Journal of Applied Microbiology ISSN 1364-5072

REVIEW ARTICLE

Prepared salads and public health

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Keywords
food safety, fresh produce, gastrointestinal disease, outbreaks, pathogens.

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2007/1465: received 10 September 2007, revised 25 January 2008 and accepted 10 February 2008

doi:10.1111/j.1365-2672.2008.03801.x

Summary

In recent years the importance of prepared salads as potential vehicles of gastrointestinal infection has been highlighted by several large outbreaks both nationally and across international boundaries. Between 1992 and 2006, 2274 foodborne general outbreaks of infectious intestinal disease were reported in England and Wales, of which 4% were associated with the consumption of prepared salads. In total, 3434 people were affected, with 66 hospitalizations and one death reported. The attribution of prepared salad types and pathogens among prepared salad associated outbreaks are presented and discussed. Findings from UK studies on salad vegetables, fruit and mixed salads from 1995 to 2007 (21 247 samples) indicate that most bacteria of concern with regard to human health are relatively rare in these products (98·6% of satisfactory quality); however, outbreaks of salmonellosis were uncovered associated with bagged salad leaves and fresh herbs during two such studies. Although it is known that fresh salad vegetables, herbs or fruit may become contaminated from environmental sources, only in recent years has the association of foods of nonanimal origin, such as salad vegetables, with foodborne illness become evident and recurrent, demonstrating that major health problems can arise from consumption of contaminated prepared salads if hygiene practices break-down.

Introduction

Prepared salads and fruits are generally considered safe to eat by consumers (Food Standards Agency 2007a), and consumption of these vegetables in the United Kingdom (UK) has increased in quantity and in variety in recent years (Mintel International Group 2002; Food Standards Agency 2007a), driven primarily by the trend towards healthier eating and in particular the Government's 5 A DAY campaign (Food Standards Agency 2007a). The development of prepared salads has also been towards salad meals – adding a variety of other ingredients (like cheese, salmon, crayfish, pasta and couscous) to produce a wide variety of combinations. Raw and minimally processed fruit and salad vegetables are typically sold to the consumer in a ready-to-use or ready-to-eat form and depend on refrigeration as the primary means of preservation (European Commission 2004). Ready-to-eat food, as defined by Regulation (EC) No. 2073/2005, 'means food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organisms of concern' (European Commission 2005).

Food production and consumption is an international business, with fresh produce and finished product being imported and exported in increasingly complex food production matrices, as a consequence of the proliferation of choice for consumers, rapid movement of goods by modern transportation, faster communication facilitating global trade and increasingly competitive markets (Hutton 2001). Salad vegetables and fruit from around the world have now therefore become available to consumers year-round throughout the European Union (EU) which underlines the need for the international application of good hygienic standards for fresh produce. Strategies for preventing fruit and vegetables being contaminated with micro-organisms of concern, such as salmonellas, campylobacters, verocytotoxin-producing *Escherichia coli* and *Listeria monocytogenes*, during production have to rely on control measures taken during pre- and postharvest operations (Fresh Produce Consortium 1999; Knight and Stanley 2000; Chilled Food Association 2007; Codex Alimentarius 2003). Food safety controls, underpinned by the application of good agricultural practices, good manufacturing practices, good hygiene practices and implementation of a hazard analysis critical control point (HACCP) system, are therefore aimed to ensure that food reaching the consumer is safe to eat and thus in line with EU food hygiene legislation (European Commission 2004, 2005).

Advances in farming and horticultural practices, processing and distribution have enabled the raw vegetable and fruit industry to supply high-quality produce to consumers all year-round. With changing food production and eating habits, new pathogens and newly recognized vehicles of infection have emerged (Beuchat 1998).

Ready-to-eat fruit and vegetables requiring minimal or no further processing prior to consumption have been implicated as vehicles for transmission of infectious micro-organisms although the frequency of foodborne outbreaks of gastrointestinal illness associated with fruit and vegetables appears to be low compared to products of animal origin (European Commission 2002b). Nonetheless, foodborne illnesses associated with fruit and vegetables appear to be increasing in many countries (European Commission 2002b) either due to improved recognition or reporting, increased consumption, changes in commodities or production practices or a combination of these factors. The extensive outbreak of *E. coli* O157 infection in Japan in 1996 linked to sprouted radish (Michino *et al.* 1999), and the more recent high profile North American outbreak of *E. coli* O157 infection associated with bagged fresh spinach in 2006 (International Food Safety Authorities Network 2007), illustrates the potential for serious public health problems arising from the contamination of ready-to-eat fruit and vegetables, and have heightened concerns that these foods may be an increasing source of illness (Tauxe 1997).

In the UK, the monitoring of microbiological food safety and the prevention of foodborne disease are the responsibility of a number of different interlinked public bodies [e.g. local authorities, Health Protection Agency (HPA), Food Standards Agency (FSA)], the food industry and other related organizations. This review describes general outbreaks of infectious intestinal diseases (IIDs) linked with prepared salads reported in England and Wales from 1992 to 2006, European outbreaks associated with prepared salads in international trade, national data on the prevalence of pathogens and indicator organisms from UK food studies and the outcomes and benefits of surveillance programmes.

General outbreaks of foodborne IID associated with the consumption of prepared salads in England and Wales, 1992–2006

Methods

The current scheme for the surveillance of general outbreaks of IID in England and Wales commenced in 1992 (Cowden *et al.* 1995). General outbreaks are those affecting members of more than one household or residents of an institution. Upon notification of an outbreak (from a variety of sources including public/environmental health staff, hospital microbiologists, government and, occasionally, the media) a standardized surveillance form is sent to the lead investigator with a request that it is completed once the outbreak investigation has ended. Designed to elicit a standard response, the questionnaire seeks to capture information about the setting of the outbreak (in foodborne disease outbreaks defined as the place where food was prepared), the mode of transmission, causative organism(s) and the results of epidemiological and environmental investigations. Up to three reminders are sent to nonresponders and the response rate is consistently over 70% (Gillespie *et al.* 2003).

