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Microbiological Hazards in the Vegetable Industry - a review

Robert Premier and Julia Behrsing

Department of Primary Industries, Victoria

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APPENDIX 2

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A review

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SUMMARY

The purpose of this review was to identify the human microbial pathogens that have been associated with fresh vegetables and which are known to pose a risk or are suspected of doing so. Three classes of organisms were examined, bacteria, parasites and viruses.

Bacterial contamination was identified as originating from 3 major routes, the soil, manure (faecal material) and contaminated water and handling by an infected individual. Organisms which can be found in soil and which have caused disease outbreaks in vegetables include *Bacillus cereus, Clostridium botulinum* and *Listeria monocy to genes.* Contaminants that can be spread from faecal matter and contaminated water and which have been associated with vegetable borne disease outbreaks include *Salmonella* spp., *E. coli* 0157:H7, enterotoxigenic *Escherichia coli* and *Campylobacter* spp. *Yersinia enterocolitica* and *Aeromonas* spp. have been isolated from vegetables but have not been associated with vegetable borne disease outbreaks. Disease outbreaks caused through direct contamination via an infected food handler have been linked to *Shigella* spp. and *Staphylococcus aureus.*

Three main parasites of concern were identified, *Cryptosporidium, Giardia* and *Cyclospora. Cryptosporidium* is ubiquitous in the environment and has been responsible for many large waterborne disease outbreaks. It is possible that it may find it's way onto vegetables via irrigation water or run-off from surface waters. *Giardia* has also been the cause of many waterborne outbreaks and has been linked to foodborne outbreaks. *Cyclospora* is a newly recognised parasite that may contaminate fresh vegetables and was recently responsible for a large outbreak in the U.S. associated with fresh raspberries as well as some smaller outbreaks involving basil and mesclum mix.

Foodborne viral disease is as common in Australia as bacterial disease outbreaks. Those of concern include hepatitis A, enteroviruses, Norwalk-like viruses and small round shaped viruses.

In Australia there is little published information on foodborne outbreaks but a recent review concluded that foodborne disease patterns are similar to those seen in other developed countries.

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1. **INTRODUCTION**

Food safety is becoming an important feature of food production at all levels. Many countries including Australia have began introducing quality assurance programs based on Hazard Analysis Critical Control Points (HACCP). This procedure for quality assurance in the food industry has been adapted by different quality programs and modified to fit the different requirements of horticultural food production. In order to make these programs work there needs to be careful consideration of the possible hazards associated with the consumption of the intended food. Microbiological hazards are a relatively new concept in horticulture and a general lack of information makes this type of hazard difficult to identify and control.

Food poisoning outbreaks involving the consumption of vegetables and fruit are not as common as those caused by the consumption of meat products. Nevertheless there have been a number of well-documented cases where whole or minimally processed vegetables/fruit have been clearly implicated. Furthermore, produce related outbreaks appear to be on the increase (Tauxe et al. 1997). Bacteria, viruses and parasites are all potential contaminants of horticultural produce and their mode of contamination is made possible through the very nature of plant production. Another complicating feature is the microbiology of fruit and vegetables. They have very high natural bacterial populations. It is not uncommon to find total aerobic counts up to 10^8 organisms per gram and the interaction of normal bacteria with human pathogens is still unclear.

It is the object of this review to examine the known microbiological hazards in the horticultural industry. The microbiological hazards examined include bacterial, viral M parasitic.

2. BACTERIOLOGICAL CONTAMINANTS

Bacteria, yeasts and moulds are the predominant microflora found on horticultural produce. Random surveys of Australian produce clearly show that the bacterial load is very high. The type of bacteria present may well constitute innocuous plant bacteria with no implications in human disease. However, disease causing bacteria have been linked to a range of fresh produce. Bacteria can contaminate fresh produce by three primary routes, the soil, contaminated water or manures and direct handling by infectious humans.

2.1 Bacterial contaminants from the soil

Organisms found in soil are renowned for their resilience and longevity. Some are spore forming bacteria, which means that they can survive for many years. Soil borne human pathogens that have been implicated in disease outbreaks associated with the consumption of vegetables include *Bacillus cereus, Clostridium botulinum* and *Listeria monocytogenous.*

2.1.1 Bacillus cereus

A gram positive spore forming bacterium with similarities to the Anthrax disease. The spores of these bacteria can be found universally in soils and can contaminate vegetables. *B. cereus* can grow in the absence and in the presence of oxygen. Upon growth two enterotoxins are produced, one associated with diarrhoea, the other associated with convulsive vomiting (emetic toxin). The former has been linked to toxins produced from growth on vegetables. Growth of this bacteria is limited as most strains cannot grow at temperatures of less than 10 °C and high level toxin production requires temperatures in the mid 20's.

Outbreaks of this type of food poisoning have been documented after consumption of cress, soy and mustard sprouts (Portnoy et al. 1976). In Australia, there were 5 known foodborne outbreaks between 1980-1995 affecting 27 people and in one of them salad and/or vegetables were implicated (Crerar et al. 1996, Table 2). This organism has been shown to be almost universally present in grains including wheat, maize and rice and spices are often contaminated with this organism.

2.7.2 *Clostridium botulinum.*

A gram positive spore forming bacterium responsible for a highly fatal afebrile intoxication. The illness is not due to an infection but is due to the actions of a powerful enterotoxin that is neurotoxic when ingested. Humans are highly susceptible to the actions of this toxin and the symptoms include headaches, intestinal pain, dizziness, weakness, constipation and possible paralyses. Death can occur by cardiac or respiratory paralysis within 3-7 days. The severity of the symptoms is related to the dose of toxin ingested and the body weight. *Clostridium botulinum* spores are found universally in soils and they have been found in a variety of vegetables including asparagus, beans, carrots, celery, com. potatoes, turnips, frozen spinach, mushrooms, onion and garlic skins and cabbage (McClure et al. 1994).

C. botulinum has been implicated in a number of outbreaks after the consumption of processed vegetables (Solomon et al. 1990, D'Argenio et al. 1993). Consumption of coleslaw made from shredded cabbage was linked to symptoms of botulism. The cabbage appeared to be packaged in a modified atmosphere (Soloman et al. 1990). Two outbreaks of *C. botulinum* in Italy were associated with the consumption of roasted eggplant in oil (D'Argenio et al. 1993) and outbreaks in the US implicated commercially bottled chopped garlic in soybean oil (De Roever 1998). One outbreak has been reported in Australia but it was not associated with any particular food type (Crerar et al. 1996). The toxin is produced by actively growing cells and thus there needs to be a favourable step for the growth of the bacteria. As spores require anaerobic conditions to actively grow into toxin producing cells, this step can only occur in oxygen depleted storage conditions. Refrigeration is also a good safeguard against this growth step. Petran et al. (1995) reported no growth of spores in cut romaine lettuce or cabbage at 4.4 or 12.7°C after 28 days storage in polyester bags. However, toxin production was found after 7 days and 14 days in non-vented packages of cabbage and lettuce respectively. This demonstrates the importance of ensuring that the modified atmospheres in packaged vegetables such as fresh-cuts do not become anaerobic. The time requirement to produce toxin at 4° C, in good growth media, is about 29 to 58 days (Snyder 1996).

