/Val heterozygotes. Eight of eleven of the more recent cases of kuru were Met/Val heterozygotes, which supports the hypothesis that the Met/Val genotype delays but does not prevent the onset of kuru in all individuals, because exposure of these individuals almost certainly ended more than 40 years ago when funerary cannibalism was outlawed (Mackay et al. 2011).

The majority of vCJD cases in the UK affected people less than 40 years of age. Possible explanations include a higher rate of dietary exposure, increased susceptibility to infection or a reduced incubation period in this age group. Greater susceptibility could be conferred by the volume of GALT, which declines with age (Mackay et al. 2011). In addition, Peyer's patches that are thought to be involved in intestinal update of prions, decline during adulthood (St Rose et al. 2006).

Infective dose

It appears that ingestion of less than 1 mg of infected brain material may be sufficient to transmit infection between cattle (Harman and Silva 2009). Transmission of BSE to macaques has been accomplished by oral administration of 5 g of infective brain homogenate, but the infective dose of bovine PrP^{Sc} to human beings is unknown (Mackay et al. 2011).

Recommended reading and useful links

European Commission Food and Feed Safety page on BSE:
http://ec.europa.eu/food/food/biosafety/tse_bse/index_en.htm

World Health Organization Media Centre page on vCJD:
http://www.who.int/mediacentre/factsheets/fs180/en/

FSANZ Consumer Information on BSE
http://www.foodstandards.gov.au/industry/bse/Pages/default.aspx

UK National Creutzfeldt-Jakob Disease Research & Surveillance Unit website
http://www.cjd.ed.ac.uk

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Salmonella (non-typhoidal)

Salmonella spp. are bacteria that cause salmonellosis, a common form of foodborne illness in humans. Outcomes from exposure to *Salmonella* spp. can range from mild symptoms to severe disease and can be fatal. *Salmonella* spp. are carried by a range of domestic and wild animals and birds and have been widely isolated from the environment.

Description of the organism

Salmonella spp. are Gram-negative, non-spore forming rod-shaped bacteria and are members of the family *Enterobacteriaceae* (Jay et al. 2003). The genus *Salmonella* is divided into two species: *S. enterica* (comprising six subspecies) and *S. bongori*. Over 99% of human *Salmonella* spp. infections are caused by *S. enterica* subsp. *enterica* (Bell and Kyriakides 2002; Crum-Cianflone 2008).

Strains of *Salmonella* can be characterised serologically (into serovars) based on the presence and/or absence of O (somatic) and H (flagella) antigens. Phage typing is used to subtype *Salmonella* serovars. The phage type is determined by the sensitivity of the bacterial cells to the lytic activity of selected bacteriophages (Bell and Kyriakides 2002; Jay et al. 2003).

The formal names used to describe types of *Salmonella* are rather cumbersome, for example *S. enterica* subsp. *enterica* serovar Typhimurium. For practical reasons, the abbreviated versions of these names using just the serovar are commonly used, such as *S.* Typhimurium (Crum-Cianflone 2008).

Some *Salmonella* serovars are host-adapted to individual animal species and may differ vastly in the severity of the disease they cause; others such as *S.* Typhimurium have a broad host range, with an ability to infect a wide range of animals, including humans (Jay et al. 2003; Wallis 2006).

S. Typhi and *S. Paratyphi* are specifically associated with infections in humans, leading to severe disease called enteric fever. *S. Typhi* and *S. Paratyphi* produce clinical syndromes referred to as typhoid and paratyphoid fever, respectively. Enteric fever is rare in developed countries, with the majority of cases associated with overseas travel (Darby and Sheorey 2008). In Australia 97.9% of notified cases of typhoid fever were likely to have been acquired overseas in 2010 (OzFoodNet 2012).

Growth and survival characteristics

Salmonella spp. have relatively simple nutritional requirements and can survive for long periods of time in foods and other substrates. The growth and survival of *Salmonella* spp. is influenced by a number of factors such as temperature, pH, water activity and the presence of preservatives (refer to Table 1).

The temperature range for growth of *Salmonella* spp. is 5.2–46.2°C, with the optimal temperature being 35–43°C (ICMSF 1996). Although freezing can be detrimental to *Salmonella* spp. survival, it does not guarantee destruction of the organism. There is an initial rapid decrease in the number of viable organisms at temperatures close to the freezing point as a result of the freezing damage. However, at lower temperatures *Salmonella* spp. have the ability to survive long term frozen storage (Jay et al. 2003). Strawn and Dayluk (2010) showed that *Salmonella* was able to survive on frozen mangoes and papayas stored at -20°C for at least 180 days.

Heat resistance of *Salmonella* spp. in food is dependent on the composition, pH and water activity of the food. The heat resistance of *Salmonella* spp. increases as the water activity of the food decreases. Foods which are high in fat and low in moisture, such as chocolate and peanut butter, may have a protective effect against heat. In low pH conditions the heat resistance of *Salmonella* spp. is reduced (Jay et al. 2003; Shachar and Yaron 2006; Podolak et al. 2010).

Salmonella spp. will grow in a broad pH range of 3.8–9.5, with an optimum pH range for growth of 7–7.5 (ICMSF 1996). The minimum pH at which *Salmonella* spp. can grow is dependent on temperature, presence of salt and nitrite and the type of acid present. Volatile fatty acids are more bactericidal than organic acids such as lactic, citric and acetic acid. Outside of the pH range for growth, cells may become inactivated, although this is not immediate and cells have been shown to survive for long periods in acidic products (Bell and Kyriakides 2002; Jay et al. 2003).

Water activity (a_w) has a significant effect on the growth of *Salmonella* spp., with the optimum a_w being 0.99 and the lower limit for growth being 0.93. *Salmonella* spp. can survive for months or even years in foods with a low a_w (such as black pepper, chocolate, peanut butter and gelatine) (ICMSF 1996; Podolak et al. 2010).

Salmonella spp. are similar to other Gram negative bacteria in regard to susceptibility to preservatives commonly used in foods. Growth of *Salmonella* spp. can be inhibited by benzoic acid, sorbic acid or propionic acid. The inhibition of *Salmonella* spp. is enhanced by the use of a combination of several preservative factors, such as the use of a preservative in conjunction with reduction in pH and temperature (ICMSF 1996; Ha et al. 2004; Banerjee and Sarkar 2004).

Salmonella spp. are classed as facultative anaerobic organisms as they do not require oxygen for growth (Jay et al. 2003).

Table 1: Limits for growth of *Salmonella* spp. when other conditions are near optimum (ICMSF 1996; Podolak et al. 2010)

	Minimum	Optimum	Maximum
Temperature (°C)	5.2	35–43	46.2
pH	3.8	7–7.5	9.5
Water activity	0.93	0.99	>0.99

Symptoms of disease

Outcomes of exposure to non-typhoidal *Salmonella* spp. can range from having no effect, to colonisation of the gastrointestinal tract without symptoms of illness (asymptomatic infection), or colonisation with the typical symptoms of acute gastroenteritis. Gastroenteritis symptoms are generally mild and may include abdominal cramps, nausea, diarrhoea, mild fever, vomiting, dehydration, headache and/or prostration. The incubation period is 8–72 hours (usually 24–48 hours) and symptoms last for 2–7 days (WHO/FAO 2002; Darby and Sheorey 2008). Severe disease such as septicaemia sometimes develops, predominantly in immunocompromised individuals. This occurs when *Salmonella* spp. enter the bloodstream, leading to symptoms such as high fever, lethargy, abdominal and chest pain, chills and anorexia; and can be fatal. A small number of individuals develop a chronic condition or sequelae such as arthritis, appendicitis, meningitis or pneumonia as a consequence of infection (Hohmann 2001; WHO/FAO 2002; FDA 2012).

Salmonella spp. are shed in large numbers in the faeces of infected individuals at the onset of illness. In the case of non-typhoid disease, bacterial shedding continues for about 4 weeks after illness in adults and 7 weeks in children. It is estimated that 0.5% of individuals with non-typhoid salmonellosis become long-term carriers and continue shedding the bacteria on an ongoing basis (Jay et al. 2003; Crum-Cianflone 2008).

Virulence and infectivity

Once ingested, *Salmonella* spp. must survive the low pH of the stomach, adhere to the small intestine epithelial cells and overcome host defence mechanisms to enable infection (Jay et al. 2003).

Salmonella spp. possess a number of structural and physiological virulence factors, enabling them to cause acute and chronic disease in humans. The virulence of *Salmonella* spp. varies with the length and structure of the O side chains of lipopolysaccharide molecules at the surface of the bacterial cell. Resistance of *Salmonella* spp. to the lytic action of complement (part of the immune response) is directly related to the length of the O side chain (Jay et al. 2003). Other important virulence factors include the presence and type of fimbriae, which is related to the ability of *Salmonella* spp. to attach to host epithelium cells, as well as the expression of genes responsible for invasion into cells (Jones 2005). Some of these virulence genes are encoded on *Salmonella* pathogenicity islands (SPI). SPI-1 is required for bacterial invasion into intestinal epithelial cells, while systemic infections and intracellular accumulation of *Salmonella* spp. are dependent on the function of SPI-2 (Valle and Guiney 2005).

Salmonella spp. produce a heat labile enterotoxin, resulting in the loss of intestinal fluids (causing diarrhoea). This enterotoxin is closely related functionally, immunologically and genetically to the toxin of *Vibrio cholerae* and the heat labile toxin of pathogenic *Escherichia coli* (Jay et al. 2003). Most *Salmonella* strains also produce heat labile cytotoxin which may cause damage to the intestinal mucosal surface and results in general enteric symptoms and inflammation. Infection with non-typhoidal *Salmonella* is generally limited to a localised intestinal event. However, the presence of virulence plasmids has been associated with non-typhoidal *Salmonella* spp. surviving in phagocytes and spreading from the small intestine to the spleen and liver (Jay et al. 2003; Hanes 2003).