For the purpose of this study, outbreaks of foodborne origin were selected initially (a proportion of foodborne outbreaks are followed by person-to-person transmission) and the reported food vehicles scrutinized by both authors. It is important to note that more than one vehicle of infection can be reported in any given outbreak. Outbreaks linked to prepared salads were identified and subsequently subclassified into 'salad', 'salad meal' and 'fruit' outbreaks. Comparisons between and within prepared salad outbreaks were undertaken. Statistical analysis was undertaken in Microsoft Excel and Stata Corporation (StataCorp, College Station, TX, USA). Proportions were compared using the chi-squared test or Fisher's exact test, and means were compared using Student's *t*-test. Changes in proportion with time were assessed using the chi-squared test for trend.

Results

Between 1992 and 2006, 9891 general outbreaks of IID were reported to the HPA Centre for Infections and, of these, 2274 (23%) were of foodborne origin (henceforth termed 'foodborne outbreaks'). Eighty-two of these outbreaks (4%) were linked to prepared salad. This proportion ranged from 1% to 6% over the surveillance period,

Table 1 Classification of general outbreaks of infectious intestinal disease (IID) associated with prepared salads

Classification (number of outbreaks)		
Category	Subcategory	Vehicle description
Salad (49)	Carrot (1)	Carrot (1)
	Lettuce (11)	Lettuce (7), iceberg lettuce (3), rocket (1)
	Pepper (1)	Pepper (1)
	Salad (35)	Salad (23), lettuce/tomato (5), green salad (3), bagged salad leaves (1), prepacked salad (1), side salad (1), lettuce/salad (1)
	Watercress (1)	Watercress (1)
Salad meal (24)	Salad meal (24)	Coleslaw (7), potato salad (4), rice salad (4), cheese salad (1), chicken salad (1), pasta salad (1), coleslaw/pasta salad (1), coleslaw/rice salad (1), savoury salad (1), mixed bean salad (1), three bean salad (1), tuna salad (1)
Fruit (8)	Fruit (8)	Fruit salad (4), melon (2), avocado (1), fresh fruit (1)
Mix (1)	Mix (1)	Coleslaw/salad (1)

but no consistent trend was observed. The classification of the prepared salad outbreaks is summarized in Table 1. 'Salads' were reported most often (49/82; 60%) and in most of these instances the salad was not described further or the salad item was lettuce. Salad meals (24/82; 29%) and fruit outbreaks (8/82; 10%) were reported less frequently.

Evidence implicating food vehicles

In most prepared salad outbreaks (63; 77%) one form of evidence (analytical epidemiology, descriptive epidemiology or microbiology) was provided to implicate the salad item in the outbreak. In 10 outbreaks (12%) two forms were reported, in two (2%) three types were provided and in seven outbreaks (9%) no evidence was provided. Analytical epidemiological information (i.e. from a cohort or case–control study) was more often provided in support of the vehicles identified in prepared salad outbreaks (49/82; 60%) than in other foodborne outbreaks (427/2192; 19%; $\chi^2 = 77.4$; $P < 0{\cdot}001$).

Dynamics and impact

A total of 3434 people were affected in the 82 prepared salad outbreaks (range 2–361), with 66 people admitted to hospital (range 0–18) and one death reported. Prepared salad outbreaks were, on average, larger than other foodborne outbreaks in terms of the number affected (mean 42 *vs* 22; *t*-test $P < 0{\cdot}001$) or at risk (564 *vs* 121; *t*-test $P < 0{\cdot}001$) but were no different in terms of reported hospital admission or death. Within the prepared salad outbreaks those attributed to lettuce were larger in terms of numbers affected (90 *vs* 34; *t*-test $P = 0{\cdot}01$) and at risk (6684 *vs* 383; *t*-test $P < 0{\cdot}001$) compared to those attributed to other vehicles. Lettuce outbreaks were also more prolonged in their duration (63 *vs* 5 days; *t*-test $P = 0{\cdot}005$).

Pathogens/toxins

The relationship within and between prepared salads and the different aetiological agents reported in foodborne outbreaks is presented in Table 2. Just under half (48%) of prepared salad outbreaks were attributed to bacteria – a proportion lower than that observed in other foodborne outbreaks (71%; $\chi^2 = 20{\cdot}31$; $P < 0{\cdot}001$). Salmonellas accounted for just under half of these outbreaks (48%) which was also at a lower frequency than that observed for other foodborne outbreaks (69%; $\chi^2 = 8{\cdot}89$; $P = 0{\cdot}03$). Viral pathogens (exclusively norovirus) were reported in 17% of prepared salad outbreaks and viruses were suspected in 72% of the prepared salad outbreaks of unknown aetiology. Combined therefore, viruses were the confirmed or suspected agent in 43% of prepared salad outbreaks and 16% of other foodborne outbreaks ($\chi^2 = 39{\cdot}44$; $P < 0{\cdot}001$). Within the prepared salad outbreaks, a viral aetiology was observed/suspected more frequently in outbreaks linked to prepared fruit (20%) compared with other prepared salad types (2%; Fisher's exact test, $P = 0{\cdot}02$).

Outbreak setting

Prepared salad outbreaks were more likely to take place in commercial food service premises (65%) than other foodborne outbreaks (35%; $\chi^2 = 4{\cdot}13$; $P = 0{\cdot}04$; Table 3), with restaurants and hotels accounting for almost three quarters of outbreaks. Whilst the proportion of prepared salad outbreaks linked to restaurants was no higher than for other outbreaks (38% *vs* 46%; $\chi^2 = 1{\cdot}40$; $P = 0{\cdot}24$) prepared salad outbreaks occurred more frequently in hotels compared with other foodborne outbreaks (20% *vs* 11%; $\chi^2 = 5{\cdot}33$; $P = 0.02$). Regardless of premises type, prepared salad outbreaks were more likely to take place in premises where functions were taking place (51/82; 62%) compared with other foodborne outbreaks

Table 2 The relationship and inter-relationship between prepared salads and aetiological agents in foodborne general outbreaks of infectious intestinal disease (IID) reported in England and Wales, 1992–2006