2.1.3 Listeria monocytogenes.

A gram positive to gram variable aerobic and microaerophilic non spore forming bacteria. It is widespread in soils throughout the world. However, it can also be found in a large number of animals and birds including cattle, sheep and poultry

(Beuchat 1996). It causes the disease called listeriosis. Symptoms can be severe and include bacterial meningitis, endocarditis (inflammation of lining membrane of heart), peritonitis (inflammation of membrane lining cavity of abdomen). Perinatal infections can cause abortion or stillbirth. It can affect all age groups but can be fatal in neonates and foetuses leading to extensive warnings to pregnant women as to the danger of this disease. It is also a major problem in immunosuppressed individuals. The prevalence of listeriosis has been increasing worldwide. This increase is due to an increase in the rate of predisposing factors in the community. These predisposing factors include neoplastic disease, drug induced immunosuppression, alcohol abuse, diabetes mellitus, cardio-renal diseases, old age and disease induced immunosuppression i AIDS). Foods have been established as a major vector for the spread of listeriosis and it is associated with a mortality rate of 30% in food outbreak situations.

Some outbreaks of *L. monocytogenes* associated with fresh vegetables have been reported. During the summer of 1979, 23 patients admitted to hospital in Boston had systemic *L. monocytogenes* infection (Ho et al. 1986). It was concluded that raw celery, tomatoes and lettuce contaminated with *L. monocytogenes* may have been responsible, although no attempt was made to isolate *L. monocytogenes* from the vegetables. An epidemic in 1981 involving both adult (7 cases) and perinatal (34 cases) infection, was linked to raw cabbage in coleslaw (Schlech et al. 1983). An investigation into the sources of the cabbage found that one farmer had used composted and fresh sheep Manure. Two of his sheep had died of listeriosis in 1979 and 1981. Crerar et al. (1996) reported two outbreaks which occurred in Australia, affecting 13 people, one of which was linked to seafood and in the other outbreak the food vehicle was unknown. Recently six people died from eating fruit salad contaminated with *L. monocytogenes* in NSW, three of whom were immunocompromised (Australian Associated Press, 1999).

A number of studies have examined various fresh vegetables for the presence of L. *monocytogenes*. Sizmur and Walker (1988) looked at 60 samples of prepacked salads purchased from two leading supermarkets in the UK. Four samples, representing 2 salad varieties were found to contain *L. monocytogenes.* One of the salads contained cabbage, celery, sultanas, onion and carrots whilst the other one consisted of lettuce, cucumber, radish, fennel, watercress and leeks. Heisick et al. (1989) conducted 1000 tests on 10 types of fresh produce from two supermarkets in the Minneapolis area in the USA. *L. tmonocytogenes* was isolated from cabbage, potatoes, cucumbers and radishes, although only potatoes and radishes contained significant amounts. Other studies have isolated *L. monocytogenes* from bean sprouts, leafy vegetables, prepacked salads, salad vegetables and tomatoes in Malaysia, the USA, Germany, Ireland and Pakistan (Beuchat 1996) and leeks, potatoes, lettuce, celery and cabbage in Spain (de Simon et al. 1992). A study of food in Taiwan detected *L. monocytogenes* in 12% of vegetable samples tested (Wong *et al.* 1990). *L. monocytogenes* has also been isolated from frozen products including vegetable soup, asparagus and cultivated mushrooms (Gola et al. 1990). A study was conducted in NSW during 1988 to 1993 on different types of foods including 54 ready-to-eat vegetable and salad samples (Arnold and Coble 1995). Only 1 sample was found to be positive for *L. monocytogenes.* Carrots have been demonstrated to be negative for this organism. They produce an unknown factor or factors, which inhibit *Listeria* growth (Beuchat 1990b). Other studies have reported on the effect of controlled atmosphere (CA) storage on the fate of *L. monocytogenes* inoculated onto lettuce, cabbage and tomatoes (Steinbruegge et al. 1988, Beuchat and Brackett 1990a, Beuchat an*d* Bracken 1991, Kallander et al. 1991). Berrang et al. (1989a) found CA storage to increase the storage life of asparagus, broccoli and cauliflower, whilst having no effect on the growth of *L. monocytogenes* which grew as well in air as in CAS both at 4 and 15°C. However, the atmosphere used (10 % $C0_2$,11 % 0_2) would not be biostatic towards *L. monocytogenes.* Kallander et al. (1991) employed an atmosphere of 70 % $C0_2$, 30 % N_2. The increased level of $C0_2$ was ineffective in controlling L. *monocytogenes* at 5 °C. Lettuce subjected to an atmosphere of 3 % 0_2,97 % N_2 at 5 and 10 °C also supported the growth of L. *monocytogenes.* The generation time at 5° C is approximately 40 hours and at 10° C it is approximately 22 hours (Snyder 1996).

2.2 Bacterial contaminants from faeces and contaminated water

There are a large number of contaminants that can be spread from faecal matter or contaminated water onto vegetables. The microorganisms reviewed here are those which have been previously implicated in disease following the consumption of vegetables. Contamination can occur from contaminated irrigation water, the application of fertilisers containing animal or human wastes that have not been composted or treated properly or from faeces of feral or domestic animals. Postharvest contamination can occur during processing by use of contaminated water or ice, from the presence of vermin in the processing environment or during transportation from contaminated trucks. *Listeria*, covered in the previous section, can also be spread from human and animal manure and it is commonly found in sheep manure.

2.2.1 Salmonella spp.

Bacteria of this genus consist of gram negative non-spore forming mesophilic bacteria. They are widely distributed in nature and there are over 2300 serotypes. Animals (including humans) and birds are the natural reservoirs. Symptomatic and asymptomatic carriers spread the bacteria primarily from the faecal route. In the vegetable industry contamination can occur from manure (eg poultry manure) and from handling by carriers during harvesting, processing and packing. Normally it is thought that large numbers of cells need to be ingested to cause illness. Symptoms usually develop between 12-36 hours after ingestion and include nausea, vomiting, diarrhoea, abdominal pain, fever, headaches and weakness.
Salmonella species have been isolated from a number of vegetables (Ercolini 1976, Tamminga et al. 1978, Rude et al. 1984, Garcia-Vullanova Ruiz et al. 1987, Arumuyaswamy et al. 1995). These include artichokes, cabbage, celery, chilli, fennel, lettuce, cauliflower, eggplant, carrots, spinach, leafy vegetables, bean sprouts and peppers. The study by Garcia-Vullanova Ruiz et al. (1987) showed a close correlation between isolates found in irrigation waters and those found on the vegetables. The contamination of vegetables with *Salmonella* is probably more widespread than believed. Studies in Italy have shown that 68% of 120 random samples of lettuce and 72% of 89 random samples of fennel (all from retail outlets) contained *Salmonella* (Ercolani 1976). A survey of fresh and salad vegetables in wholesale and retail markets in the US revealed that 4 of 50 samples (8%) contained *Salmonella* spp. (Rude *et al.* 1984). Monitoring of international airline foods in Australia showed high levels of *Salmonella* spp. in vegetable and fruit salads (Fain 1996).