Multiple antibiotic resistant strains of *Salmonella* have emerged, an example being *S.* Typhimurium definitive phage type 104 (DT104). Multi-resistant *S.* Typhimurium DT104 infects both humans and animals, such as cattle and sheep. To date, this organism is not endemic in Australia, although it is a significant health problem in European countries, North America, the Middle East, South Africa and South-East Asia (Jay et al. 2003).

Mode of transmission

Salmonella spp. are transmitted by the faecal-oral route by either consumption of contaminated food or water, person-to-person contact, or from direct contact with infected animals (Jay et al. 2003).

Incidence of illness and outbreak data

Salmonellosis is one of the most commonly reported enteric illnesses worldwide, being the second most frequently reported cause of enteric illness in Australia (behind campylobacteriosis). It is a notifiable disease in all Australian states and territories, with a notification rate in 2012 of 49.8 cases per 100,000 population (11,273 cases). This was an increase on the previous 5 year mean of 46.9 cases per 100,000 population per year (ranging from 38.6–54.2 cases per 100,000 population per year) (NNDSS 2013).

The salmonellosis notification rate varied between jurisdictions from 40.5 cases per 100,000 population in New South Wales to 180.1 cases per 100,000 population in the Northern Territory in 2012 (NNDSS 2013). Children between 0–4 years had the highest notification rate, with 218.3 and 160.2 notifications per 100,000 population for males and females, respectively, in 2010 (OzFoodNet 2012) The higher rate of notified cases in this age group may reflect an increased susceptibility upon first exposure, but may also be a result of other factors such as an increased likelihood of exposure and increased likelihood to seek medical care.

The distribution of *Salmonella* serovars in Australia varies geographically, however *S.* Typhimurium was the most commonly reported serovar in 2010, representing 44% of all notified *Salmonella* infections in Australia. Internationally, *S. Enteritidis* is frequently reported as causing human illness; however it is not endemic in Australia. In 2010, 93% of *S. Enteritidis* cases reported in Australia were acquired overseas (Greig and Ravel 2009; OzFoodNet 2012).

The notification rate for salmonellosis in New Zealand in 2011 was 24 cases per 100,000 population (1,056 cases). This was a slight decrease from the 2010 rate of 26.2 cases per 100,000 population (Lim et al. 2012). In the United States (US) 17.73 cases of salmonellosis were notified per 100,000 population in 2010. This was a slight increase from the 2009 rate of 16.18 cases per 100,000 population (CDC 2012). In the European Union the notification rate for salmonellosis was 20.7 cases per 100,000 population in 2011 (ranging from 1.6–80.7 cases per 100,000 population between countries). This was a 5.4% decrease in the number of cases from 2010 (EFSA 2013).

Outbreaks attributed to *Salmonella* spp. have predominantly been associated with animal products such as eggs, poultry, raw meat, milk and dairy products, but also include fresh produce, salad dressing, fruit juice, peanut butter and chocolate (Jay et al. 2003; Montville and Matthews 2005) (refer to Table 2).

Table 2: Selected major outbreaks associated with *Salmonella* spp. (>50 cases and/or ≥1 fatality)

Year	Serovar	Total no. cases (fatalities)	Food	Country	Comments	Reference
2010	S. Typhimurium PT9	170	Aioli	Australia	Aioli was made with raw eggs. *S.* Typhimurium PT9 isolated from aioli and chopping boards	(OzFoodNet 2010)
2009-2010	S. Montevideo	272	Salami containing red or black pepper	US	Pepper was added to the salami after the kill step, pepper samples were positive for S. Montevideo	(CDC 2010)
2006-2007	S. Tennessee	628	Peanut butter	US	Environmental samples from the plant were positive for S. Tennessee	(CDC 2007)
2005-2006	S. Oranienburg	126	Alfalfa	Australia	Alfalfa at the production facility were positive for S. Oranienburg	(OzFoodNet 2006)
2005	S. Typhimurium PT135	63	Eggs used in bakery products	Australia	*S.* Typhimurium PT135 isolated from cream piping bag and bench of bakery. Issues with handling raw eggs, inadequate hygiene practices and cross-contamination. Eggs were dirty (externally) and from the same farm	(Stephens et al. 2007)
2001-2002	S. Oranienburg	>439	Chocolate	Germany	The high fat content of chocolate increases the heat resistance of *Salmonella* spp.	(Werber et al. 2005)
1999	S. Typhimurium PT135a	507	Unpasteurised fruit juice	Australia	*S.* Typhimurium PT135a was found on the oranges. It was also found in the fungicide tank and wax tank (through which the oranges passed) of the packing shed	(Federal Court of Australia 2003)
1985	S. Typhimurium	16,284 (7)	Pasteurised milk	US	Potential cross-contamination between the unpasteurised milk and pasteurised milk tank	(Ryan et al. 1987; Montville and Matthews 2005)

Occurrence in food

The primary reservoir of *Salmonella* spp. is the intestinal tract of warm and cold-blooded vertebrates, with many animals showing no sign of illness. Unlike diseased animals which can be removed from production and/or treated, these asymptomatic (carrier) animals can shed large numbers of *Salmonella* spp. in their faeces and are therefore an important source of contamination. Faecal shedding of *Salmonella* spp. leads to contamination of the surrounding environment including soil, crops, plants, rivers and lakes. A wide range of foods have been implicated in foodborne salmonellosis, particularly those of animal origin and foods that have been subject to sewage pollution (ICMSF 1996; Jay et al. 2003).

At the time of slaughter, *Salmonella* infected animals may have high numbers of organisms in their intestines as well as on the outside of the animal (faecal contamination of hides, fleece, skin or feathers) (Bryan and Doyle 1995; Jay et al. 2003). In Australia, *Salmonella* spp. have been isolated from 3% of chilled cattle carcass samples (n=100) (Fegan et al. 2005). The distribution of *Salmonella* spp. on contaminated meat carcasses is not uniform. For example, a US study by Stopforth et al. (2006) found that the prevalence of *Salmonella* spp. on fresh beef ranged from 0.8% (rib eye roll, n=133) to 9.6% (strip loins, n=52) depending on the cut of meat. Cross-contamination during processing may also lead to increased prevalence of *Salmonella* in finished products (Bryan and Doyle 1995).

Salmonella spp. have been detected in a range of foods. The prevalence of *Salmonella* spp. in bulk tank milk internationally is 0–11.8% (FSANZ 2009a). In shellfish (mussels, clams, oysters and cockles) collected off the coast of Spain, *Salmonella* spp. were detected in 1.8% samples (n=2980) (Martinez-Urtaza et al. 2003). Boughton et al. (2004) isolated *Salmonella* spp. from 2.9% of retail pork sausages samples in Ireland (n=921), and in Spain *Salmonella* spp. were detected in 2% of cooked ham samples (n=53) and 11.1% of cured dried pork sausage samples (n=81) (Cabedo et al. 2008).

An Australian survey found 43.3% of chicken meat at retail (n=859) was positive for *Salmonella* spp. The most prevalent serovar was S. Sofia, with 30.5% of chicken meat samples positive for this serovar (Pointon et al. 2008). Although S. Sofia accounted for a large proportion of isolates, it appears to be a non-virulent serovar and has been rarely associated with human or animal illness (Gan et al. 2011). The predominance of S. Sofia in poultry is unique to Australia as S. Sofia is essentially geographically isolated to Australia (Mellor et al. 2010).

S. Enteritidis (in particular phage type 4) is a globally important *Salmonella* serotype that can infect the reproductive tract of poultry and contaminate the internal contents of eggs. However, it is not endemic in Australian egg layer flocks (FSANZ 2009b).

Host factors that influence disease

People of all ages are susceptible to *Salmonella* spp. infection. However, the elderly, infants and immunocompromised individuals are at a greater risk of infection and generally have more severe symptoms (Jay et al. 2003; FDA 2012).

Dose response

Human feeding trials were undertaken during the 1950s to determine the relationship between the dose of *Salmonella* spp. ingested and whether illness occurred. These studies showed that ingestion of $1.3 \times 10^5 - 2.4 \times 10^7$ organisms could cause illness; however, for some strains 1×10^{10} organisms were required for illness to occur (McCullough and Eisele 1951a; McCullough and Eisele 1951b; McCullough and Eisele 1951c; McCullough and Eisele 1951d). However, there are a number of limitations on the use of this feeding trial data. Firstly, the volunteers selected were all healthy adult males, so the results may underestimate the risk to the overall population. Secondly, low doses which are more likely to exist in real food contamination events were not considered (Kothary and Babu 2001; Bollaerts et al. 2008). Investigations of salmonellosis outbreaks have estimated a wide range in the dose of organisms that has caused disease. Ranges reported vary from <10 to 10^9 depending on the food. As such, doses resulting in illness may be much lower than those reported in the feeding trials (Todd et al. 2008). The WHO/FAO (2002) developed a dose-response model based on outbreak data and estimated a 13% probability of illness from consumption of 100 *Salmonella* organisms.

Recommended reading and useful links

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Shiga toxin-producing *Escherichia coli* (STEC)

Escherichia coli are bacteria that form part of the normal gut flora of humans and other warm-blooded animals. Although most *E. coli* are considered harmless, certain strains can cause severe illness in humans, particularly Shiga toxin-producing *E. coli* (STEC). Infection with STEC is the main cause of haemolytic uraemic syndrome, a condition which can be fatal in humans.

Description of the organism

E. coli are Gram-negative, rod-shaped bacteria and are members of the family *Enterobacteriaceae*. Other species of the genus *Escherichia* include *E. adecarboxylata*, *E. blattae*, *E. fergusonii*, *E. hermanii* and *E. vulneris* (Meng and Schroeder 2007).

Pathogenic *E. coli* are classified into specific groups based on the mechanisms by which they cause disease and clinical symptoms. These categories include enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and diffusely adhering *E. coli* (DAEC) (Montville and Matthews 2005). STEC are Shiga-toxin producing *E. coli*, also known as verocytotoxin-producing *E. coli* (VTEC). The STEC strains that cause haemorrhagic colitis (bloody diarrhoea) belong to the EHEC group of pathogenic *E. coli* (Yoon and Hovde 2008). In developed countries EHEC is the most serious of the pathogenic *E. coli*, however, in developing countries EPEC is a major disease causing agent in children (Meng and Schroeder 2007; Ochoa et al. 2008).