Pathogen/toxin	Number of outbreaks (%)						
			Prepared salad subclassification				
	Other foodborne vehicles	Prepared salad	Salad	Salad meal	Fruit	Mix	Lettuce*
Salmonella Enteritidis PT 4	565 (26)	8 (10)	3 (6)	5 (21)	0	0	0
Salm. Enteritidis Non-PT 4	266 (12)	1 (1)	1 (2)	0	0	0	0
Norovirus	233 (11)	14 (17)	9 (18)	4 (17)	1 (13)	0	0
Clostridium perfringens	238 (11)	1 (1)	0	1 (4)	0	0	0
Salm. Typhimurium	131 (6)	4 (5)	3 (6)	1 (4)	0	0	3 (27)
Other salmonellas	104 (5)	5 (6)	5 (10)	0	0	0	2 (18)
Campylobacter spp.	75 (3)	5 (6)	4 (8)	1 (4)	0	0	2 (18)
Verocytotoxin-producing *Escherichia coli* O157	68 (3)	2 (2)	2 (4)	0	0	0	0
Bacillus spp.	66 (3)	3 (4)	3 (6)	0	0	0	1 (9)
Scombrotoxin	61 (3)	0	0	0	0	0	0
Staphylococcus aureus	31 (1)	4 (5)	1 (2)	3 (13)	0	0	0
Shigella sonnei	4 (0·2)	2 (2)	2 (4)	0	0	0	1 (9)
Rotavirus	5 (0·2)	0	0	0	0	0	0
Astrovirus	4 (0·2)	0	0	0	0	0	0
Cryptosporidium	4 (0·2)	0	0	0	0	0	0
Shigella flexneri	1 (0·05)	2 (2)	1 (2)	0	1 (12)	0	0
Other	7 (0·3)	2 (2)	2 (4)	0	0	0	0
Mixed aetiology	2 (0·1)	0	0	0	0	0	0
Unknown	327 (15)	29 (35)	13 (27)	9 (38)	6 (75)	1 (100)	2 (18)
Total	2192	82	49	24	8	1	11

*Lettuce is a subset of 'salad' outbreaks in the subclassification and therefore the total number can exceed the number of prepared salad outbreaks.

(970/2192; 44%; $\chi^2 = 10{\cdot}28$; $P = 0{\cdot}001$). Prepared salad outbreaks were also more likely to occur in the community (5%) compared with other foodborne outbreaks (2%; Fisher's exact test, $P = 0{\cdot}0003$).

Within the prepared salad outbreaks, outbreaks associated with prepared fruit (63%) were more likely to occur in hotels compared to outbreaks attributed to other prepared salad vehicles (16%; Fisher's exact test, $P = 0{\cdot}008$). Furthermore, four lettuce outbreaks occurred in the community compared with none of the prepared salad outbreaks not linked to lettuce (Fisher's exact test, $P < 0{\cdot}001$). Prepared fruit outbreaks (seven of eight; 88%) and salad meal outbreaks (21/24; 88%) were both more commonly linked to premises holding functions than other prepared salad outbreaks (23/50; 46% $\chi^2 = 14{\cdot}29$; $P < 0{\cdot}001$).

Contributory faults

In most foodborne outbreaks (1452; 64%) the faults thought to have contributed to the outbreak were reported and, when this was the case, one (802/1452; 55%) or two faults (467; 32%) were reported most commonly. In 148 outbreaks (10%), three faults were reported, in 30 (2%) four faults were reported and in five outbreaks (0·3%) five faults were reported.

Cross-contamination was the most commonly reported fault (28/80; 35%) in prepared salad outbreaks, followed by an infected food handler (22; 28%) and inappropriate storage (13; 16%). Whilst the reported frequency of cross-contamination and inappropriate storage in prepared salad outbreaks was higher than for other outbreaks (571/2239; 26%; $\chi^2 = 0{\cdot}64$; $P = 0{\cdot}06$ and 542/2192; 25%; $\chi^2 = 3{\cdot}37$; $P = 0{\cdot}07$, respectively), significantly an infected food handler was more commonly reported as a contributory fault in prepared salad outbreaks than other outbreaks (262/39; 12%; $\chi^2 = 17{\cdot}93$; $P < 0{\cdot}001$).

Within the different types of prepared salad outbreaks there was little variation in the reported contributory faults. An infected food handler was reported in four of nine prepared fruit outbreaks (44%), but this was no different from the other prepared salad outbreaks (18/71; 25%; Fisher's exact test, $P = 0{\cdot}25$). Likewise cross-contamination was reported no more frequently in 'salad' outbreaks (15/36; 42%) than other prepared salad outbreaks (13/44; 30%; $\chi^2 = 1{\cdot}26$; $P = 0{\cdot}26$). However, within the prepared salad outbreaks an infected food handler was more commonly reported where a viral aetiology was confirmed or suspected (15/35; 43%) compared with those attributed to other pathogens (7/41; 17%; $\chi^2 = 6{\cdot}02$; $P = 0{\cdot}01$).

Table 3 The relationship and inter-relationship between prepared salads and outbreak setting in foodborne general outbreaks of infectious intestinal disease (IID) reported in England & Wales, 1992–2006

Outbreak setting		Number of outbreaks (%)						
				Prepared salad subclassification				
Category	Subcategory	Other foodborne vehicles	Prepared salad	Salad	Salad meal	Fruit	Mix	Lettuce
Food service	Restaurant	537 (24)	20 (24)	18 (37)	1 (4)	1 (13)	0	3 (8)
	Hotel	246 (11)	16 (20)	7 (14)	4 (17)	5 (63)	0	1 (3)
	Private	211 (10)	6 (7)	1 (2)	5 (21)	0	0	0
	Pub/bar	154 (7)	6 (7)	3 (6)	2 (8)	0	1 (100)	0
	Hall/caterers	105 (5)	7 (9)	4 (8)	3 (13)	0	0	1 (3)
	Club/centre	87 (4)	4 (5)	1 (2)	3 (13)	0	0	0
	Shop caterer	24 (1)	0	0	0	0	0	0
Institutional/residential	Residential	287 (13)	1 (1)	1 (2)	0	0	0	0
	Canteen	88 (4)	5 (6)	2 (4)	3 (13)	0	0	0
	School	81 (4)	0	0	0	0	0	0
	Hospital	59 (3)	3 (4)	2 (4)	1 (4)	0	0	0
	Armed services	43 (2)	1 (1)	1 (2)	0	0	0	0
	University/college	19 (1)	2 (2)	0	1 (4)	1 (13)	0	0
	Holiday camp	13 (1)	0	0	0	0	0	0
	Workplace	8 (0·4)	2 (2)	1 (2)	1 (4)	0	0	0
Retail	Shop retailer	118 (5)	3 (4)	2 (4)	0	1 (13)	0	1 (3)
	Mobile	15 (1)	0	0	0	0	0	0
Other	Community	41 (2)	4 (5)	4 (8)	0	0	0	4 (11)
	Farm	27 (1)	0	0	0	0	0	0
	Other	29 (1)	2 (2)	2 (4)	0	0	0	1 (3)
Total		2192	82	49	24	8	1	11

Seasonal trends

The date of the first reported onset date in each outbreak was available for 2159/2274 outbreaks (95%), and this was used to define the month of the outbreak. Over half (44/79; 56%) of the prepared salad outbreaks occurred between the months of May and August but this frequency was no different for other foodborne outbreaks (931/2080; 45%; $\chi^2 = 3{\cdot}67$; $P = 0{\cdot}06$). No differences were observed in the season pattern of prepared salad outbreaks attributed to the different salad types.