There have been a number of outbreaks associated with beansprouts (Puohiniemi et al. 1997), sliced raw tomatoes (Fain 1996), mustard cress (Beuchat 1995), alfalfa sprouts (Tauxe et al.1997), fresh tomatoes (De Roever 1998) and cantaloupes (Tamplin 1997). Studies have shown that *Salmonella* spp. are able to grow on the surface of intact tomatoes at ambient temperature and growth can be rapid in chopped tomatoes, reaching 10^8 CFU/g within 24 hours (Zhuang et al. 1995). In Australia, the greatest number of bacterial outbreaks have been caused by this organism, 27 in total, affecting 2053 people, with one death (Table 2). Thirteen of these outbreaks involved meat, seafood, poultry and eggs and one was linked to imported coconut. Two outbreaks involved deep-fried ice cream and fruit salad and the source of the rest were not determined (Crerar et al. 1996). There was an outbreak of *S. typhimurium* associated with orange juice in SA that affected around 500 people (Bates & Associates, 1999). The source was traced to a single packing shed where the grower had failed to adequately clean his orange washing facilities. Internationally there has been an increase in cases of salmonellosis in the last two decades, caused principally by *S. enteritidis* (Kraa 1995, Crerar et al. 1996). This increase has not been seen in Australia because *S. enteritidis* is not endemic in Australian layer flocks. The most common serovar isolated in Australia is *S. typhimurium* (Kraa 1995).

The survival time of *Salmonella* species at 2-4° C is about 4 weeks. At ambient temperatures *Salmonella* can survive for up to 60 days. *Salmonella* have relatively uncomplicated nutrient requirements and can grow on a number of substrates. The minimum infective dose is highly variable and depends on a number of factors - eluding the species of *Salmonella* present, as some are more virulent than others. levels of 10 have been mentioned in the literature as causing disease. This means that there needs to be a substantial growth phase of the bacteria to reach infectious levels or a large initial inoculum for the disease to manifest. The generation times for *Salmonella* are quite variable, with generation times of 66 hour at 5° C and 13 hours at 10° C reported for some foods (Snyder 1996). The generation times in raw vegetable are unknown.

2.2.2 Escherichia coli.

An aerobic, gram negative non-spore forming bacteria which is widely spread in the environment. *E. coli* species are normal inhabitants of the intestinal tract of man and many warm-blooded animals and generally they form the predominant flora. Some strains are pathogenic to humans and cause a wide range of illnesses, which are determined by the types of toxins that are produced by a particular strain. There are four recognised categories of disease causing *E. coli*. These are divided into enteropathogenic, enterotoxigenic, enteroinvasive and enterohaemorrhagic. Enteropathogenic *E. coli* consist of many serotypes and are the leading cause of paediatric diarrhoea. Enterotoxigenic *E. coli* produce a cholera like syndrome. Enterohaemorrhagic *E. coli* can result in haemolytic-uraemic syndrome (HUS). Enteroinvasive *E. coli* is responsible for bloody diarrhoea syndrome. *E. coli* strain •157:H7 has been well documented as causing HUS. On average 2-7% of patients with HUS die, but some outbreaks in old peoples institutions have resulted in 50% mortality (Featherstone 1997). This organism colonises the gut of cattle, sheep and chickens so that manure from these sources should be treated with suspicion when used to grow vegetables (Cieslak et al. 1993). Some *E. coli* can be passed on by handlers or packers but rarely do these cause problems. The minimum infective dose for *E. coli* infection varies and depends a lot on previous exposure. The term traveller's diarrhoea was coined as visitors to new places often have no resistance to local strains of *E. coli*.

The first recognised outbreak of *E. coli* 0 157:H7 in the UK was strongly linked to the consumption of locally produced potatoes (Morgan et al. 1988). More recently outbreaks associated with eating alfalfa sprouts occurred in the US (U.S. Department of Health and Human Services 1997) and a prolonged outbreak in Japan affecting 9500 people was linked to radish sprouts (Little et al. 1997). Infection has also been found due to consumption of garden vegetables fertilised with cow manure (Cieslak et al. 1993). Infections of enterotoxigenic *E. coli* from the ingestion of airline salad containing shredded carrots and iceberg, romaine and endive lettuce and in a separate incident, tabouleh salad have been reported (Benoit et al. 1994). Investigation of the airline passengers affected implicated carrots as the infection source. There has been one well-publicised outbreak in Australia in 1995 caused by *E. coli* 0111:H- and associated with mettwurst (Crerar et al. 1996).

Diaz and Hotchkiss (1996) studied the growth of E. coli 0157:H7 on shredded iceberg lettuce stored under different atmospheres at 13 and 22°C. They found that because of the extended shelf life achieved by some of the modified atmospheres *E. coli* was able to grow to higher numbers. A study by Abdul-Raouf et al. (1993) found that *E. coli* growth on salad vegetables declined at 5°C but increased at 12 and 21°C. They also concluded that unknown factors in carrots could inhibit their growth. Generation times for *E. coli* at 10° C are approximately 5 hours and *E. coli* can survive in soils at 20° C for up to 28 days (Snyder 1996).

2.2.3 Yersinia enterocolitica.

A gram negative non spore forming microbe which has the ability to grow at very low temperatures and is a facultative anaerobe which means that it is capable of growing both in aerobic environments and in anaerobic environments. It is a common inhabitant of the intestinal tract of animals including pigs, horses, cattle, sheep, humans and dogs. The bacterium is found worldwide and can be more prominent in colder months. These bacteria have been isolated from fresh produce including leafy vegetables (Gilmour and Walker 1988, dos Reis Tassinari et al. 1994). In random surveys they have been found in shredded carrots and mixed vegetables. An outbreak occurred in the U.S. caused by bean sprouts (Little et al. 1997). Although disease outbreaks due to these bacteria have been few, the presence of this pathogen in vegetables is a major cause of concern particularly when considering the relatively fast generation time at very low temperatures. At -1° C the organism has a generation time of 32 hours and at 5° C it has a generation time of less than 10 hours (Snyder 1996).