Strains of *E. coli* can be characterised serologically based on the detection of specific O (somatic), H (flagella) and K (capsule) antigens. For most *E. coli* strains the O and H antigens are sufficient to identify the strain. For example, *E. coli* O157:H7 is the leading cause of STEC infections internationally (Meng and Schroeder 2007; Gyles 2007).

Growth and survival characteristics

The growth and survival of *E. coli* depends on a number of environmental factors such as temperature, pH, water activity (a_w) and the composition of the food (refer to Table 1).

The temperature range for growth of *E. coli* is 7–8°C to 46°C, with an optimum temperature of 35–40°C (ICMSF 1996). Heat resistance of *E. coli* in food is dependent on the composition, pH and a_w of the food. The heat resistance of *E. coli* increases as the a_w decreases. Also, *E. coli* is more resistant to heat when it is in its stationary phase of growth compared to its log phase of growth (Desmarchelier and Fegan 2003). Low temperature has little effect on *E. coli* survival. Strawn and Danyluk (2010) showed that *E. coli* O157:H7 was able to survive on mangoes and papayas stored at -20°C for at least 180 days.

E. coli grow in a broad pH range of 4.4–10.0, with an optimum pH of 6–7 (Desmarchelier and Fegan 2003). A study by Molina et al. (2003) demonstrated that STEC are tolerant to acidic conditions, with many STEC strains able to survive at pH 2.5–3.0 for over 4 hours. *E. coli* O91:H21 was able to survive at pH 3.0 for more than 24 hours. Arnold and Kaspar (1995) found that *E. coli* O157:H7 is more tolerant to acid when it is in stationary growth phase or starved during its log-phase of growth. Therefore STEC may be able to survive and grow in food products previously considered too acidic to support the survival of foodborne pathogens. The effect of pH on *E. coli* survival, however,

is dependent on the type of acid present. For example, *E. coli* O157:H7 can survive in a growth medium adjusted to pH 4.5 with hydrochloric acid but not when adjusted to the same pH with lactic acid (ICMSF 1996).

The minimum a_w required for growth of *E. coli* is 0.95. In sub-optimal temperature or pH conditions, a higher a_w value is required for growth of *E. coli* (Desmarchelier and Fegan 2003).

E. coli are facultative anaerobic organisms so do not require oxygen for growth. However, they grow better in aerobic conditions (Meng and Schroeder 2007).

Table 1: Limits for growth of *E. coli* when other conditions are near optimum (ICMSF 1996; Desmarchelier and Fegan 2003)

	Minimum	Optimum	Maximum
Temperature (°C)	7–8	35–40	46
pH	4.4	6–7	10.0
Water activity	0.95	0.995	–

Symptoms of disease

Infection with STEC can result in no clinical symptoms (asymptomatic infection) or can cause diarrhoea (may progress to bloody diarrhoea), abdominal cramps, vomiting and fever. The onset of illness is 3–8 days (median of 3–4 days). Most patients recover within 10 days of the initial onset of symptoms (Meng and Schroeder 2007; WHO 2011). In some cases, patients develop haemolytic uraemic syndrome (HUS). HUS is characterised by haemolytic anaemia, thrombocytopenia (decrease in blood platelets) and kidney failure. HUS can also have neurological effects and cause seizures, stroke and coma (WHO 2011). Approximately 6.3% of STEC infected individuals develop HUS, with a fatality rate of 4.6%. Children are more susceptible, with 15.3% of children under five years of age developing HUS following STEC infection (Gould et al. 2009).

STEC are shed in the faeces of infected individuals for several weeks. In children the median shedding time is 13 days (range of 2–62 days) for individuals with diarrhoea. In people who develop HUS, the median shedding time is 21 days (range 5–124 days) (Meng and Schroeder 2007; Pennington 2010).

Virulence and infectivity

STEC strains produce two types of Shiga toxins (Stx1 and Stx2). Stx1 is virtually identical to the toxin produced by *Shigella dysenteriare* serotype 1. The presence of Stx2 is significantly associated with human disease (Spears et al. 2006). Stx are toxic to Vero cells (African green monkey kidney cells) and so are also known as verotoxins (VT). The term STEC is used interchangeably with VTEC. In the laboratory, Vero cells can be used to detect Stx activity, as Stx causes Vero cell death (Desmarchelier and Fegan 2003; Meng and Schroeder 2007).

Due to the acid resistance of STEC, when ingested it is able to survive in the stomach environment and attach to the cells of the intestine. Some STEC strains form a characteristic attaching and effacing lesion on the intestinal cells. The presence of these lesions is a risk factor for the development of HUS (Gyles 2007). Stx produced by STEC is able to bind to specific receptors on susceptible host cells, resulting in the death of these cells. Vascular endothelial cells are a primary target for Stx. Hence production of sufficient Stx results in damage to the blood vessels in the colon and subsequent bloody diarrhoea. If sufficient Stx is taken up by the blood and circulated

through the body, this can lead to impaired kidney and neurological function and the development of HUS (Desmarchelier and Fegan 2003; Gyles 2007).

Mode of transmission

STEC are transmitted by the faecal-oral route by either consumption of contaminated food or water, from direct contact with infected animals or via person-to-person contact. It is estimated that 85% of STEC infections are transmitted by food (Meng and Schroeder 2007; Gyles 2007).

Incidence of illness and outbreak data

Infection with STEC is a notifiable disease in all Australian states and territories. The incidence of STEC infections notified in Australia in 2012 was 0.5 cases per 100,000 population (112 cases), which includes both foodborne and non-foodborne cases. This is the same as the previous 5 year mean of 0.5 cases per 100,000 population per year (ranging from 0.4–0.6 cases per 100,000 population per year) (NNDSS 2013). *E. coli* O157 was the most common STEC identified in Australia in 2010 (58.8% of cases), the next most common was *E. coli* O111. There was 1 case of STEC-associated HUS reported in Australia in 2010 (OzFoodNet 2012). Notified cases of STEC infection are influenced by different jurisdictional practices. South Australia routinely tests all bloody stools for STEC via PCR and subsequently they have the highest notification rate in the country (2.8 cases per 100,000 population compared to 0.0–1.4 cases per 100,000 population for the other jurisdictions in 2012) (OzFoodNet 2012; NNDSS 2013).

The notification rate for STEC in New Zealand in 2011 was 3.5 cases per 100,000 population (154 cases). This was a slight increase from the 2010 rate of 3.2 cases per 100,000 population (Lim et al. 2012).

In the United States (US) the notification rate for STEC in 2010 was 1.78 cases per 100,000 population. This was a slight increase from the 2009 rate of 1.53 cases per 100,000 population (CDC 2012). In the European Union there were 1.93 cases of STEC infection per 100,000 population in 2011 (ranging from 0–6.80 cases per 100,000 population between countries). This was a 159.4% increase in the number of cases from 2010 due to the *E. coli* O104:H4 outbreak that affected nearly 4,000 people (EFSA 2013).

The incidence of STEC infections has a seasonal association, with the number of cases increasing during the warmer months. In Australia STEC is most prevalent from November to April (OzFoodNet 2010).

Foods associated with outbreaks of STEC include undercooked ground beef, fresh produce, unpasteurised juices, salami, cheese and raw (unpasteurised) milk (Yoon and Hovde 2008; FDA 2012) (refer to Table 2).

Table 2: Selected major foodborne outbreaks associated with STEC (>50 cases and/or ≥1 fatality)

Year	Strain	Total no. cases (fatalities)	Food	Country	Comments	References
2011	O104:H4	3842 (53)	Fenugreek sprouts	Germany	Imported fenugreek seeds likely source of STEC contamination. Fenugreek sprout related STEC illness in France linked to outbreak (same sprout seeds)	(EFSA 2011; Robert Koch Institut 2011)
2009	O157:H7	80	Raw pre-packaged cookie dough	US	STEC isolated from sample of cookie dough at the factory, however, it was different to the outbreak strain	(CDC 2009)
2006	O157:H7	205 (3)	Pre-packaged spinach	US	STEC isolated from river, cattle and wild pig faeces near spinach field	(California Food Emergency Response Team 2007)
1996-1997	O157:H7	490 (20)	Cooked meat products	Scotland	Either inadequate cooking or cross-contamination from raw meat to cooked products	(Bell and Kyriakides 1998)
1996	O157:H7	7966 (3)	White radish sprouts	Japan	Suspected product supplied in school meals	(Montville and Matthews 2005)
1995	O111:H⁻	161 (1)	Uncooked fermented mettwurst	Australia	No starter culture used, pH drop during fermentation and water activity during drying not monitored. Product released before maturation was completed	(Chivell 1995)
1993	O157:H7	731 (4)	Hamburgers	US	Insufficient cooking of hamburgers	(Meng et al. 2007)

Occurrence in food

The major animal reservoir of STEC is ruminants, in particular cattle and sheep (Gyles 2007). Individual animals can carry more than one serotype of STEC (Barlow and Mellor 2010). Meat derived from these animals may become contaminated with STEC organisms if the meat is exposed to faecal material during processing. A study of faecal samples from Australian beef cattle showed 10% of samples (n=300) were STEC positive, with *E. coli* O157 isolated from 1.7% of all samples (Barlow and Mellor 2010). Barlow et al. (2006) isolated STEC from 16% of ground beef (n=285) and 40% of lamb cuts (n=275) sampled in Australia, although the serotypes isolated were not associated with reported human cases in Australia. The detection of STEC at a substantially higher rate in lamb is consistent with the higher concentration and prevalence of *E. coli* on sheep carcasses compared to beef carcasses (Phillips et al. 2001a; Phillips et al. 2001b). The reported prevalence of STEC in bulk tank milk internationally is 0–33.5% (FSANZ 2009).