European outbreaks associated with prepared salads in international trade

There is increasing globalization of trade in foods with the EU the world's main importer of fruits and vegetables. Intra-EU trade represents 17 million tons per year of fruits and vegetables (and represents on average two-thirds imports in the EU; this proportion varies from 40% to 90% according to the country) (European Commission 2002b). Currently there is concern that food in international trade may carry pathogenic micro-organisms that could result in outbreaks of illness. Movement of goods between countries can be effective ways of swiftly and widely distributing disease internationally. Events within one country may now potentially affect many other nations, demonstrating the importance of robust national and international control programmes and networks to combat these threats (Threlfall *et al.* 2003; Fisher and Threlfall 2005). The international surveillance network for enteric pathogens (Enter-net) does this by undertaking surveillance of foodborne pathogens internationally, predominantly within Europe (Fisher and Threlfall 2005). The possibility of introducing new or emerging pathogens into countries (e.g. new *Salmonella* strains, multi-drug resistant organisms) or spreading pathogens across boundaries from endemic areas to nonendemic areas (e.g. *Vibrio cholerae*) contributes to the concern regarding imported food and was a consideration in an European Commission ban in 1998 on fruits and vegetables from Uganda, Kenya, Tanzania and Mozambique (European Commission 1998).

There are many examples of prepared salads in international trade causing human illness in Europe and beyond (Table 4). Strikingly a large number (11/23; 49%) have involved lettuce or salad leaf products contaminated with various *Salmonella* spp. and in particular multi-drug resistant *Salmonella* serotype Typhimurium definitive phage type (DT) 104 and DT 204b (Table 4). Multi-drug resistant salmonella infections are associated with an

Table 4 European outbreaks of foodborne disease associated with prepared salads and fruit in international trade, 1994–2007

Year	Prepared salad	Organism	Country of origin	Countries involved	No. cases	Reference
2007	Baby corn	*Shigella sonnei*	Thailand	Denmark	120	Lewis *et al.* (2007)
2007	Fresh basil	*Salm.* Senftenberg	Israel	Denmark, the Netherlands, UK, USA	49	Pezzoli *et al.* (2007)
2007	Frozen raspberries	Norovirus	China	Denmark	9	Anon (2007)
2006	Frozen raspberries	Norovirus	China	Sweden	43	Hjertqvist *et al.* (2006)
2005	Lettuce (iceberg)	*Escherichia coli* O157 VT2	Sweden	Norway, Sweden	120+	Soderstrom *et al.* (2005)
2005	Lettuce	*Salm.* Typhimurium DT104 (ACSSuT)	Spain	UK	96	Health Protection Agency (2005)
2005	Lettuce (iceberg)	*Salm.* Typhimurium DT104b (ACSSuT)	Spain	Finland, Sweden	60+	Takkinen *et al.* (2005)
2005	Frozen raspberries	Norovirus	Not known	France	75	Cotterelle *et al.* (2005)
2005	Frozen raspberries	Norovirus	Poland	Denmark	1043+	Falkenhorst *et al.* (2005)
2004	Lettuce (rocket)	*Salm.* Thompson	Italy	Denmark, Norway, Sweden	100	Nygard *et al.* (2007)
2004	Lettuce	*Salm.* Newport	Spain	UK, Isle of Man	375	Health Protection Agency (2004)
2003	Lettuce	*Salm.* Braenderup	Spain	UK	40	Health Protection Agency (2003)
2002	Cucumber	*E. coli* O157 PT34 VT2	Belgium	UK, France	21	Duffell *et al.* (2003)
2001	Lettuce (rocket)	Hepatitis A	Spain	Sweden	54	Nygard *et al.* (2001)
2001	Salad leaf (iceberg)	*Salm.* Newport PT33	Spain	UK	19	Ward *et al.* (2002)
2000	Lettuce	*Salm.* Typhimurium DT104 (ACSSuT)	Not known (not UK)	UK	361	Horby *et al.* (2003)
2000	Lettuce	*Salm.* Typhimurium DT204b (ACGNeKSSuTTmNxCp$_L$)	Not known (not UK)	Denmark, Germany, Iceland, the Netherlands, UK	140	Crook *et al.* (2003)
1998	Baby corn	*Shigella sonnei*	Thailand	Denmark	25+	Mølbak and Neimann (1998)
1998	Beansprouts	*Salm.* Saint-Paul	Australia	UK, Sweden	143	O'Mahony *et al.* (1989)
1995	Alfalfa sprouts	*Salm.* Stanley	Not known (not USA, Finland)	Finland, USA	242	Mahon *et al.* (1997)
1995–1996	Alfalfa sprouts	*Salm.* Newport	Not known (not Canada, Denmark, USA)	Canada, Denmark, USA	133+	Van Beneden *et al.* (1999)
1994	Alfalfa sprouts	*Salm.* Bovismorbificans	Australia	Finland, Sweden	595	Ponka *et al.* (1995)
1994	Lettuce (iceberg)	*Shigella sonnei*	Spain	Norway, UK, Sweden	218	Frost *et al.* (1995)

Key to antimicrobial drugs: A, ampillicin; C, chloramphenicol; G, gentamicin; Ne, neomycin; K, kanamycin; S, streptomycin; Su, sulfonamides; T, tetracycline; Tm, trimethoprim; Nx, naladixic acid; Cp$_L$, decreased susceptibility to ciprofloxacin (MIC: 0·125–1·0 mg l^{-1}).

increased hospitalization rate, morbidity and mortality (Adak *et al.* 2002; Mølbak 2005). Vegetable crops produced in the natural environment cannot be expected to be free of microbiological agents. Decontamination practices therefore have to be considered when the possibility of contamination cannot be excluded (Beuchat 1998). Effective washing and decontamination of ready-to-eat vegetables is difficult. The decontamination efficiency of the washing system in terms of pathogen removal is generally unknown, and reductions of total viable bacterium counts on fruits and vegetables are typically 100-fold at best (Seymour 1999). Pathogens, such as *Salmonella* and *E. coli* O157, can adhere steadfastly to leaf surfaces and/or become internalized by invading the plant tissue through stomata (Everis 2004). This had led to increasing concern regarding the microbiological safety of prepared salads and the effectiveness of current decontamination methods (Seymour 1999). The importance of these critical issues is recognized by government and the industry in the UK, whom have funded a longstanding programme of research into these issues.