2.2.4 Campylobacter jejuni.

A gram negative non spore forming bacterium, common in the gastrointestinal tract of many domestic animal species including poultry, cattle, pigs and sheep. It can be spread by contaminated water and manure. It is the cause of severe enteritis in humans and its prevalence is probably underestimated. Recent studies in the USA have shown that it is the most frequent cause of bacterial diarrhoea but is often diagnosed as *Salmonella* or *E. coli*. In Australia it has been the most common cause of gastrointestinal disease in the last few years (Communicable Diseases Network - Australia New Zealand - National Notifiable Diseases Surveillance System, personal communication). *Campylobacter* enteritis has been associated with the consumption of contaminated fruit and vegetables (Harris et al. 1986). In Australia there have been five reported foodborne outbreaks identified (1980-1995) with 106 cases, one outbreak was linked to salad and/or vegetables and the other four outbreaks had unknown food vehicles (Crerar et al. 1996). *Campylobacter* spp. are not especially hardy in the environment, they are readily killed by heating and do not survive well at low temperatures. At low temperatures of 4-8°C the bacterium does not grow to any extent and requires temperatures of 32° C for reasonable growth (Advisory Committee on the Microbiological Safety of Foods, 1992).

2.2.5 Aeromonas spp.

These are gram negative bacteria, non-spore forming and facultative anaerobes. Two species have been implicated in causing disease causing in humans, *Aeromonas hydrophila* and *Aeromonas sobria.* Both have been isolated from water and sewage. They cause mild gastro-enteritis and dysentery in man. Principally a contaminant of meats and seafood, *A. hydrophila* has been isolated from parsley, celery, alfalfa sprouts, broccoli, lettuce, spinach, asparagus, cauliflower and prepared salads (Callister and Agger 1989, Little et al. 1997). Studies have shown that it can grow well on asparagus, broccoli and cauliflower (Berrang et al. 1989b). *Aeromonas* was found to be present on nearly all samples obtained from a produce market and grew to levels exceeding 10^6 cell/gram in 2 weeks at 4°C (Beuchat 1995). It can grow at temperatures of 4°C-25°C and reach infectious doses within days.

2.3 Direct Bacterial Contamination

The spread of pathogens by direct contact can occur during harvesting, processing, packing and during exposure in shop shelves. A number of the organisms discussed in the sections above can also be spread by direct means. The ones described in this section are mainly spread by direct contact by infected individuals. Although the potential exists for many diseases to be spread by these means, common sense and an understanding of the mechanisms involved in disease production rule out a large number. Respiratory diseases are unlikely to be spread by contamination of produce during packing or during sale. The reasons are that firstly the microbes responsible for the disease must find their way, in an aerosol form, into the respiratory system. Secondly there is a minimum infective dose required to begin the disease. It is unlikely that numbers of respiratory pathogens build up to significant levels on vegetables stored at low temperatures. Blood borne diseases are also unlikely to cause problems in this area since it is very unlikely that body fluids from a contaminated individual will find their way onto fruit and vegetables. Diseases like HTV and blood born viral diseases do not infect very well through the gastro-intestinal tract and, with the exception of Hepatitis B, do not survive well at low temperatures. However, a number of infectious diseases could or have caused problems in humans and vegetables have been implicated as the source. Some of these are discussed below.

2.3.1 Staphylococcus aureus.

A gram positive non spore forming bacteria, which occurs widely in warm blooded animals. Handling of food sources with contaminated hands can spread this organism. It causes an illness as a result of the ingestion of a toxin produced by the bacteria. Therefore growth on the food source is required. It has been isolated from carrots, lettuce, parsley, radish and ready to eat vegetable salads (Fowler and Koffi 1976, Beuchat 1995). However, it has a relatively slow generation time of 55-78 hours at 4-8°C and doesn't compete well with other microorganisms normally present on fresh produce. There have been nine food-related outbreaks in Australia involving 99 people, with one death. Four of the outbreaks were associated with meat, poultry and pizza noodles and the rest were unknown (Crerar et al. 1996).

2.3.2 Shigella spp.

A non spore forming, gram negative bacteria. They belong to the family of enterobacteriaceae and are related phenotypically and genetically to *E. coli.* Four main subgroups have been identified, *S. dysenteria, S. flexneri, S. boydii* and *S. sonnei.* Shigellosis is a dysentery disease that occurs in humans and apes but does not have other animal hosts. Only a small contaminating dose is required to cause illness (Martin et al. 1986). In developed countries the spread of this disease is usually by contaminated humans handling food substances but can also occur via contaminated water and foods.

Shigellosis has been linked to a major outbreak, involving 347 cases, following the consumption of shredded lettuce contaminated by an infected food handler (Davis et al. 1988). Another outbreak in the U.S.A was due to poor techniques used during preparation of salad (Dunn et al. 1995). The number of cases of *S. sonnei* infection increased in the spring of 1994 in several European countries, including the U.K., Norway and Sweden. Epidemiological evidence implicated iceberg lettuce from Spain as the source of infection and the source of contamination either irrigation water or postharvest cooling water (Kapperud et al. 1995). Two other outbreaks not associated with a food handler were linked to the consumption of iceberg lettuce (Martin et al. 1986). A multi-state outbreak involving green onions was traced to Mexico because of the serotype identified, *S. flexneri* 6A, which is very rare in the US but relatively common in Mexico (De Roever 1998).

Shigella has been shown to survive in cabbage and lettuce at refrigeration temperatures. It can survive on lettuce at 5°C for 3 days without a decrease in numbers and can increase 1000 fold when stored at 22°C (Davis et al. 1988). *S. sonnei* can survive and proliferate in shredded cabbage stored under aerobic, vacuum and modified atmosphere packaging (Satchell et al. 1990). The minimum dose required for infection will depend on a number of factors associated with the immune status of the host. No reliable information has been found on the generation times of these bacteria at 5° C although we know that they can multiply to infectious doses at low temperatures within 24 hours. This evidence comes from an outbreak of shigellosis in cold served airline food (Hedberg et al. 1994).

2.3.3 Helicobacter pylori

Helicobacter pylori is a gram variable non spore forming bacterium. It is a disease causing microorganism in humans and primates and can be spread by contact via contaminated individuals. It can be spread by sputum or poor hygiene. The disease associated with this organism is related to the inflammation of the gastric mucosa and it has now been associated with peptic ulcers and stomach cancer. This organism has been linked to the consumption of uncooked vegetables (Hopkins et al. 1993). Growth studies suggest that the organism is microaerophilic and grows best in air which has been enriched with at least 10 % CO2. The optimum growth temperature is 37°C but it can grow at temperatures as low as 30°C. There is no reported growth at lower temperatures.