STEC outbreaks have occurred due to the consumption of fruits and vegetables. Fresh produce may be contaminated due to irrigation with contaminated water or the use of soil treated with farm effluent (Fremaux et al. 2008). The presence of STEC on seafood and poultry at retail may be due to cross-contamination or harvesting seafood from contaminated waters (Desmarchelier and Fegan 2003). STEC has been found to survive for months

in soil and manure. It can survive for long periods of time in water and has been isolated from ponds, streams, wells and water troughs. Waterborne transmission of STEC has been reported, both from contaminated drinking water and from recreational water (e.g. swimming) (Fremaux et al. 2008; WHO 2011).

Host factors that influence disease

People of all ages are susceptible to infection with STEC. However, the young and the elderly are more susceptible and are more likely to develop more serious symptoms (FDA 2012).

Dose response

The dose response relationship for STEC is complicated by the number of serotypes and the association of STEC with a variety of foods. The infective dose of *E. coli* O157:H7 is estimated to be very low, in the range of 10–100 cells. The infective dose of other STEC serotypes is suspected to be slightly higher (FDA 2012).

Dose response models have been developed for *E. coli* O157:H7. Teunis et al. (2004) used data from an *E. coli* O157:H7 outbreak at a school in Japan to estimate the dose required to cause disease. In children the estimated ingested dose was 31 organisms, with 25% of exposed children becoming ill. In adults the estimated ingested dose was 35 organisms, with 16% of exposed adults becoming ill.

Haas et al. (2000) used data from a prior animal study undertaken by Pai et al. (1986) and validated their model by comparison with two human outbreaks, one foodborne and the other waterborne, that occurred in the US. This model estimated that the dose required for 50% of the exposed population to become ill was 5.9×10^5 organisms. The corresponding probability of illness for the ingestion of 100 organisms was 2.6×10^{-4}.

Human feeding trial data has been used to generate a dose response model for *E. coli* serotypes other than *E. coli* O157:H7 (*E. coli* O111 and O55) (Haas et al. 2000). The model estimated the dose required for 50% of the exposed population to become ill was 2.55×10^6 and the probability of illness for ingestion of 100 organisms was 3.5×10^{-4}.

Recommended reading and useful links

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

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Shigella species

Shigella spp. are bacteria that cause shigellosis, also known as bacillary dysentery. They are a highly infectious organism, with foodborne outbreaks often involving infected food handlers. Unlike other common foodborne pathogens, humans are the only natural hosts of *Shigella* spp.

Description of the organism

Shigella spp. are Gram-negative, non-spore forming rod-shaped bacteria and are members of the family Enterobacteriaceae. The genus *Shigella* is divided into four species based on their O antigen type and biochemical characteristics: *S. dysenteriae* (comprising 15 serotypes), *S. flexneri* (comprising 14 serotypes), *S. boydii* (comprising 20 serotypes) and *S. sonnei* (1 serotype) (Lampel and Maurelli 2003; Levine et al. 2007).

The most severe form of shigellosis is caused by *S. dysenteriae* serotype 1. *S. sonnei* causes the mildest form of disease, while *S. flexneri* and *S. boydii* can cause either severe or mild illness (FDA 2012). In Australia, *S. sonnei* was the most frequently reported species in 2010, representing 55.6% of all notified *Shigella* infections (OzFoodNet 2012). *S. dysenteriae* serotype 1 is very rare in Australia, with all reported cases acquired overseas (Lightfoot 2003).

Growth and survival characteristics

The growth and survival of *Shigella* spp. in foods is influenced by a number of factors such as temperature, pH, salt content and the presence of preservatives (refer to Table 1). For example, survival of *S. flexneri* has been shown to increase with: decreasing temperature, increasing pH, and decreasing NaCl concentration (Zaika and Phillips 2005).

The temperature range for growth of *Shigella* spp. is 6–8 to 45–47°C (ICMSF 1996). Rapid inactivation occurs at temperatures around 65°C. In contrast, under frozen (-20°C) or refrigerated (4°C) conditions *Shigella* spp. can survive for extended periods of time (Lightfoot 2003; Warren et al. 2006).

Shigella spp. grow in a pH range of 5–9 (ICMSF 1996). Zaika (2001) demonstrated that *S. flexneri* is tolerant to acid and can survive at pH 4 for 5 days in broth when incubated at 28°C. *Shigella* spp. are better able to survive lower pH conditions at reduced temperatures, with *S. flexneri* and *S. sonnei* able to survive for 14 days in tomato juice (pH 3.9–4.1) and apple juice (pH 3.3–3.4) stored at 7°C (Bagamboula et al. 2002).

S. flexneri is salt tolerant and is able to grow in media containing 7% NaCl at 28°C (Zaika 2002a). It is sensitive to organic acids typically used to preserve food. For example, lactic acid has been demonstrated to be effective at inhibiting *S. flexneri* growth, followed in order by acetic acid, citric acid, malic acid and tartaric acid (Zaika 2002b).

Shigella spp. have been shown to survive on various surfaces. *S. sonnei* has been isolated and cultured from fingers several hours after hand contamination (Christie 1968). A study by Nakamura (1962) demonstrated that *S. sonnei* was able to survive on cotton, glass, wood, paper and metal with survival times ranging from 2 days on metal to 28 days on paper at 15°C. *S. dysenteriae* serotype 1 has also been shown to survive on surfaces including plastic, glass, aluminium, wood and cloth (Islam et al. 2001).

S. sonnei, *S. flexneri* and *S. dysenteriae* serotype 1 can take on a viable but non-culturable (VBNC) state when exposed to various environmental conditions. These VBNC cells are able to survive in a dormant state while culturable cells die off (Colwell et al. 1985; Islam et al. 2001). A study by Nicolo et al. (2011) demonstrated

that *S. flexneri* lost culturability when inoculated into grapefruit juice, however, when the VBNC *S. flexneri* was inoculated into resuscitating media it was able to grow again. As the VBNC cells are potentially still virulent and able to be resuscitated, they may be involved in shigellosis transmission (Colwell et al. 1985; Islam et al. 2001; Nicolo et al. 2011).

Table 1: Limits for growth of *Shigella* spp. when other conditions are near optimum (ICMSF 1996; Lightfoot 2003)

	Minimum	Optimum	Maximum
Temperature (°C)	6–8	-	45–47
pH	5	6–8	9
NaCl (%)	-	-	4–5

Symptoms of disease

The clinical symptoms of shigellosis range from mild diarrhoea to severe dysentery, depending on the *Shigella* serotype causing infection, dose and the immunity and age of the host. The incubation period is 1–7 days (usually 3 days) and symptoms typically last for 1–2 weeks (Lampel and Maurelli 2007). Initial symptoms include watery diarrhoea, fever and fatigue. In more severe cases, as is the case for *S. dysenteriae* serotype 1 infection, patients can develop dysentery (characterised by frequent, painful stools containing blood and mucus), abdominal cramps, nausea and vomiting (Niyogi 2005; Nygren et al. 2012). All *Shigella* spp. can cause acute bloody diarrhoea (FDA 2012).

For most *Shigella* serotypes illness is generally self-limiting and fatality is very rare, however, the fatality rate for *S. dysenteriae* serotype 1 can be as high as 20% (Lampel and Maurelli 2003). Cases may develop long-term sequelae such as Reiter's syndrome (reactive arthritis) following *S. flexneri* infection, and haemolytic uremic syndrome following *S. dysenteriae* serotype 1 infection (Warren et al. 2006).

Shigella spp. are shed in large numbers (10^3–10^9 cfu/g of stool) during the acute phase of infection and to a lesser extent (10^2–10^3 cfu/g of stool) in convalescing patients. Adults who live in areas where shigellosis is endemic may become asymptomatic carriers (continue to shed the bacteria but show no sign of infection) (Lampel and Maurelli 2003).

Virulence and infectivity

Once ingested, *Shigella* spp. must survive the acidic environment of the stomach and invade the epithelial cells of the colon to enable infection. *Shigella* spp. multiply inside the colonic epithelial cells and spread to adjacent cells, leading to the death of the infected cells. The colon becomes inflamed and ulcerated and the dead mucoid cells are shed, resulting in the bloody mucoid diarrhoea often characteristic of *Shigella* infection (Lightfoot 2003; Montville and Matthews 2005; Warren et al. 2006).

Shigella spp. have a virulence plasmid that encodes genes involved in the invasion process and intra- and inter-cellular spread. Other genes involved in the invasion process are located on the chromosome (Warren et al. 2006). *S. flexneri* 2a produce the chromosome encoded *shigella* enterotoxin 1, while most *Shigella* serotypes produce the virulence plasmid encoded *shigella* enterotoxin 2. *S. dysenteriae* serotype 1 strains produce the

potent Shiga toxin. Shiga toxin is chromosomally encoded and has cytotoxic, enterotoxic and neurotoxic effects (Niyogi 2005; Warren et al. 2006).

Mode of transmission

Shigella spp. are transmitted by the faecal-oral route by either person-to-person contact, or consumption of contaminated food or water (Nygren et al. 2012).

Nygren et al. (2012) analysed 120 reported foodborne shigellosis outbreaks in the United States (US) between 1998–2008. The contributing factors identified in these outbreaks included infected food handlers (58%), bare-handed contact of the food handler with ready-to-eat food (38%), inadequate cold-holding temperatures (15%), and inadequate cleaning of food preparation equipment (15%). It should be noted that more than one factor can be involved in an outbreak.

Contaminated water is another vehicle for transmission of *Shigella* spp. This can occur due to inadequately treated contaminated water being used for drinking and food preparation, seepage of sewage through the earth, or faecal contamination of recreational water (Lightfoot 2003).

Incidence of illness and outbreak data

Shigellosis is a notifiable disease in all Australian states and territories. The incidence of shigellosis in Australia in 2012 was 2.4 cases per 100,000 population (549 cases), which includes both foodborne and non-foodborne cases. This was a decrease from the previous 5 year mean of 2.8 cases per 100,000 population per year (ranging from 2.2–3.9 cases per 100,000 population per year) (NNDSS 2013).