Several outbreaks of shigellosis have also been attributed to the consumption of contaminated salad vegetables. In 1994, a number of cases of *Shigella sonnei* infection occurred in several European countries, including Norway, Sweden and the UK. Epidemiological evidence indicated that iceberg lettuce from Spain was the likely source of infection (Table 4, Frost *et al.* 1995). In 1998 and 2007, outbreaks of shigellosis were reported in Denmark. These outbreaks were epidemiologically linked to the consumption of baby corn (Table 4, Mølbak and Neimann 1998; Lewis *et al.* 2007) and were traced to imported baby corn from Thailand. In the 2007 investigation, microbiological examination of the suspected batches of imported baby corn had high levels of *E. coli*, indicating faecal contamination. *Salmonella* serotype Weltevreden and *Salmonella* serotype Hvittingfoss were also found in two of the batches (Lewis *et al.* 2007). In the previous outbreak in 1998, *Salmonella* was also found in the baby corn (H. Lewis, Statens Serum Institut, Denmark, personal communication).

Food poisoning outbreaks throughout the world have been and continue to be associated with the consumption of sprouted seeds, although there have been none reported in the UK and the rest of Europe since 1989 (Table 4). Outbreaks are, however, particularly frequent in the United States (Beales 2004; Food Standards Agency 2004). Most reported outbreaks associated with sprouted seeds have involved either *Salmonella* species or *E. coli* O157. The largest single outbreak, involving radish sprouts contaminated with *E. coli* O157, affected more than 11 000 (6000 culture confirmed) people in Japan in 1996 (Michino *et al.* 1999). The scale of most *Salmonella* outbreaks elsewhere has been around 20 to 200 people and most were associated with alfalfa sprouts (Beales 2004; Food Standards Agency 2004). In the United States in 1998, precautionary advice was issued by the US Food and Drug Administration that high-risk groups (young, elderly, pregnant women and immunocompromised people) should avoid the consumption of raw alfalfa sprouts. In November 2002, following a number of new outbreaks, this advice was extended to raw and lightly cooked mung bean sprouts (Food and Drug Administration 2002). Sprouted seeds exhibit a unique hazard potential, since the germination stage breaches the inhibitory barrier of the seed coat, allowing pathogens, which may be present to grow on nutrients from the sprouted plant. No effective decontamination process has been identified that will substantially reduce or eliminate contaminating pathogens. Nevertheless, by washing seeds with chlorinated water a degree of reduction can be achieved (European Commission 2002b; Beales 2004).

Over the past 20 years verocytotoxin-producing *E. coli* O157 has emerged to become an important cause of severe gastrointestinal illness (O'Brien and Adak 2002). Several outbreaks associated with the consumption of raw fruit and vegetable products have been reported in recent years in countries as widely dispersed as the UK, India, Japan and the United States and Canada (Parish 1997; Michino *et al.* 1999; Duffell *et al.* 2003; Everis 2004; Food and Drug Administration 2006; International Food Safety Authorities Network 2007). While some are likely to be due to cross-contamination from meat products during food preparation, others are probably due to direct contamination with animal faeces in the field (Mead and Griffin 1998). Contamination in the field was the most likely route of contamination of cucumbers grown in Belgium implicated in an outbreak that affected people from France and the UK in 2002 (Table 4). *Escherichia coli* O157 phage type (PT) 34 is an unusual phage type in England and Wales, and accounted for only 4% of all isolates in 2001 (Health Protection Agency 2002), but was the second most common phage type in a survey of *E. coli* among Belgian cattle (Tutenel *et al.* 2002). Likewise, contamination of the iceberg lettuce in the large outbreak of *E. coli* O157 VT2 infection that occurred in Sweden during 2005 (Table 4) was caused in the field by using irrigation water from a small stream (Soderstrom *et al.* 2005).

Also of note are several recent outbreaks of gastroenteritis in a number of European countries caused by norovirus from frozen imported raspberries (Table 4). Contamination with norovirus may have occurred at farm level by faecally contaminated irrigation water, during harvesting by infected farm workers and/or during processing and freezing by infected workers at company level.

Freezing allows viruses to survive in berries for a long time. Contaminated berries have also been reported to transmit viruses other than norovirus. Frozen strawberries caused two widespread outbreaks of hepatitis A in 1990 and 1997 in the United States (Sivapalasingam *et al.* 2004) and an outbreak of hepatitis A in Scotland was linked to the consumption of mousse prepared from frozen raspberries (Reid and Robinson 1987).

One of the challenges during outbreak and food incident investigations is traceability. The complexity of the food supply chain and the lack of identifying markers on salad and fruit stuffs can make it extremely difficult to track produce back to their origin. For example, in 2000 two large outbreaks, one international, were linked with the consumption of wholesaled lettuce. The causative agents were *Salm.* Typhimurium DT 104 and *Salm.* Typhimurium DT 204b (Table 4, Long *et al.* 2002). Unfortunately, due to the lack of identifying markers on the lettuce it was impossible to trace through the stages of the food chain back to the original growers. In comparison, outbreaks linked with the consumption of retail prepacked lettuce, such as the *Salmonella* serotype Newport PT33 outbreak associated with retail bagged salad in 2001(Ward *et al.* 2002), did have sufficient identifying markers that enabled the original growers to be identified (Sagoo *et al.* 2003a). It is important to acknowledge that in this instance these data were collected as part of a national microbiological study which uncovered the outbreak. Labelling of some fresh salad produce, particularly those sold wholesale, is clearly not sufficient to allow proper tracing of some products. This has implications for public health since food alerts and product withdrawals are contingent on accurate identification of the suspect product. EU legislation [Regulation (EC) 178/2002] governing full traceability came into force on 1 January 2005 (European Commission 2002a), and requires that a food business operator at any point in the food chain can identify its suppliers and whom the business has supplied with ingredients or final food product, i.e. traceability is determined by one step forward, one step back basis. Consideration needs to be given to the measures required to improve the traceability of salad vegetables and fruit sold via wholesale.