2.4 Current disease outbreak trends in Australia

There are a number of notifiable organisms that have been discussed above including *Listeria monocy to genes, Salmonella* spp., *Yersinia* spp., *Campylobacter* spp., and *Shigella* spp. The National Notifiable Diseases Surveillance System (NNDSS) was established in its current form in 1991 under the auspices of the Communicable Diseases Network Australia New Zealand (Curran et al. 1997). In 1999 the number of cases of listeriosis was 63, with no cases reported from the Northern Territory or the Australian Capital Territory (Roche et al. 1999). Salmonellosis (not reported elsewhere as, for example, typhoid) numbered 7489 with a higher number reported in the warmer months. There were 12,643 cases of campylobacteriosis, the rate of campylobacteriosis has continued to rise since 1992. The number of cases of shigellosis and yersiniosis were 547 and 143 respectively. The report does not specify how many of these outbreaks were food related and it recognises that due to under-reporting, notified cases only represent a proportion of the total number. However, it is believed that for *Campylobacter, Salmonella, Yersinia and Listeria* spp. transmission is mainly via food. Crerar et al. (1996) reviewed 128 outbreaks of foodborne disease which occurred between 1980 and 1995 (Table 2). Those that could be related to vegetables/salads are noted for each organism above. Nine outbreaks were associated with salad/vegetables where the causative agent was unknown.

It has been suggested that there is a need in Australia for a comprehensive national surveillance system for reporting and collation of foodborne disease (Department of Primary Industries and Energy 1995, Crerar et al. 1996). This would provide a more accurate assessment of the true situation regarding foodborne outbreaks related to vegetables.

Table 2. Foodborne disease outbreaks, cases and deaths in Australia.
1980-1995 (taken from Crerar et al. 1996).

Cause	Outbreaks	Cases	Deaths
Bacterial	68 (53%)	2053	5
Salmonella spp	27 (21%)	1323	1
Clostridium perfringens	14(11%)	280	0
Staphylococcus aureus	9 (7%)	99	1
Campylobacter spp.	5 (4%)	106	0
Bacillus cereus	5 (4%)	27	0
Vibrio parahaemolyticus	4 (3%)	181	2
Listeria monocy to genes	2 (2%)	13	0
Escherichia coli 0111	1 (0.8%)	23	1
Clostridium botulinum	1 (0.8%)	1	0
Viral	13 (10%)	2329	0
Small round-structured viruses	11 (9%)	2267	0
Rotavirus	1 (0.8%)	55	0
Hepatitus A virus	1 (0.8%)	7	0
Protozoal	1 (0.8%)	13	1
Toxoplasma	1 (0.8%)	13	1
Chemical	4 (3%)	43	0
Unknown	42 (33%)	1514	0

3. PARASITES

Parasites are single-celled eukaryotic microorganisms belonging in the main to one of two taxonomic groups, protozoa and helminths (Goldsmid and Speare 1997). Although there are a large number that can cause infections in humans, many are more prevalent in developing countries and are often associated with poor sanitation and hygiene. Some of particular concern in developed countries have been associated with municipal water and food. These are the protozoan parasites *Cryptosporidium*, *Giardia* and *Cyclospora*. Protozoan parasites have been the most frequently identified etiologic agents in waterborne disease outbreaks for many years (Louis 1988, Levine et al. 1991, Moore et al. 1994, Marshall et al. 1997). Generally, the etiologic agents are only confirmed in 30 to 50% of waterborne outbreaks (Bean and Griffin 1990). Therefore, the true number may be greater from both water and food sources considering the high degree of under reporting which is thought to take place.

3.1. Cryptosporidium

3.1.1 Introduction

Cryptosporidium is ubiquitous in the environment. *Cryptosporidium parvum* is the species of concern responsible for gastroenteritis in humans (West 1991, Pontius 1993). *Cryptosporidium* can exist as a very resistant oocyst (Pontius 1993). Its lifecycle starts when it is ingested and passes into the small intestine. The oocysts split open releasing sporozoites, which invade the cells lining the intestine, disrupting their normal function. The cells ability to absorb water and nutrients is severely impaired and water and food ingested by the host passes straight through. The *Cryptosporidium* form more oocysts, which either split to release further sporozoites or are excreted in the faeces. The infective dose is unknown but thought to be 10-100 oocysts (West 1991).

Transmission is via the faecal-oral route by person-to-person or animal-to-person contact and through contaminated water or food (Fayer 1994a). Many mammals are hosts of C. *parvum* including humans, mice, rats, domestic cats, dogs, ferrets, rabbits, monkeys, pigs, cattle, sheep, goats and wild ruminants. Cattle and sheep are implicated as the most common sources of human infection (Gallaher et al. 1989). It has been estimated that 50% of dairy calves shed oocysts and it is present on >90% of dairy farms (Meng and Doyle 1997).

Cryptosporidium has been recognised as a cause of diarrhoeal disease in man since 1976 (Smith 1989). It is thought to be one of the third most common enteropathogens causing diarrhoeal illness found worldwide (Rose 1997). The illness caused is called cryptosporidiosis with symptoms of diarrhoea, nausea, abdominal cramps, vomiting and fever (Pontius 1993). It normally lasts 10 to 14 days but may linger up to 30 days. Susceptible groups include the young, elderly, malnourished and immunocompromised individuals. The disease can be fatal for persons with AIDS particularly as there is no effective, specific drug therapy available.

3.1.2 Survival and viability of oocysts

Cryptosporidium oocysts appear to be fairly resistant to low and high temperatures. *C. parvum* oocysts frozen at -10°C for up to 168 hours were shown to have 100% infectivity in all 6 mice intubated with them (Fayer and Nerad 1996). At -20°C no viable oocysts were found after 24 hours. However, viable oocysts were found after 24 hours at -15°C with all 6 mice infected but by 168 hours there was no viability.

Survival at higher temperatures has been investigated (Fayer 1994b). Heating 10^6 oocysts/ml in water at >72.4°C for 1 minute or at >64.2°C for 5 minutes resulted in loss of infectivity. Oocysts are highly resistant to chlorine at the concentrations generally used for water treatment (Moore et al. 1994).

3.1.3 Occurrence in water

Cryptosporidium oocysts are widespread in water sources in both pristine and polluted areas in the USA (Moore et al. 1994). Water analysis at 66 North American and Canadian surface water treatment plants showed low levels of *Cryptosporidium* oocysts in up to 27% of water samples. Oocysts were found in 5.6-100% of samples taken from source waters in North America at a concentration of 0.001-5800 oocysts/L. In the USA, Canada and Scotland, 3.8-40.1% of drinking water was found to be contaminated with 0.001-0.48 oocysts/L (Lisle and Rose 1995). A pilot study of surface and tap water in Spain revealed 4 out of 8 surface water samples to be positive for *Cryptosporidium* and 3 out of 9 tap water samples (De Leon et al. 1993).

In July, August and September 1998 the protozoa contamination of Sydney, Australia water resulted in the recommendation that it was unfit for consumption. *Cryptosporidium* and *Giardia* were found in the water. Although it is believed that there was not an increase in diarrhoeal illness during this time it was a timely reminder that Australia is not exempt from waterborne contamination.