The Northern Territory had the highest notification rate in 2012 with 46.9 cases per 100,000 population (NNDSS 2013). This was a significant reduction from the 2005–2009 average annual notification rate of 70.1 cases per 100,000 population. The decline in cases may be attributed to a marketing campaign to raise awareness about the importance of hand washing implemented in 2007/2008 targeting both Indigenous and non-Indigenous people, including remote communities (OzFoodNet 2012).

Children between 0–4 years had the highest notification rate in 2010, with 7.5 and 8.3 notifications per 100,000 population for males and females, respectively (OzFoodNet 2012). The higher rate of notified cases in this age group could be due to increased susceptibility or may be the result of other factors such as reduced personal hygiene practices, an increased likelihood of exposure and increased likelihood to seek medical care.

The notification rate for shigellosis in New Zealand in 2011 was 2.3 cases per 100,000 population (101 cases). This was similar to the 2010 rate of 2.4 cases per 100,000 population (Lim et al. 2012).

In the US, 4.82 cases of shigellosis were notified per 100,000 population in 2010. This was a slight decrease from the 2009 rate of 5.24 cases per 100,000 population (CDC 2012). In the European Union there was three strong evidence foodborne outbreaks of shigellosis in 2011 and one outbreak reported in 2010 (EFSA 2012; EFSA 2013).

Foods generally associated with outbreaks of *Shigella* spp. are those that are consumed raw or ready-to-eat foods that have substantial handling during production, such as salads (refer to Table 2).

Table 2: Selected major foodborne outbreaks associated with *Shigella* spp. (>50 cases and/or ≥1 fatality)

Year	Strain	Total no. cases	Food	Country	Comments	Reference
2007	*S. sonnei*	270	Baby corn	Australia and Denmark	Corn from a common packing shed in Thailand, sub-hygienic practices at collection houses and packing shed	(Lewis et al. 2009)
2004	*S. sonnei* 1	63	Raw carrot	US	Food hygiene deficiencies of caterer, chlorine vegetable sanitiser malfunctioning	(Gaynor et al. 2009)
2001	*S. flexneri* 2a	886	Tomatoes	US	Contamination likely to have occurred during hand sorting of tomatoes	(Reller et al. 2006)
2000	*S. sonnei*	406	Commercially prepared dip	US	Contamination thought to be from infected employee at the production facility	(Kimura et al. 2004)
1998	*S. sonnei*	486	Parsley	US and Canada	Most parsley sourced from a common farm in Mexico which used inadequately chlorinated water that was vulnerable to contamination	(Naimi et al. 2003)
1995–1996	*S. sonnei*	279	Fresh cheese made from pasteurised milk	Spain	Infected food handler at the cheese factory, unhygienic practices at the factory	(Garcia-Fulgueiras et al. 2001)
1988	*S. sonnei*	3175 (estimated)	Uncooked tofu salad	US	Food handlers recently had shigellosis	(Lee et al. 1991)

Occurrence in food

There is very little published surveillance data on the presence of *Shigella* in food. Some international surveys have been performed in which *Shigella* spp. have been found in a range in foods. For example, Ghosh et al. (2007) isolated *Shigella* spp. from 15% of coconut slices (n=150), 9% of ready-to-eat salads (n=150) and 7% of samples of coriander sauces (n=150) from Indian street vendors. *Shigella* spp. have also been detected in 11% of raw meat samples (n=250) from retail outlets in Pakistan (Hassan Ali et al. 2010). In Mexico, *Shigella* spp. have been isolated from 6% of freshly squeezed orange juice samples (n=100) and from the surface of 17% of oranges sampled (n=75). All four *Shigella* spp. were isolated from the surface of the oranges, whereas only *S. sonnei* and *S. dysenteriae* were isolated from the orange juice samples (Castillo et al. 2006).

Although *Shigella* can be isolated from a range of food, outbreaks often occur due to an infected food handler contaminating food that is served cold or raw. A study of foodborne shigellosis outbreaks in the US demonstrated that 20% of outbreaks were due to exclusively raw food (e.g. lettuce based salads) and 30% of outbreaks were from partially raw food (e.g. potato salad) (Nygren et al. 2012).

Host factors that influence disease

People of all ages are susceptible to *Shigella* spp. infection. However, infants, the elderly and immunocompromised individuals are most at risk (FDA 2012).

Protective immunity against *Shigella* infection can occur as a result of repeated exposure to the organism (Barnoy et al. 2010). A study by Ferreccio et al. (1991) tracked shigellosis in a cohort of children in Chile over 30 months. A previous case of shigellosis was found to confer 72% protection against illness with the same *Shigella* serotype. However, prior infection did not protect against illness due to other *Shigella* serotypes. This serotype-specific immunity is mediated, at least in part, by antibodies directed at the O antigen of the lipopolysaccharide that forms part of the bacterial cell wall. As the O antigen varies between serotypes, the immunity is serotype-specific (Levine et al. 2007; Kweon 2008).

Research into candidate vaccines against shigellosis has been performed for many years. Various live attenuated *S. flexneri* 2a vaccines have been trialled in animals and humans, and whilst shown to protect vaccinated individuals from *S. flexneri* 2a infection, immunity appears to be serotype-specific (Mel et al. 1965; Coster et al. 1999; Ranallo et al. 2012). Non-replicating vaccines including inactivated whole cell and subunit vaccines have also been trialled (Kaminski and Oaks 2009). A *S. sonnei* conjugate vaccine provided significant protection against shigellosis in the field; however it was only effective against *S. sonnei* infection (Cohen et al. 1997).

An experimental trivalent vaccine has been constructed which expressed the O antigens of *S. flexneri* 2a and *S. sonnei* and the Vibrio cholera toxin B subunit antigen. The trivalent vaccine was able to protect mice and rhesus monkeys from infection with *S. flexneri* 2a and *S. sonnei* (Wang et al. 2002). A pentavalent vaccine has been proposed consisting of *S. flexneri* 2a and 3a (cross-protection between most *S. flexneri* serotypes has been achieved in guinea pigs due to a common O antigen carbohydrate backbone), *S. flexneri* 6 (which does not cross-react with the other *S. flexneri* serotypes), *S. dysenteriae* serotype 1 and *S. sonnei*. Hypothetically, this could protect against the majority of the causes of shigellosis in the world (Noreiga et al. 1999; Levine et al. 2007).

Dose response

Very little data is available on the dose-response relationship for *Shigella* spp. During the 1960s and 1970s, human feeding trials using strains of *S. dysenteriae* serotype 1, *S. flexneri*, and *S. sonnei* were performed to determine the dose required to cause shigellosis. The dose response varied between strains; illness was caused by *S. dysenteriae* serotype 1, *S. flexneri*, or *S. sonnei* with ingestion of 10, 100 and 500 organisms, respectively (DuPont et al. 1989).

Recommended reading and useful links

FDA (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 25–28.
http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

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Staphylococcus aureus

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans.

Description of the organism

S. aureus is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus. The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* produces staphylococcal enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning (Montville and Matthews 2008; FDA 2012). *S. intermedius*, a *Staphylococcus* species which is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning (Talan et al. 1989; Khambaty et al. 1994; Le Loir et al. 2003).

Growth and survival characteristics

The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity (a_w), pH, the presence of oxygen and composition of the food (refer to Table 1). These physical growth parameters vary for different *S. aureus* strains (Stewart 2003).

The temperature range for growth of *S. aureus* is 7–48°C, with an optimum of 37°C. *S. aureus* is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C. *S. aureus* is readily killed during pasteurisation or cooking. Growth of *S. aureus* occurs over the pH range of 4.0–10.0, with an optimum of 6–7 (ICMSF 1996; Stewart 2003).

S. aureus is uniquely resistant to adverse conditions such as low a_w, high salt content and osmotic stress. In response to low a_w, several compounds accumulate in the bacterial cell, which lowers the intracellular a_w to match the external a_w (Montville and Matthews 2008). As such, most *S. aureus* strains can grow over a a_w range of 0.83 to >0.99 (FDA 2012). *S. aureus* is a poor competitor, but its ability to grow under osmotic and pH stress means that it is capable of thriving in a wide variety of foods, including cured meats that do not support the growth of other foodborne pathogens (Montville and Matthews 2008).

S. aureus is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart 2003).

For a non-sporing mesophilic bacterium, *S. aureus* has a relatively high heat resistance (Stewart 2003). The observed average decimal reduction value (D-value, the value at which the initial concentration of bacterial cells would be reduced by 1 $\log_{10}$ unit) was 4.8–6.6 min at 60°C when heated in broth (Kennedy et al. 2005). The bacteria has a higher heat resistance when it is encapsulated in oil, with a D-value at 60°C of 20.5 min for *S. aureus* in fish and oil (Gaze 1985). An extremely heat resistant strain of *S. aureus* (D-value at 60°C of >15 min in broth) has been recovered from a foodborne outbreak in India (Nema et al. 2007).

Several chemical preservatives, including sorbates and benzoates, inhibit the growth of *S. aureus*. The effectiveness of these preservatives increases as the pH is reduced. Methyl and propyl parabens are also effective (Stewart 2003; Davidson and Taylor 2007).

Table 1: Limits for growth of *S. aureus* and enterotoxin production when other conditions are near optimum (ICMSF 1996)

	Bacterial Growth		Enterotoxin Production	
	Optimum	Range	Optimum	Range
Temperature (°C)	37	7–48	40–45	10–48
pH	6–7	4–10	7–8	4.5–9.6
Water activity	0.98	0.83–>0.99	0.98	0.87–>0.99

Symptoms of disease

Staphylococcal food poisoning symptoms generally have a rapid onset, appearing around 3 hours after ingestion (range 1–6 hours). Common symptoms include nausea, vomiting, abdominal cramps and diarrhoea. Individuals may not demonstrate all the symptoms associated with the illness. In severe cases, headache, muscle cramping and transient changes in blood pressure and pulse rate may occur. Recovery is usually between 1–3 days (Stewart 2003; FDA 2012). Fatalities are rare (0.03% for the general public) but are occasionally reported in young children and the elderly (4.4% fatality rate) (Montville and Matthews 2008).