The microbiological quality of prepared salads in the UK

The Local Authorities Coordinators of Regulatory Services (LACORS) and the HPA implement a national programme of coordinated food studies in partnership with Local Authority Food Liaison Groups. The programme focuses on foods of concern associated with food production, and the food service and retail sectors. These studies produce national data on the microbiological quality of ready-to-eat foods, such as prepared salads, examined within the UK.

Studies on mixed salads, salad vegetables, fresh herbs and fruit carried out by HPA and LACORS between 1995 and 2007 in the UK are summarized in Table 5. Based on published microbiological guidelines (Gilbert *et al.* 2000), information from these studies has indicated that the majority of ready-to-eat salads and fruit sold from food service and retail premises in the UK were of satisfactory/acceptable microbiological quality (Tables 5 and 6). *Escherichia coli* O157 and *Campylobacter* spp. were not detected in any of these microbiological food studies, but *Salmonella* spp. was detected in a study of bagged salad vegetables and another one on fresh herbs. Prepared salad and fruit samples of unsatisfactory microbiological quality in the studies were mainly due to *L. monocytogenes* and *E. coli* (a faecal indicator organism) counts in excess of 10^2 CFU g^{-1} (Table 5). Both are common environmental micro-organisms that are found in soil and water, and salad vegetables, fresh herbs and fruit may therefore easily become contaminated with these bacteria. Levels of these micro-organisms in raw ready-to-eat salad vegetables, herbs and fruit, however, should be kept to a minimum by applying good hygiene practices.

From 2006, microbiological criteria for certain foodstuffs were introduced in the EU by implementation of Regulation (EC) No. 2073/2005 (European Commission 2005) which will also be used in determining the microbiological quality of foods in future UK studies. This regulation stipulates that levels of *L. monocytogenes* must be no greater than 10^2 CFU g^{-1} during the shelf-life of ready-to-eat foods. Regulation (EC) No. 2073/2005 also contains criteria for *Salmonella* and *E. coli* in precut vegetables, herbs and fruit. Precut vegetables, herbs and fruit (in general these are categorized as those undergoing a size reduction stage after initial harvest) are covered by the food safety criteria (precut fruit and vegetables and *Salmonella* spp.) that applies for products placed on the market. *Salmonella* spp. should be absent in all ready-to-eat salad vegetables, fresh herbs and fruit and those not falling within the category of precut vegetables and fruit in Regulation (EC) No. 2073/2005 will be covered by the published public health Microbiological Guidelines for ready-to-eat foods sampled at the point of sale (Gilbert *et al.* 2000) and Regulation (EC) No. 178/2002 (General Food Law Regulation, European Commission 2002a). Regulation (EC) No. 2073/2005 contains process hygiene criteria for *E. coli* in precut fruit and vegetables (n = 5, c = 2, m = 100 CFU g^{-1}, M = 1000 CFU g^{-1}), however these apply only at the manufacturing process. Action taken in the case of unsatisfactorily *E. coli* results would include improvements in production hygiene and selection of raw materials (European Commission 2005).

Table 5 Microbiological results of prepared salads and fruit examined in the UK

Organism	Year	Prepared salad type	No. Samples	No. positive (%)	Reference
Escherichia coli O157	2002	Precut fruit	997	0	Little and Mitchell (2004)
	2002	Sprouted seeds	808	0	Little and Mitchell (2004)
	2001	Open salad vegetables	2950	0	Sagoo *et al.* (2003b)
	2001	Bagged salad vegetables	3852	0	Sagoo *et al.* (2003a)
	2000	Organic vegetables	3200	0	Sagoo *et al.* (2001)
	1999	Retail whole lettuce	151	0	Little *et al.* (1999)
Salmonella	2002	Precut fruit	997	0	Little and Mitchell (2004)
	2002	Sprouted seeds	808	0	Little and Mitchell (2004)
	2001	Open salad vegetables	2950	0	Sagoo *et al.* (2003b)
	2001	Bagged salad vegetables	3852	5 (0·1)	Sagoo *et al.* (2003a)
	2000	Organic vegetables	3200	0	Sagoo *et al.* (2001)
	1999	Retail whole lettuce	151	0	Little *et al.* (1999)
Campylobacter	2001	Open salad vegetables	2950	0	Sagoo *et al.* (2003b)
	2001	Bagged salad vegetables	3852	0	Sagoo *et al.* (2003a)
	2000	Organic vegetables	3200	0	Sagoo *et al.* (2001)
	1999	Retail whole lettuce	151	0	Little *et al.* (1999)
Listeria monocytogenes	2005	Prepacked mixed vegetable salads	2686	130 (4·8); 2 > 10^2 CFU g^{-1}	Little *et al.* (2007)
	2002	Precut fruit	997	86 (8·6); 1 > 10^2 CFU g^{-1}	Little and Mitchell (2004)
	2002	Sprouted seeds	808	28 (3·5); 1 > 10^2 CFU g^{-1}	Little and Mitchell (2004)
	2001	Open salad vegetables	2950	89 (3·0); 1 > 10^2 CFU g^{-1}	Sagoo *et al.* (2003b)
	2001	Bagged salad vegetables	3852	90 (2·3); 1 > 10^2 CFU g^{-1}	Sagoo *et al.* (2003a)
	2000	Organic vegetables	3200	3200 < 20 CFU g^{-1}	Sagoo *et al.* (2001)
	1999	Retail whole lettuce	151	151 < 20 CFU g^{-1}	Little *et al.* (1999)
	1995	Prepacked salad vegetables	2276	66 (3·0); 1 > 10^2 CFU g^{-1}	Little *et al.* (1997)
	1995	Prepacked crudités	247	2 (0·8)	Little *et al.* (1997)
E. coli	2002	Precut fruit	997	3 (0·3); 3 > 10^2 CFU g^{-1}	Little and Mitchell (2004)
	2002	Sprouted seeds	808	32 (4·0); 16 (2·0) >10^2 CFU g^{-1}	Little and Mitchell (2004)
	2001	Open salad vegetables	2950	198 (6·7); 87 (3·0) >10^2 CFU g^{-1}	Sagoo *et al.* (2003b)
	2001	Bagged salad vegetables	3852	52 (1·4%); 19 (0·5%) >10^2 CFU g^{-1}	Sagoo *et al.* (2003a)
	2000	Organic vegetables	3200	48 (1·5); 11(0·3) >10^2 CFU g^{-1}	Sagoo *et al.* (2001)
	1999	Retail whole lettuce	151	151 < 20 CFU g^{-1}	Little *et al.* (1999)
	1995	Prepacked salad vegetables	2276	307 (13·5); 33 (1·5%)>10^2 CFU g^{-1}	Little *et al.* (1997)
	1995	Prepacked crudités	247	33 (13·7); 5 (2·0) >10^2 CFU g^{-1}	Little *et al.* (1997)