Sewage discharges and/or run-off from cattle grazing lands are significant sources of viable oocysts (Lisle and Rose 1995). The faecal material seems to protect the oocyst from desiccation, prolonging its survival in the environment.

3.1.4 Waterborne outbreaks

There were twelve outbreaks of cryptosporidiosis in drinking water supplies in North America between 1984 and 1995 (Rose 1997) and eight in the UK between 1983 and 1991 (Lisle and Rose 1995). The largest occurred in Milwaukee in 1993 with 403,000 cases. Of these 4,400 were hospitalised and costs for hospital days reached $US 760,000 (Kramer et al. 1996). Three of the outbreaks in the US showed cryptosporidiosis to be a serious problem for AIDS sufferers (Rose 1997). The mortality rate was 52 to 68% within 6 months to 1 year after the outbreaks. Another large outbreak occurred in Georgia in 1987, affecting 13,000 people (Levine et al. 1991).

Failures in the water treatment process that were thought responsible for the outbreaks were inadequate filtration of paniculate matter, inadequate flocculation, placing filters off and on-line without backwashing, recycling backwash water and seepage of contaminated water. For many of the outbreaks the water systems met the current state and federal drinking water standards in force at the time (Levine et al. 1991, Moore et al. 1994, Kramer et al. 1996). Therefore, coliform counts, as an indicator of contamination by protozoa does not appear to be adequate. *Clostridium perfringens* has been suggested as a more suitable indicator (Payment and Franco 1993). It is an anaerobic bacterium that produces a resistant spore, is found in the faeces of animals and humans and is easily enumerated by membrane filtration.

Outbreaks of cryptosporidiosis appear to be on the increase but this is probably due to an increased awareness of the organisms involvement in waterbome disease outbreaks (Moore et al. 1994). In May 1992 a mixed outbreak of cryptosporidiosis and giardiasis occurred at a school camp in the eastern suburbs of Melbourne (Lester 1992). All 89 of the party of students and staff were affected. A pump failure in the septic tank resulted in leakage into the inground water tank. There have been a number of outbreaks associated with recreational swimming in the UK, US and Australia (Joce et al. 1991, Sorvillo et al. 1992, Moore et al. 1994, Kramer et al. 1996, Rose 1997, Beers et al. 1998). Many of these were caused by faecal accidents. Other causes were a plumbing defect allowing ingress of sewage and a malfunctioning filter.

3.1.5 Foodborne Cryptosporidiosis

In Costa Rica a study was carried out on eight different types of vegetables to determine the presence of *Cryptosporidium* spp. (Monge and Chinchilla 1995). Oocysts were found in 5% (4 samples) of cilantro leaves, 8.7% (7 samples) of cilantro roots, 2.5% (2 samples) of lettuce and a 1.2% incidence was found in other vegetables (radish, tomato, cucumber and carrot). A higher percentage of positive samples were found in the rainy season.

Foodborne cryptosporidiosis appears to be rare however, there have been a few documented incidents (Smith 1993). Suspect foods implicated were raw cows milk, raw goats milk, frozen tripe, salad and the etiology of the other one could not be determined although raw cows milk and sausage were suspected. The salad and cows milk incidents occurred in US travellers returning from Mexico. The goats milk incident occurred in Australia and the other two in England. In a study in England and Wales, 9% of infected persons drank raw milk in the month before onset (Public Health Laboratory Service Group 1990). This again emphasises that cattle are important hosts.

There have not been any reported outbreaks of cryptosporidiosis in developed countries associated with fruits or vegetables. However, considering the ubiquitous nature of this organism in many water supplies, the number of outbreaks for which an etiologic agent is unknown and the fact that it can be transmitted via foods, infection by eating raw produce contaminated by irrigation water is a real possibility.

3.2. *Giardia*

3.2.1 Introduction

Giardia is the most frequently isolated enteric protozoan worldwide (Rose et al. 1991). It is widespread among domestic and wild animal hosts but no animal to human infection has been reported (Buret et al. 1990, Fayer 1994a). *Giardia lamblia* (also known as G. *duodenalis* and G. *intestinalis)* is a flagellate and alternates between trophozoite and cyst stages in its life cycle (Wolfe 1992). The dormant cyst infects the host and the trophozoite causes disease. After the cyst is ingested it splits open in the stomach under the acid environment releasing trophozoites (Smith 1993). These attach to the intestinal cell membrane where they feed and multiply. As detached trophozoites move downwards encystation takes place and the cyst is then excreted. As few as 10 cysts are infective (Steiner et al. 1997).

Infection is spread from person to person by faecal-oral contamination with cysts or through contamination of food or water by faecal contamination of vegetable gardens, water sources or food during preparation (Goldsmid and Speare 1997). Healthy children and adults as well as immunocompromised persons are susceptible (Smith 1993). Symptoms of giardiasis usually begin with intestinal uneasiness, followed by nausea and develop into explosive watery diarrhoea, abdominal gurgling, distension and perhaps cramps. The acute stage lasts for 3 or 4 days but a chronic infection may develop which can involve up to 2 years of intermittent diarrhoea. During this chronic phase, lassitude, headache, myalgia, malabsorption and continued weight loss can occur.

3.2.2 Survival and viability of oocysts

Giardia oocysts are not as resistant to temperature changes as *Cryptosporidium* oocysts. Oocyst viability decreases with increasing temperature (Bingham et al. 1979). At 37°C cysts did not retain their viability for longer than 4 days and freezing followed by thawing resulted in 99% loss of viability. In contrast, oocyst survived for 77 days at 8°C.

3.2.3 Occurrence in water

Surveys carried out on cyst levels in different waters in the US showed that 26-43% of the surface waters were contaminated with *Giardia* cysts with concentrations of 0.3 to 100 cysts per 100L (Rose et al. 991). Pristine watersheds protected from all human activity had much lower levels of 0.6 to 5 cysts per 100L. Sixty-six water treatment plants in 14 states in the US and 1 Canadian province were tested for *Giardia* cysts (Smith 1993). Eighty-one percent of incoming raw water samples were found to be positive although microscopic evaluation indicated that many were non-viable. Plants with highly contaminated source waters were more likely to have contaminated finished drinking water. A recent study by Wallis et al. (1996) collected samples from 72 municipalities across Canada at monthly intervals between 1991 and 1995. In total 1,760 samples were collected and *Giardia* cysts were found in 73% of raw sewage, 21% of raw water and 18.2% of treated water. Twenty-five percent of the samples were tested for viability by inoculation into gerbils, with an observed infection rate of 2.2% for raw water, 7.6% for treated water and 22.2% for sewage.