S. aureus can cause various non-food related health issues such as skin inflammations (e.g. boils and styes), mastitis, respiratory infections, wound sepsis and toxic shock syndrome (Stewart 2003; Montville and Matthews 2008).

Virulence and infectivity

Staphylococcal food poisoning is an intoxication that is caused by the ingestion of food containing pre-formed SE (Argudin et al. 2010). There are several different types of SE; enterotoxin A is most commonly associated with staphylococcal food poisoning. Enterotoxins D, E and H, and to a lesser extent B, G and I, have also been associated with staphylococcal food poisoning (Seo and Bohach 2007; Pinchuk et al. 2010).

SEs are produced during the exponential phase of *S. aureus* growth, with the quantity being strain dependent. Typically, doses of SE that cause illness result when at least $10^5 – 10^8$ cfu/g of *S. aureus* are present (Seo and Bohach 2007; Montville and Matthews 2008). Most genes for SEs are located on mobile elements, such as plasmids or prophages. As such, transfer between strains can occur, modifying the ability of *S. aureus* strains to cause disease and contributing to pathogen evolution (Argudin et al. 2010; Pinchuk et al. 2010).

S. aureus produces SEs within the temperature range of 10–48°C, with an optimum of 40–45°C (refer to Table 1). As the temperature decreases, the level of SE production also decreases. However, SEs remain stable under frozen storage. SEs are extremely resistant to heating and can survive the process used to sterilise low acid canned foods. SE production can occur in a pH range of 4.5–9.6, with an optimum of 7–8. Production of SE can occur in both anaerobic and aerobic environments; however, toxin production is optimum in aerobic conditions (ICMSF 1996; Stewart 2003).

SEs are resistant to the heat and low pH conditions that easily destroy *S. aureus* bacteria. The SEs are also resistant to proteolytic enzymes, hence SEs retain their activity in the gastrointestinal tract after ingestion. SEs range in size from 22–28 kDa and contain a highly flexible disulphide loop at the top of the N-terminal domain that is required for stable conformation and is associated with the ability of the SE to induce vomiting (Argudin et al. 2010).

It has been suggested that SEs stimulate neuroreceptors in the intestinal tract which transmit stimuli to the vomiting centre of the brain via the vagus nerve (Montville and Matthews 2008; Argudin et al. 2010). In addition, SEs are able to penetrate the lining of the gut and stimulate the host immune response. The release of inflammatory mediators, such as histamine, causes vomiting. The host immune response also appears to be responsible for the damage to the gastrointestinal tract associated with SE ingestion, with lesions occurring in the stomach and upper part of the small intestine. Diarrhoea that can be associated with staphylococcal food poisoning may be due to the inhibition of water and electrolyte re-absorption in the small intestine (Argudin et al. 2010).

Mode of transmission

Staphylococcal food poisoning occurs when food is consumed that contains SE produced by *S. aureus*. Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination via direct contact or through respiratory secretions (Argudin et al. 2010).

Incidence of illness and outbreak data

Staphylococcal food poisoning is not a notifiable disease in Australia or New Zealand. There were two reported outbreaks of staphylococcal food poisoning in Australia in 2011 and two outbreaks reported in 2010. In New Zealand there were no outbreaks of staphylococcal food poisoning in 2011 and two outbreaks reported in 2010 (Lim et al. 2012; OzFoodNet 2012a; OzFoodNet 2012b). It is generally recognised that there may be significant under reporting of staphylococcal food poisoning due to the short duration of illness and self-limiting symptoms. In Australia it is estimated that *S. aureus* accounts for 1% of foodborne illness caused by known pathogens (Hall et al. 2005).

In the European Union there were 0.07 reported cases of staphylococcal food poisoning per 100,000 population in 2011 (ranging from <0.01–0.45 per 100,000 population between countries). This was similar to the 2010 rate of 0.06 cases per 100,000 population (EFSA 2012; EFSA 2013).

In the United States (US) the notification rate for vancomycin-intermediate *S. aureus* was 0.04 cases per 100,000 population in 2010, which was as increase from the 2009 rate of 0.03 (CDC 2012). It is estimated that in the US, *S. aureus* accounts for 2.6% of foodborne illness caused by 31 major pathogens (Scallan et al. 2011).

The incidence of staphylococcal food poisoning is seasonal. Most cases occur in the late summer when temperatures are warm and food is stored improperly (Montville and Matthews 2008).

Foods associated with outbreaks of staphylococcal food poisoning include meat and meat products, poultry and egg products, milk and dairy products, salads, cream-filled bakery products and sandwich fillings. Foods that require extensive handling during preparation and are kept above refrigeration temperature (4°C) for extended periods after preparation are often involved in staphylococcal food poisoning (Argudin et al. 2010; FDA 2012) (refer to Table 2). Foods high in starch and protein are believed to favour SE production (Stewart 2003).

Table 2: Selected major outbreaks associated with *S. aureus* (>50 cases and/or ≥1 fatality)

Year	No. of cases (fatalities)	Food	Country	Comments	References
2007	400 (1)	UHT milk	Paraguay	Production line operator identified as source of post-pasteurisation contamination	(Weiler et al. 2011)
2006	113	Chicken and rice	Austria	Kitchen worker positive for *S. aureus*	(Schmid et al. 2007)
2000	13,420	Powdered skim milk	Japan	Production of powdered skim milk stopped midway and delayed for 9 hours due to power cut, this permitted *S. aureus* growth and SE production. SE survived the pasteurisation process that destroyed *S. aureus* bacteria	(Asao et al. 2003)
1998	4000 (16)	Chicken, roast beef, rice and beans	Brazil	Food preparation began over 48 hours before food served, food left at room temperature for one day. All food handlers positive for *S. aureus*	(Do Carmo et al. 2004)
1990	100	Ham	US	Food handler positive for *S. aureus* removed ham casings without gloves. Improper refrigeration, prolonged handling and inadequate reheating of ham	(Richards et al. 1993)
1989	99	Canned mushrooms	US	Mushrooms picked and sorted by hand. Mushrooms stored without refrigeration in plastic bags, this permitted *S. aureus* growth and SE production. Deficiencies in sanitation. Imported product	(Levine et al. 1996)
Mid-1980s	>850	Chocolate milk	US	Milk stored for several hours in inadequately cooled tank prior to pasteurisation, conditions permitted *S. aureus* growth and SE production	(Evenson et al. 1988)

Occurrence in food

Despite *S. aureus* colonising a wide range of animals, people are the main reservoir of food contamination (Montville and Matthews 2008). Prevalence of enterotoxigenic *S. aureus* in food handlers is variable between industries and countries. Prevalence estimates from several small studies range from 2% of food handlers in Italy (n=545) (Talarico et al. 1997), 12% of flight-catering staff in Finland (n=136) (Hatakka et al. 2000), 19% of restaurant workers in Chile (n=102) (Figueroa et al. 2002) to 62% of fish processing factory workers in India (n=87) (Simon and Sanjeev 2007).

The udders and teats of cows are known sources of enterotoxigenic *S. aureus*, and the occurrence of *S. aureus* in unpasteurised milk and cheese is common. The tonsils and skin of pigs, chickens and turkeys often harbour *S. aureus*, and are also potential sources of *S. aureus* contamination (Stewart 2003).

A survey of food from retail markets and dairy farms in Turkey was performed between 2007 and 2008. Enterotoxigenic *S. aureus* was found in 11.3% of meat (n=115), 10.2% of unpasteurised milk (n=303), 8.0% of dairy products (n=452), 3.5% of bakery products (n=141) and 2.3% or ready-to-eat products (n=44) (Aydin et al. 2011).

An Italian survey performed between 2003 and 2005 indicated that 9.2% of dairy products (n=641) and 5.0% of meat products (n=993) were positive for enterotoxigenic *S. aureus* (Normanno et al. 2007). In Japan a retail survey performed between 2002 and 2003 found 17.6% of raw chicken meat (n=444) were positive for enterotoxigenic *S. aureus* (Kitai et al. 2005).

Host factors that influence disease

All people are believed to be susceptible to staphylococcal food poisoning. However, the severity of symptoms may vary depending on the amount of SE consumed in the food and the general health of individuals. The young and elderly are more likely to develop more serious symptoms (FDA 2012).

Dose response

A human feeding trial in the 1960s demonstrated that 20–25 µg (0.4 µg/kg body weight) of enterotoxin B caused illness (Raj and Bergdoll 1969). However, more recently it has been reported that less than 1.0 µg of SE is sufficient to cause staphylococcal food poisoning (FDA 2012). In fact evidence from outbreaks indicates that ingestion of less than 200 ng of enterotoxin A is sufficient to cause illness in susceptible individuals (Evenson et al. 1988; Asao et al. 2003).

Recommended reading and useful links

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Toxoplasma gondii

Toxoplasma gondii is a protozoan parasite that causes the disease toxoplasmosis. It is a very common parasitic infection in humans and other warm-blooded animals, with approximately a third of the world's human population estimated to have been exposed to the parasite. Toxoplasmosis can be asymptomatic (no clinical symptoms) or can have more severe consequences such as congenital birth defects, eye disease, or potentially fatal toxoplasmic encephalitis in immunocompromised individuals.

Description of the organism

T. gondii is a protozoan parasite which belongs to the phylum *Apicomplexa*, subclass *Coccidiasina* and family *Sarcocystidae* (Hill et al. 2007; Pereira et al. 2010). The infective stages of the parasite can take three different forms – sporozoites, tachyzoites and bradyzoites. Following sporulation in the environment, oocysts containing sporozoites are infective, and give rise to tachyzoites when ingested by an intermediate host. Most species of mammals and birds are susceptible to infection and may serve as an intermediate host (Thompson et al. 2009). Tachyzoites are the rapidly replicating stage of the parasite and are disseminated around the body via the bloodstream and infect a variety of tissues. The rapid replication and release of tachyzoites from host cells causes tissue damage and provokes a strong inflammatory response and therefore is responsible for the clinical manifestations of disease. Bradyzoites are structurally similar to tachyzoites but replicate slowly in tissue cysts that form intracellularly in brain, cardiac and skeletal muscle tissue of the host. Bradyzoites are not responsible for acute clinical disease; and can persist for the life of the host without causing a host inflammatory response (Dubey et al. 1998; Montoya and Liesenfeld 2004).