Table 6 Microbiological quality of prepared salads and fruit examined in the UK, 1995–2005

Year	Study	No. Samples	No. Satisfactory quality* (%)	Reference
2007	Fresh herbs	3760	3698 (98·3)	
2005	Prepacked mixed salads	2686	2682 (99·9)	Little *et al.* (2007)
2002	Precut fruit	997	993 (99·6)	Little and Mitchell (2004)
2002	Sprouted seeds	808	792 (98·0)	Little and Mitchell (2004)
2002	Unpasteurized juices	291	291 (100)	Little and Mitchell (2004)
2001	Bagged salad vegetables	3852	3826 (99·3)	Sagoo *et al.* (2003a)
2001	Open salad vegetables	2950	2862 (97·0)	Sagoo *et al.* (2003b)
2000	Organic salad vegetables	3200	3185 (99·5)	Sagoo *et al.* (2001)
1998	Imported whole lettuce	151	151 (100)	Little *et al.* (1999)
1995	Salads and crudites	2552	2471 (96·8)	Little *et al.* (1997)
Total		21 247	20 951 (98·6%)	

*Based on HPA microbiological guidelines (Gilbert *et al.* 2000).

Food studies also provide a resource for linking data on food and data on human disease. For example, a study of retail bagged ready-to-eat salad vegetables carried out during 2001 uncovered a national outbreak of *Salm.* Newport PT33 (Sagoo *et al.* 2003a). Five of 3853 bagged salad samples in 2001 were contaminated with *Salmonella Salm.* Newport PT33 (one sample), *Salmonella* serotype Umbilo (three samples) and *Salmonella* serotype Durban (one sample), which indicates a health risk. Nineteen cases of *Salm.* Newport PT33 infection were subsequently identified throughout England and Wales. The outbreak strain of *Salm.* Newport PT33 isolated from the salad and from humans had a unique plasmid and pulsed field gel electrophoresis (PFGE) profile (Ward *et al.* 2002). Investigations of the products contaminated with *Salmonella* spp. appeared to link contamination of the different lettuce implicated (iceberg, organic rocket) with a grower in Spain and another in Italy, suggesting that these were two isolated incidents. In the case of *Salm.* Umbilo, contamination was traced directly to the field lizard population at source. In each case, the retailer involved and the UK Food Standards Agency were immediately informed. Comprehensive investigations revealed no further contaminated product.

A 6-month study of fresh herbs on retail sale in the UK commenced in May 2007. At the time of writing (August 2007) eight samples taken in different parts of the UK during May from different retailers revealed *Salmonella* serotype Senftenberg contamination of fresh basil grown in Israel (Pezzoli *et al.* 2007). Concurrently 31 cases of *Salm*. Senftenberg infection in England and Wales were identified by the HPA Laboratory of Enteric Pathogens. Basil and human isolates were indistinguishable by plasmid and PFGE profile. Cases of *Salm.* Senftenberg with an indistinguishable PFGE pattern were also reported by Scotland (3), Denmark (3), the Netherlands (2) and the United States (10). On 25 May 2007 the UK retailers affected publicly recalled product and the FSA advised consumers who may have bought certain batches of fresh packets of basil not to eat them (Pezzoli *et al.* 2007). Comprehensive investigations along the supply chain revealed no further contamination of the product had occurred.

A case–control study of potential sources of sporadic illness of *L. monocytogenes* infection from 2000 to 2003 in the United States demonstrated an association with eating melons at commercial establishments (Varma *et al.* 2007). Data from UK food studies indicate that more precut fruits (8·0%) contained *L. monocytogenes* compared to prepared salads (sprouted seeds, 3·0%; open salads, 3·0%; bagged salads, 2·3%) and unpasteurized juices (<1%) (Sagoo *et al.* 2003a,b; Little and Mitchell 2004). Most of the precut fruit sampled were precut melon, or mixed fruits that contained a high proportion of melon. Unlike most other fruits melon has a near neutral pH and if pathogens, such as *L. monocytogenes,* are introduced they are likely to survive. Reducing the burden of foodborne listeriosis may require interventions directed at foods, such as melons, that hitherto are not commonly recognized as high risk.

Although the incidence of major pathogens on raw vegetables and fruit was generally low, given the very large quantities consumed and the fact that further cooking is often minimal or absent, prepared salads are a potential public health risk. Minimally processed cut and packaged salad vegetables, herbs and fruit are exposed to a range of conditions during growth, harvest, preparation and distribution, and it is possible that these conditions may increase the potential for microbial contamination, highlighting the necessity for the implementation of good hygiene practices from farm to fork to prevent contamination and/or bacterial growth in these prepared salad products.

Conclusions

The microbiological safety of prepared salads has gained ever-increasing attention as a result of outbreaks of infection. Information from outbreaks of IID reported in England and Wales between 1992 and 2006 suggests that consumption of prepared salads account for a small proportion (4%) of all foodborne general outbreaks reported to the HPA. Although a variety of pathogens was reported, viruses appeared to play a major role in the aetiology of these outbreaks. Infected food handlers were often implicated in outbreaks of known or suspected viral aetiology and might well have been the cause of many of the outbreaks rather than the victim. The food handler faults reported suggest that further food hygiene training is needed in the commercial food service sector.