G. *lamblia* has been found in domestic ruminants such as sheep and cattle with a higher incidence found in calves and lambs (Buret et al. 1990). Run-off from cattle and sheep grazing lands can therefore become a significant source of viable oocysts. Two adjacent watersheds in Canada with similar topographical features were studied (Ong et al. 1996). Ranch cattle had access to creeks on one of the watersheds (A) whereas in the other one (B) access was restricted. In the watershed A intake, significantly higher levels of *Giardia* cysts were detected than in the B watershed intake. In both watersheds downstream samples had significantly higher levels of both *Giardia* and *Cryptosporidium* cysts than those upstream of the cattle ranches. Highest levels coincided with calving activity. Cattle from both ranches were found to be *Giardia* positive when faecal samples were tested.

3.2.4 Waterborne outbreaks

In 1985, *G. lamblia* was the most frequently identified cause of waterborne disease for the seventh consecutive year (Louis 1988). There were 16 community system outbreaks of giardiasis in North America between 1985 and 1994 as well as 5 non-community system outbreaks (Louis 1988, Levine et al. 1991, Moore et al. 1994, Kramer et al. 1996). The community system outbreaks affected 2329 people, with the largest occurring in Massachusetts in 1985 affecting more than 700. Failures in the treatment process which probably contributed to the outbreaks were improper operation of a chlorinator, cross-connections resulting in contaminated surface water or sewage water entering a system and inadequate chlorine contact times in systems where this was the only treatment.

There have been several outbreaks associated with recreational swimming in the U.S., either in a swimming pool, lake or river (Louis 1988, Levine et al. 1991, Moore et al. 1994, Kramer et al. 1996). One pool outbreak lasted for 3 months, caused by intermittent breakdown of the pools filter (Kramer et al. 1996).

3.2.5 Foodborne Giardia **outbreaks**

There have been five documented outbreaks of giardiasis associated with food (Smith 1993). However, it has been estimated that 3,850 cases occur in Canada and 7,000 in the US each year. In the five outbreaks the suspect foods involved were fruit salad, sandwiches, noodle salad, home canned salmon and either lettuce, onions or tomatoes. In four of the outbreaks the foods were contaminated by handling. In the fifth outbreak in New Mexico, which involved lettuce, onion and tomatoes, the route of contamination was not clear. Analysis indicated that lettuce and onions in particular were correlated with illness and each of these ingredients was chopped on the same board, which was not washed in between. Potable water may also have been contaminated but plumbing changes were made before this could be tested (Grabowski et al. 1989).

3.3. *Cyclospora*

3.3.1 Introduction

Cyclospora is a newly recognised protozoan parasite first described as causing diarrhoea in humans in Nepal (Goldsmid and Speare 1997). Its complete lifecycle is unknown but it does require time outside the host to sporulate and thus become infectious. Therefore, transmission via food handlers is unlikely. The minimum time required for sporulation is unknown but it is favoured by warm temperatures and high humidity. The infective dose is unknown.

Poultry may serve as a source of infection (Garcia-Lopez et al. 1996). A *Cyclospora* spp. with the same diagnostic features as C. *cayetanensis* was found in chicken faeces. Generally, it is unknown as to whether animals are sources of infection. Transmission is more likely from water and food.
Symptoms of the disease are similar to those of Cryptosporidiosis; watery stools, abdominal pain, nausea and fatigue (Meng and Doyle 1997). However, unlike cryptosporidiosis, it can be treated with trimethoprim-sulfamethoxazole (Jackson et al. 1997). If untreated the duration can be prolonged with an average of 43 days and symptoms may relapse.

3.3.2 Waterborne outbreaks

The first reported illness in the US associated with *Cyclospora* occurred in 1990 at a hospital in Chicago with tap water implicated as the source of infection (Meng and Doyle 1997). An outbreak among British soldiers in Nepal associated with water was reported in 1994 (Rabold et al. 1994). The drinking water was a mixture of river and municipal water treated with chlorine. It was also checked regularly for coliform bacteria.

3.3.3 Foodborne Cyclospora

In 1996 there was a large outbreak of cyclosporiasis associated with fresh raspberries involving 20 states in the USA (Herwaldt and Ackers 1997). In total, 1465 cases were reported with 978 of these being laboratory confirmed. The raspberries implicated were imported from Guatemala but it was unclear how they became contaminated. Irrigation water or water used for spray applications were considered the most likely routes particularly as the outbreak took place in the rainy season when there is an increase in surface-water runoff. Transmission of *Cyclospora* seems to be seasonal, at least where it has been studied in temperate, seasonal climates (Colley 1996).

Basil was implicated in twenty-six clusters of cases of cyclosporiasis occurring in the US in June and July 1997 (Pritchett et al. 1997). It was either served fresh or prepared as pesto sauce. The mode of contamination is being investigated. Another outbreak in the same year in the US was caused by imported mesclun mix (DeGraw and Heber 1997, Little et al. 1997).

4. VIRUSES

Foodborne viruses are a common but probably under-recognised cause of gastroenteritis (IFST 1997). The most common mode of transmission is from person to person but transmission is also possible via food and water. Food and waterborne viruses are enteric and specific for humans. Foodborne viruses cannot grow on food (Appleton et al. 1988, IFST 1997), but can survive to cause human disease. Viruses are resistant against sewage treatment processes and can survive in waterways, sludge, sediment, soil, shellfish, on crops irrigated by recycled effluent and on inanimate surfaces (Grohmann, et al. 1994, Appleton 1994, Grohmann 1997). They can survive pickling and fermentation processes because they are generally resistant to acidic environments. They can also survive drying, refrigeration and freezing (Appleton 1994, Grohmann 1997). Chlorination is not always effective in deactivating viruses depending on the concentration of free chlorine (West 1991. Appleton 1994). All these factors combined allow viruses to survive well in the environment and in food.

Many outbreaks of foodborne viral disease are due to uncooked or partially cooked foods. Most viruses will generally not survive temperatures greater than $60^{o}C$ (Larkin 1981, Caul 1993, Patterson et al. 1997). Horticultural produce such as fruit, vegetables and salads have the potential to transmit foodborne viruses, because they are often eaten raw or only partially cooked. In the United States, about half of reported foodborne viral infections are associated with salad dishes (Appleton. et al. 1988). Very few virus particles are needed to cause illness.

Horticultural produce has been implicated in several outbreaks of viral disease. The types of viruses implicated are mainly from the families Picornaviridae *and* Caliciviridae. However, there are others that are less commonly associated with foodborne viral outbreaks such as astroviruses, rotaviruses, reoviruses and adenoviruses.

4.1 Picornaviridae

The Picornaviridae family has been implicated in many foodbome outbreaks of disease, and includes Hepatitis A and enteroviruses (coxsackie, polio and echo-viruses). Foodborne disease outbreaks associated with horticultural produce are not common but do occur.