Growth and survival characteristics

Depending on environmental conditions, the time taken for oocysts to sporulate and become infectious ranges from one day to several weeks. Conditions such as aeration and temperature affect the length of time required for sporulation to occur, with lower temperatures slowing the sporulation rate (Lindsay et al. 2002; Jones et al. 2003; Hill et al. 2007). A study conducted by Lindsay (2002) demonstrated that unsporulated oocysts can survive in the environment at 4°C and retain their ability to sporulate for at least 3 months. Sporulated oocysts are more resilient than unsporulated oocysts. Once sporulated, oocysts maintain infectivity in moist soils for up to 18 months (Frenkel et al. 1975) and in water and seawater for several years at 4°C (Dubey 1998a; Lindsay and Dubey 2009). The duration of infectivity, however, decreases with increasing temperatures. Infectivity is maintained for at least 200 days in the temperature range of 10–25°C, for 1 month at 35°C, for 1 day at 45°C and sporulated oocysts become non-infective after 1 minute at 60°C (Dubey 1998a). Unsporulated oocysts die within 24 hours when stored at 37°C, whereas sporulated oocysts can survive for over a month at 35°C and 9 days at 40°C (Lindsay et al. 2002). Constant freezing at -21°C kills unsporulated and sporulated oocysts within 1 and 28 days, respectively (ESR 2010).

T. gondii tissue cysts remain viable in infected meat stored at refrigeration temperatures of 4°C for up to 19 days. Cooking infected meat to internal temperatures of 67°C or higher inactivates the tissues cysts (Dubey et al. 1990; Dubey 2004). Freezing meat at -10°C for 3 days or -20°C for 2 days or treatment with gamma irradiation at a dose of 75 krad is also sufficient to kill tissue cysts (El-Nawawi et al. 2008). Tachyzoites that may be found in the milk of intermediate hosts are inactivated by pasteurisation (Tenter et al. 2000).

Bradyzoites found in tissue cysts are resistant to gastric digestion, whereas tachyzoites are usually destroyed by the acid and proteolytic enzymes of the stomach (Tenter 2009). Experimental evidence indicates that tachyzoites may survive in acid-pepsin solution for up to 2 hours (Dubey 1998b) and the type of meal eaten may also increase the pH of the stomach and allow tachyzoites to traverse the stomach into the small intestine in an infective state (Tenter 2009).

Symptoms of disease

Most human infections with *T. gondii* are asymptomatic, but infection may result in severe clinical disease and on occasion be fatal. Infection in humans may be acquired postnatally or *in utero* and may result in fetal death, congenital toxoplasmosis, toxoplasmic encephalitis, ocular toxoplasmosis or less severe acute self-limiting disease (Montoya and Liesenfeld 2004).

In healthy adults and children the majority of postnatally acquired infections are asymptomatic with only 10–20% of individuals developing a self-limiting and non-specific illness (Montoya and Liesenfeld 2004; Pereira et al. 2010). Symptoms of disease may include mild, flu-like illness with low grade fever, muscular pain, swollen lymph nodes, lethargy and headache (Abu-Madi et al. 2010; ESR 2010). Enlarged lymph nodes are the most commonly observed clinical manifestation of human toxoplasmosis (Hill and Dubey 2002). The onset of illness is 3–25 days (mean of 11 days) (Hill et al. 2007; Ayi et al. 2009; ESR 2010).

Toxoplasmic retinochoroiditis (inflammation of the retina and choroid) can be associated with congenital or postnatally acquired disease as a result of acute infection or reactivation of a latent infection (Montoya and Liesenfeld 2004). In humans, the parasite multiplies in the retina causing inflammation in the choroid; the parasite does not multiply in the choroid (Dubey et al. 2012). Typical findings of both postnatally acquired and congenital retinochoroiditis include white-appearing lesions with overlying severe inflammation of the viscous fluid at the back of the eye (Montoya and Liesenfeld 2004; Delair et al. 2011). These symptoms occur as a consequence of active retinal lesions, leading to retinal scarring. Toxoplasmic retinochoroiditis is a significant cause of vision loss. The natural course of ocular toxoplasmosis and the long term impact on vision depends on the frequency of recurrences, with retina destruction minimised if active disease is treated early. Recurrence of retinochoroiditis can occur for both postnatally acquired and congenital toxoplasmosis. Severe complications associated with ocular toxoplasmosis may include fibrous bands, retinal detachment, cataracts and inflammation and damage to the optic nerve (Delair et al. 2011). Ocular disease is one of the most important clinical manifestations of acute, postnatally acquired toxoplasmosis, particularly in countries such as Brazil. The majority of cases of ocular toxoplasmosis are postnatally acquired (Dubey et al. 2012).

Congenital toxoplasmosis occurs when a woman becomes infected with *T. gondii* during pregnancy. Tachyzoites circulating in the mother's bloodstream can invade and multiply in the placenta and subsequently infect the foetus. Transmission of the parasite *in utero* can cause congenital defects or spontaneous abortion. These congenital defects can include ocular toxoplasmosis, hydrocephalus (big head), mental retardation and intracranial calcifications (Hill et al. 2007; Zhou et al. 2011). Although the risk of transmission is less common in the first trimester, congenital infections acquired during the first trimester are more severe than those acquired in the second or third trimester of pregnancy (Montoya and Liesenfeld 2004; Hill et al. 2007; Ayi et al. 2009).

In infected immunocompromised individuals, the parasite may be uncontrollably released due to the rupture of tissue cysts in the brain (Feustel et al. 2012). This leads to symptoms that affect the central nervous system, including headache, altered mental status, seizures, hemiparesis (muscle weakness on one side of the body),

ataxia and/or facial weakness. If left untreated; the infection may progress to fatal toxoplasmic encephalitis (Walker and Zunt 2005; Feustel et al. 2012). Immunocompromised individuals are susceptible to toxoplasmic encephalitis from either acquired infection or reactivation of a latent infection, however it is believed the majority of toxoplasmic encephalitis cases are due to the latter (Montoya and Liesenfeld 2004). Reactivation occurs if the bradyzoites are released from the cysts and convert into tachyzoites due to the suppression of the host immune response that previously inhibited parasite activity. This rupture of the cysts in immunocompromised individuals generally occurs in the brain (Feustel et al. 2012). In Australia the rate of hospitalisations due to toxoplasmic encephalitis declined substantially from a peak in 1993 due to prophylactic treatment of human immunodeficiency virus (HIV) patients (Huppatz et al. 2009).

Virulence and infectivity

T. gondii virulence and infectivity are reliant on factors that control parasite-host cell interactions and/or moderate the host immune response (Dubremetz and Lebrun 2012). The population structure of *T. gondii* is comprised of three highly abundant and overrepresented genetic lineages, commonly referred to as genotypes I, II and III, amongst a diverse array of related genotypes (Su et al. 2012). The three clonal lineages are very closely related but the small genetic differences result in distinct phenotypic differences in infectivity and virulence (Sibley and Ajioka 2008).

Most virulence studies have involved genotypes I, II and III and virulence has typically been assessed in a mouse pathogenicity model, with comparatively little known about human infection (Dubremetz and Lebrun 2012). In the mouse model, highly virulent strains are typically genotype I whereas the vast majority of non-virulent strains are genotype II and III (Sibley and Boothroyd 1992). Little is known about atypical or recombinant genotypes (Dubremetz and Lebrun 2012). In humans, the evidence for strain specific virulence is less well studied and relies predominantly on epidemiological evidence. The majority of human cases have been attributed to genotype II (Howe and Sibley 1995) which is likely to be an artefact of an overrepresentation of this genotype in animals in Europe and the United States (US) where most human cases have been documented (Boothroyd and Grigg 2002). The virulent nature of genotype I strains in mice may, however, extend to humans as severe ocular disease in otherwise immunocompetent adults have been attributed to genotype I strains (Boothroyd and Grigg 2002). Furthermore, non-genotype II strains have been associated with more severe disease at birth in congenitally infected newborns in the US (McLeod et al. 2012). More recently, highly virulent atypical genotypes in French Guiana and Brazil have caused severe disease in immunocompromised individuals, foetuses and otherwise healthy individuals (Carme et al. 2009; Dubey et al. 2012). In Australia, genotype II strains have been reported from a human isolate (Sibley and Boothroyd 1992) and a dog isolate (Al-Qassab et al. 2009) and atypical and type II-like strains have been isolated from native Australian wildlife (Parameswaran et al. 2010). Of the few Australian isolates examined thus far, all have been avirulent in mouse bioassays.

Mode of transmission

The principal modes of *T. gondii* transmission are ingestion of faecal oocysts or tissue cysts, and the transplacental transmission of tachyzoites from mother to unborn child. Infection with faecal oocysts may occur by accidentally ingesting contaminated soil (e.g. not washing hands after gardening or eating unwashed fresh produce), drinking untreated contaminated water, eating shellfish grown in contaminated water, or contact with cat faeces (e.g. a cat litter box). Infection from tissue cysts may occur by consuming raw or undercooked meat, by accidentally consuming tissue cysts after handling raw meat and not washing hands thoroughly, or by cross-contamination of food prepared using unwashed utensils and chopping boards that have had contact

with raw meat (Abu-Madi et al. 2010; CDC 2010; Pereira et al. 2010). Oocyst-acquired infections in humans are clinically more severe than tissue cyst-acquired infections (Dubey 2004). As tachyzoites are sensitive to environmental conditions they are usually killed rapidly outside the host and so are rarely involved in foodborne transmission of *T. gondii* (Tenter 2009).