Infected food handlers and cross-contamination were the two contributory factors most commonly reported. Pathogens with a human reservoir and a low infectious dose, e.g. norovirus, can be readily transferred onto salad vegetables and fruit by infected food handlers. A feature of norovirus infection is projectile vomiting, which enables aerosols with high infectivity to be carried over a wide area (Advisory Committee on the Microbiological Safety of Food 1998). Virus particles are difficult to eliminate from kitchens, and can easily get onto foods from contaminated surfaces, utensils or hands. In contrast, cross-contamination was more commonly associated with bacterial pathogens. The source of these pathogens is most likely to be of animal origin, but prepared salads can also become cross-contaminated through poor handling or storage practices. Therefore the application of good basic food hygiene would greatly reduce the risk of transmission via infected food handlers or cross-contamination.

In seeking to interpret the role of prepared salads in IID in England and Wales it is important to bear in mind outbreak ascertainment and investigation and their potential to distort the role of certain food vehicles. Outbreaks rely on individuals recalling a common exposure and/or experiencing a similar illness and, with regard to the former, outbreak identification becomes simpler when individuals report a single distinct food service premises or a chain of food service premises, rather than, for example, shopping at a common national retail chain. Furthermore, when a large number of individuals are exposed to a single event at a single time then this adds weight to investigators' hypotheses that this exposure is the likely source of the observed illness. This phenomenon may explain the observed association between prepared salad outbreaks and commercial food service premises in general and hotels in particular, and the strong statistical relationship with these outbreaks and functions. Similarly, these outbreaks by their nature represent natural cohorts which facilitate the application of analytical epidemiology, especially as guests often have a particular interest in what has 'spoiled the party'. Nevertheless, there is no denying that these outbreaks in England and Wales took place, and our analyses demonstrate that the main breakdown in control which precipitates outbreaks in the commercial food service setting is the role of the infected food handler. Furthermore, prepared salad outbreaks, and lettuce-associated outbreaks in particular, occurred more often in the community and these outbreaks would not be subject to the same ascertainment or investigation bias.

Outbreaks associated with prepared salads might become more common with the growing global distribution of foodstuffs. The outbreaks of infection associated with prepared salads that have occurred in Europe over the past 14 years associated with international trade illustrate the potential risks that exist. The European perspective adds to a growing body of evidence (Tauxe *et al.* 1997; European Commission 2002b; Everis 2004; Sivapalasingam *et al.* 2004) demonstrating that the contamination of salad vegetables and fruit during production and processing can result in major, geographically widespread, outbreaks of infection with serious public health consequences. This highlights the role and importance of international surveillance systems, such as Enter-net (Fisher and Threlfall 2005), which can be vital mechanisms in recognizing and investigating such epidemics. This becomes a matter of particular importance when the potential for disseminating multi-drug resistant strains of pathogens is taken into consideration. Several epidemiological studies have demonstrated that infections with drug-resistant non-Typhi *Salmonella* serotypes and *Campylobacter* species are associated with excess mortality and morbidity (Mølbak 2005). This requires international collaboration to address this food safety issue.

Microbiological food surveillance studies contribute to a greater understanding of the microbiological and food hygiene problems associated with food, and of how food safety may be improved. The results from such studies can be used to monitor trends, assess risks in food safety and judge the effectiveness of regulation. Information from food studies can also form part of the science base for the development of food policy and informing microbiological risk assessments. Such studies therefore help to establish sound evidence on which to base advice. Food studies in the UK and elsewhere have indicated that raw vegetables and fruit may harbour potential pathogens (Tables 5 and 6, Beuchat 1998; Nguyen-the and Carlin 1994; Tauxe *et al.* 1997; Everis 2004). *Listeria monocytogenes*, *Salmonella*, *Shigella* and *E. coli* have been isolated from mixed salads, salad vegetables and fruit. This has been further illustrated by the number of high profile product recalls in the UK during the first half of 2007 (Food Standards Agency 2007b,c,d,e,f). These have included recalls of retail prepared salad products due to contamination with *Salmonella*; watercress and *Salm.* Tallahassee; leaf salad and *Salm.* Newport, lambs lettuce and *Salm.* Napoli, fresh basil and *Salm*. Senftenberg; bagged salads and prepared mixed salads and *Salm*. Typhimurium DT 92 (E.J. Threlfall and E. de Pinna, Laboratory of Enteric Pathogens, HPA. personal communication).

Food studies carried out by LACORS and the HPA in the UK have provided a great deal of useful data on the sources of food poisoning organisms, and two such studies of prepared salads have even uncovered outbreaks of salmonellosis in the UK and elsewhere (Ward *et al.* 2002; Sagoo *et al.* 2003a; Pezzoli *et al.* 2007). Expansion of global trade, the free movement of foods within Europe, the larger and more intensive farming and manufacturing processes and the continuing rise in reported food poisoning cases, continue to offer a challenge to public health professionals. Although improved production and processing methods, application of good manufacturing practices and the HACCP system are important in ensuring that the number of pathogens present in finished produce is minimized, well-organized food studies can nevertheless provide useful explanations to many of the hygiene, safety and other questions relating to the safety of food.

Consumption of prepared salads is increasing in the UK (Mintel International Group 2002; Food Standards Agency 2007a) and the possibility of contamination with foodborne pathogens is of concern for the safety of prepared fruits and vegetables. Stringent hygienic conditions during cultivation and processing may limit, but not exclude, contamination. Compliance with good manufacturing practices and the HACCP system are needed to control factors related to contamination, survival and growth of micro-organisms at every stage of the food chain. A sound approach to food safety management cannot be overemphasized.

Acknowledgements

The authors gratefully acknowledge the assistance of Ian Fisher, International Surveillance Network for the Enteric Infections Salmonella and VTEC O157 (Enter-net), and John Threlfall, Health Protection Agency Centre for Infections, for their assistance in the preparation of this paper. Thanks are also extended to the public health, environmental health, hospital and laboratory staff who contribute to the: LACORS/HPA coordinated food liaison group microbiological sampling programme; the surveillance scheme for general outbreaks of IID, and to Mrs S. Le Baigue, Mrs S. Hopcroft and Miss C. Penman who maintain the database at the HPA Centre for Infections.

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