4.1.1 Hepatitis A

Hepatitis A causes symptoms such as loss of appetite, malaise, fever and vomiting followed by jaundice (IFST 1997). It has a long incubation period of 3-6 weeks. In the USA most cases result from contact with infected persons and *~3%* are associated with food or waterborne modes of transmission (Niu et al. 1992). Outbreaks have been linked to frozen strawberries (Niu et al. 1992), frozen raspberries (Ramsay and Upton 1989) and lettuce (Rosenblum et al. 1990). In each case it was suggested that contamination could have been from infected pickers or packers, it was also suggested that contaminated water used in irrigation or faeces used as fertiliser may have contaminated the lettuce (Rosenblum et al. 1990). Two outbreaks in Finland were linked to imported salad ingredients (Pebody et al. 1998).

4.1.2 Enteroviruses

These cause gastrointestinal disease symptoms such as malaise, abdominal pain, nausea, diarrhoea and/or vomiting (Appleton et al. 1988). Examples of disease associated with horticultural produce are not common, but coxsackie, polio and echo viruses have all been isolated from raw vegetables and cherries (Larkin 1981) and were found to survive quite well on inoculated surfaces of vegetables including cucumbers, tomatoes, lettuce and radishes (Bagdasaryan 1964). They were also found to survive in soils for up to 150-170 days. An outbreak of echovirus disease was found to have been transmitted via coleslaw, however there was no evident source of contamination (Cliver 1994b). Sadovski et al. (1978) found that enteroviruses were below detectable limits on vegetables irrigated with sewage effluent but they were still concerned because of their low infectious dose.

4.2 Caliciviridae

The Caliciviridae family includes Norwalk and Norwalk-like viruses, Small Round Structured Viruses (SRSV's) and others such as human calici virus and Hepatitis E. These viruses generally cause gastrointestinal disease with symptoms such as vomiting, diarrhoea and abdominal pain (EFST 1997).

4.2.1 Norwalk and Norwalk-like viruses

Detailed surveillance data from Minnesota (1990 to 1996) cited fresh produce items as the most frequently identified food vehicle, accounting for 30% of the outbreaks (De Roever 1998). The most common source of contamination was handling by a sick person during preparation and the most common agent was Norwalk-like virus accounting for 54% of the outbreaks. Fresh-cut fruit (Herwaldt et al. 1994) and celery in chicken salad (Warner et al. 1991) were implicated in Norwalk virus disease outbreaks. The source for the fresh-cut fruit outbreak was traced to food preparation by contaminated food handlers. The celery had been exposed to non-potable water. Orange juice reconstituted with contaminated water was the cause of a Norwalk virus outbreak on domestic air flights in Australia (Grohmann 1997).

4.2.2 Small Round Shaped Viruses (SRSV's).

Vegetable soup (Evans and Maguire 1996) and potato salad (Patterson et al. 1997) were implicated in SRSV disease outbreaks. In these cases the disease was linked to contamination by food processors during preparation. Caul (1993) reported that salads were implicated in 4, mashed potato in 1 and fruit salad in 2 of the 16 reported SRSV outbreaks.

4.3 Current trends in Australia

Hepatitis A is a notifiable disease. In 1996 there were 2150 cases reported but there was no indication of how the disease was contracted (Curran et al. 1997). A review by Crerar et al. (1996) identified 13 foodborne viral outbreaks between 1980-1995 involving 2329 cases (Table 2). Small round-structured viruses caused the most outbreaks (11) and rotavirus and Hepatitis A were responsible for the other two. The rotavirus outbreak, involving 55 cases, was linked to salad vegetables. Although the number of outbreaks was far less than those linked with bacterial pathogens the number of persons affected was greater. In fact, viruses were responsible for the highest number of deaths related to foodborne disease.

5. CONCLUSION

A number of different organisms have been isolated from vegetables in random overseas surveys including *Listeria monocytogenes. Salmonella* spp., *Yersinia enterocolitica, Aeromonas* spp., *Staphylococcus aureus*, *Cryptosporidium* spp. and enteroviruses. This is not to say that other organisms might not be present, rather that they may not have been discovered. There is little information published on contaminants of Australian vegetables. One in NSW conducted during 1988 to 1993 on different types of foods including 54 ready-to-eat vegetables and salads found 1 sample to be positive for *L. monocytogenes*. More studies of this type need to be carried out, in particular for those organisms considered as posing the highest risk. There are many potential sources of contamination starting in the field via soil or inputs such as animal manures and irrigation water or from handling or use of contaminated **water** at various stages during production and distribution. The magnitude of risk needs to be carefully and scientifically investigated in the Australian vegetable industry.

There have been several foodborne disease outbreaks in developed countries in winch horticultural produce has been implicated. Organisms identified in these outbreaks include *B. cereus, C. botulinum, L. monocytogenes, Salmonella* spp.. *E.coli* 0157:H7, enterotoxigenic *E. coli, Shigella* spp., *Cyclospora,* hepatitis A, Norwalk-like viruses and SRSV's. Usually it is difficult to trace the source of contamination, particularly for smaller outbreaks. Identified sources include use of animal manures, use of contaminated irrigation water, poor hygienic practices and handling by infected individuals. The true number of outbreaks is probably more than realised because of under reporting and shortfalls in food classification schemes such as that used m the US. Fraser et al. (1995) suggested an alternative food classification where, 'salads with raw ingredients' for example form a separate category. Presently salads would fall under the category 'other' and fail to be recognised. When categorised under the alternative classification, a 1992 study showed they accounted for 851 reported foodborne disease outbreaks, fourth in ranking after ‘sandwich’ (15%), ‘chicken' (11%) and 'Mexican foods' (10%). This implies that fresh vegetables may be a more significant cause of food poisoning than previously recognised. Detailed surveillance data from a Minnesota, USA study cited fresh produce items as the most frequently identified food vehicle accounting for 30% of the outbreaks (De Roever 1998). However, 22% of these originated from salad bars.

The World Health Organisation estimates that in developed countries, reported cases of foodborne illness may be underestimated by a factor of ten (Kraa *1995)*. In Australia there is little published information on foodborne disease outbreaks but a recent review showed that foodborne disease patterns are similar to those seen in other developed countries. There is an urgent need in Australia for a comprehensive national surveillance system for reporting and collation of foodborne disease.

Detailed data for foodborne disease outbreaks linked to fresh vegetables are severely lacking in Australia. However, this review has shown that there is enough evidence to suggest that they could be a significant vehicle for disease outbreaks. Consequently, the vegetable industry needs to adopt good production practices at ail stages of the production and handling chain. Preharvest practices include the use of properly treated manures, clean water for irrigation and provision *of* suitable facilities for workers, such as toilets and hand washes. Postharvest it encompasses the use of clean equipment (processing and transport), adequately sanitised water and adoption of good personal hygiene practices. Other good food handling practices need to be adopted where appropriate. These include proper protective clothing and a basic knowledge of food safety practices. The Australian vegetable industry needs to be proactive in developing its own food safety guidelines. These are urgently needed to minimise the risk of foodborne disease from vegetables.

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