Organ transplant recipients can develop toxoplasmosis due to transmission of the parasite with the transplanted organ from a *Toxoplasma*-seropositive donor to a *Toxoplasma*-seronegative recipient. Heart transplantation is the most common type of organ transplantation procedure when this occurs, as cysts form in the cardiac muscles (Martina et al. 2011; Derouin and Pelloux 2012). However, toxoplasmosis is an uncommon outcome from organ transplantation as only 5% of human pathogenic parasites have reportedly caused significant illness in transplant recipients (Barsoum 2006). It is also possible that parasite transmission could occur as the result of blood transfusion or haematopoietic stem cell transplantation. The chance of either of these occurring is very low and could only occur if the donor was recently infected with *T. gondii* and so had tachyzoites present in their blood and bone marrow (Derouin and Pelloux 2012).

Infection of the feline definitive host occurs when a cat consumes an intermediate host (such as a mouse or bird) infected with tissue cysts. Upon ingestion of a tissue cyst by a susceptible cat, the walls of the cyst are digested by proteolytic enzymes and bradyzoites are released. The bradyzoites undergo asexual reproduction followed by sexual reproduction in intestinal epithelial cells to produce microgametocytes and macrogametocytes. The microgametocytes fertilise the macrogametocytes, leading to the production of zygotes. The zygotes differentiate into unsporulated oocysts and are shed in the faeces of the definitive host (Ortega 2007; Jones and Dubey 2010). After a prepatent period of up to 10 days following primary infection with tissue cysts, a cat may shed more than 100 million oocysts into the environment over a 2-3 week period (Tenter et al. 2000).

Figure 1: Life cycle of *T. gondii* (CDC–DPDx 2009)
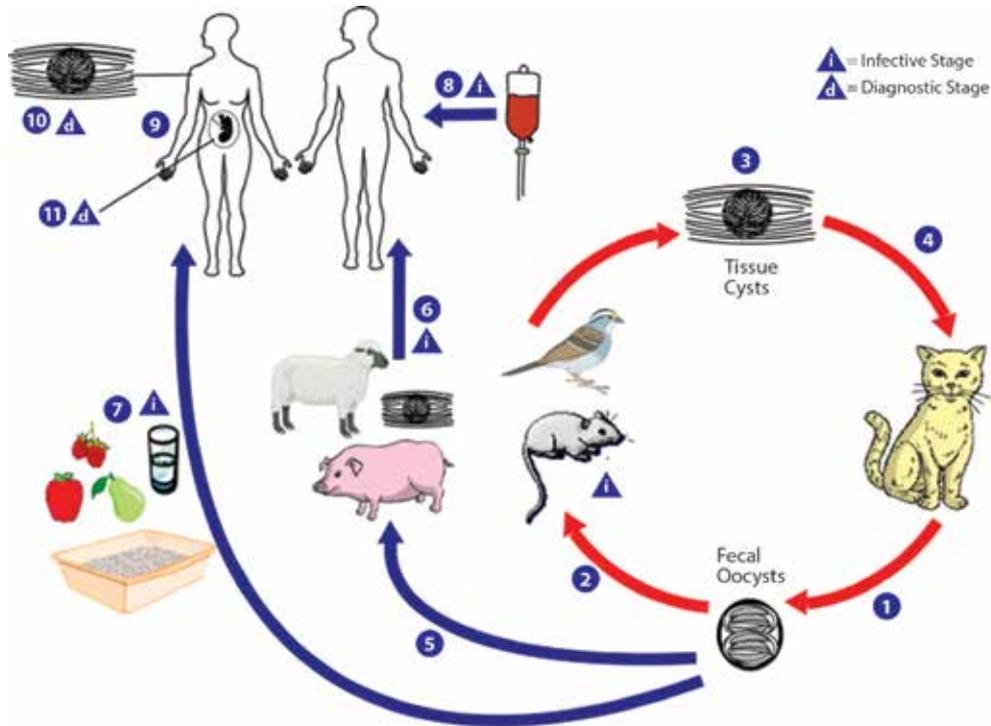
(1)   Unsporulated oocysts are shed in cat's faeces.

(2)   Intermediate hosts in nature (including birds and rodents) become infected after ingesting sporulated oocysts in contaminated soil, water or plant material.

(3)   Oocysts transform into tachyzoites shortly after ingestion. These tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites.

(4)   Cats become infected after consuming intermediate hosts harbouring tissue cysts. Cats may also become infected directly by ingestion of sporulated oocysts.

(5)   Food animals and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment. Humans can become infected by multiple routes:

(6)   eating undercooked meat of animals harbouring tissue cysts;

(7)   consuming oocysts in food or water contaminated with cat faeces or by contaminated environmental samples (such as faecally contaminated soil or changing the cat litter box);

(8)   blood transfusion or organ transplantation; or

(9)   transplacental transmission of tachyzoites from mother to unborn child.

(10) Diagnosis is usually achieved by serology, although tissue cysts may be observed in stained biopsy specimens.

(11) Diagnosis of congenital infections can be achieved by detecting *T. gondii* DNA in amniotic fluid using molecular methods.

(CDC–DPDx 2009)

Incidence of illness and outbreak data

Toxoplasmosis is one of the most common parasitic zoonoses worldwide. It is estimated that around a third of the world's population have the parasite, with the majority of cases being asymptomatic (Pereira et al. 2010; Innes 2010). Despite a large proportion of the population being seropositive for *T. gondii*, scientific literature indicates that the seroprevalence is decreasing in several countries including France, Belgium, the United Kingdom and the US (Rosso et al. 2008).

The incidence and prevalence of toxoplasmosis in Australia is difficult to estimate since toxoplasmosis is not a notifiable disease (DOHA 2005; AWHN 2009) and most *T. gondii* infections are asymptomatic. Reliable estimates of incidence tend to come from high risk groups such as newborn infants. However, not all new cases can be attributed to foodborne exposure during pregnancy since environmental, water and cat exposure also result in transmission to the mother. Similarly, incidence of toxoplasmosis during pregnancy is not necessarily representative of the wider population. In a small study from south eastern Australia, incidence of congenital toxoplasmosis from 2001–2009 was estimated at 0.17 cases per 10,000 live births (Jayamaha et al. 2012).

International estimates of incidence or prevalence at birth tend to be higher than Australia, but caution should be exercised in drawing conclusions since many European countries have prenatal screening programs. Incidence or prevalence at birth of congenitally acquired toxoplasmosis, both reported as cases per 10,000 live births, have been reported for France (2.9/10,000) (Villena et al. 2010), Poland (11/10,000) (Paul et al. 2001), Sweden (0.7/10,000) (Evengard et al. 2001), Denmark (1.6/10,000) (Roser et al. 2010), Brazil (10-13/10,000) (Vasconcelos-Santos et al. 2009; Bichara et al. 2012), Columbia (9.8/10,000) (Gomez-Marin et al. 2011) and Mexico (20/10,000) (Vela-Amieva et al. 2005). In Poland, when only susceptible women (i.e. non-immune mothers) were taken into account, the incidence of congenital toxoplasmosis increased to 20 cases per 10,000 live births (Paul et al. 2001). A study of birth prevalence in non-immune mothers in Western Australia found 2.3 cases per 10,000 live births (Walpole et al. 1991).

It is widely accepted that outbreaks of toxoplasmosis involving more than a single family or small group are rare and infrequently reported (Demar et al. 2007). Water and undercooked meat have been associated in *T. gondii* outbreaks (refer to Table 1).

Table 1: Selected major foodborne outbreaks associated with *T. gondii* (≥5 cases and/or ≥1 fatality)

Year	Total no. cases	No. congenital cases	Food	Country	Comments	Reference
2001-2002	176	0	Water and ice cream	Brazil	Kittens lived on top of the water reservoir tank. Rainfall may have carried oocysts into water reservoir. Ice cream prepared from contaminated water	(de Moura et al. 2006)
1995	5	0	Pork liver	Korea	Consumption of raw pork offal from a domestic pig	(Choi et al. 1997)
1994	13	1	Kangaroo meat	Australia	Consumption of undercooked meat	(Robson et al. 1995)
1993	17	0	Mutton	Brazil	Consumption of raw mutton	(Bonametti et al. 1997)

Occurrence in food

The type of food most often associated with toxoplasmosis is raw or undercooked meat, including lamb, pork, venison, free-range poultry and game meat (Jones et al. 2009; Jones and Dubey 2012; Chumpolbanchorn et al. 2013). Beef consumption is not considered important since cattle are a poor intermediate host (Jones and Dubey 2012). Ingestion of unfiltered water contaminated with *T. gondii* oocysts has also been associated with toxoplasmosis (Jones et al. 2003; Bahia-Oliveira et al. 2003; Pereira et al. 2010). Tachyzoites of *T. gondii* have been found in unpasteurised milk of sheep, goats and cows; however, only consumption of unpasteurised goat's milk has been associated with human toxoplasmosis (Tenter et al. 2000).

Poor hygiene is also a major contributor towards food contamination. Contamination can occur due to a person failing to wash their hands prior to food preparation after contact with plants or soil in the garden, a cat, cat faeces, or the cat litter box. Infrequent washing of kitchen utensils used to prepare raw meat or other contaminated foods also represent a potential cause of food contamination (Jones et al. 2003).

Host factors that influence disease

People most at risk of developing clinical symptoms include immunocompromised individuals, pregnant women who acquire (or have a reactivation of) the infection during gestation, foetuses that are congenitally infected and individuals who have previously been infected *in utero* (Tenter et al. 2000; Jones et al. 2003; Montoya and Liesenfeld 2004; ESR 2010). Factors that increase the risk of acquiring a *T. gondii* infection include owning a pet cat, undercooking meat and maintaining poor personal hygiene.

Dose response

The number of faecal oocysts or tissue cysts required to cause *T. gondii* infection in humans has not been established (ESR 2010). However, using a pig animal model Dubey et al. (1996) demonstrated that as few as one sporulated oocyst was able to cause infection.

Recommended reading and useful links

CDC (2008) Parasites - Toxoplasmosis (*Toxoplasma* infection).
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