ACM/476

DISCUSSION PAPER

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD (ACMSF)

MICROBIOLOGICAL STATUS OF READY-TO-EAT FRUIT AND VEGETABLES

1. Members will recall that they asked the Secretariat to commission a briefing paper to enable them to take a view of the potential risks associated with ready-to-eat fruit and vegetables and the need for further work in this area by the ACMSF.

2. The attached paper has been prepared by the Public Health Laboratory Service and will be presented by Dr Bob Mitchell and Dr Bob Adak.

Secretariat
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THE MICROBIOLOGICAL STATUS OF READY TO EAT FRUIT AND VEGETABLES

Sarah J O'Brien, Robert T Mitchell, Iain A Gillespie, Goutam K Adak
PHLS Communicable Disease Surveillance Centre

INTRODUCTION

This paper examines the role of Ready to Eat (RTE) fruit and vegetables in infectious intestinal disease in terms of epidemiological investigations and microbiological analysis both in the UK and abroad. The expression "Ready To Eat" is used to describe those foods that are eaten raw, i.e. without cooking. It includes products that are eaten with or without washing or peeling.

The paper is presented in five sections:

Section 1. General Outbreaks of foodborne infectious intestinal disease associated with the consumption of salad items, fruit and vegetables in England & Wales 1992-1999.
Section 2. Outbreaks associated with RTE Fruit And Vegetables: An International Perspective.
Section 3. Products of particular concern.
Section 4. The Microbiological Status of RTE Fruit And Vegetables.
Section 5. Conclusions and areas requiring further work

The epidemiological and microbiological information available for interpretation can be subject to the following limitations:

1. In some instances it is possible that the food was the vehicle of infection rather than the source *per se*. It is not always possible to discriminate between these two possibilities based on the information available.

2. The number of reports associated with a particular country can be as much a reflection of the extensiveness of their surveillance systems and the frequency with which they publicise their data.

3. Reports will tend to be skewed towards those organisms that are responsible for outbreaks that can be readily identified. Organisms that cause severe illness, e.g. Enterovirulent *E. coli* are more likely to feature in reports than organisms that do not. On the other hand, organisms that cause sporadic cases, e.g. Campylobacter, a less likely to feature than those that cause well-defined outbreaks.

4. Any foodborne pathogen is potentially capable of being transmitted by RTE fruit and vegetables. The extent to which the information received to date reflects surveillance and reporting systems as opposed to relating to the properties of the organisms themselves remains to be seen.

SECTION 1. GENERAL OUTBREAKS OF FOODBORNE INFECTIOUS INTESTINAL DISEASE ASSOCIATED WITH THE CONSUMPTION OF SALAD ITEMS, FRUIT AND VEGETABLES, ENGLAND AND WALES 1992 - 1999

Purpose

The purpose of this section is to describe the features of foodborne general outbreaks of infectious intestinal disease in England and Wales associated with the consumption of salad items, fruit and vegetables.

Method

Outbreaks of infectious intestinal disease (IID) are reported to the PHLS Communicable Disease Surveillance Centre from a variety of sources, including the national laboratory reporting system, Consultants in Communicable Disease Control (CsCDC), Environmental Health Officers (EHOs), microbiologists, and general practitioners.

A standard, structured questionnaire is posted to the appropriate CCDC, with a request that the lead investigator completes it when the outbreak investigation is concluded. The questionnaire seeks a minimum set of data on all outbreaks, including details of the setting, mode of transmission and causative organism. Details of epidemiological and laboratory investigations are also captured.

Outbreaks were included in the analysis where salad items, fruit or vegetables were implicated as a vehicle of infection on the basis of microbiological evidence, a statistical association, or descriptive epidemiology (strong circumstantial evidence).

Outbreaks excluded from the analysis were:

- salmonella outbreaks where the implicated vehicle contained raw shell eggs,
- *Clostridium perfringens* outbreaks where the vehicle was cooked
- scombrotoxin food poisoning outbreaks.

Results

Between 1992 and 1999 60 outbreaks of foodborne infectious intestinal disease associated with the consumption of salad items, fruit and vegetables were reported from England and Wales (Table 1). This represents 4.3% (60/1408) of the total of foodborne outbreaks reported during that period.

Morbidity
Two thousand one hundred and seventy people were affected (at risk = 19650) and 27 were admitted to hospital. No deaths were reported. In 17 of the outbreaks more than 50 people were affected (range 4 to 193).

Table 1: General outbreaks of IID associated with the consumption of salad items, vegetables or fruit* reported to CDSC, England & Wales, 1992-1999

Year	General outbreaks	Foodborne outbreaks	Salad/fruit/veg outbreaks	Percent
1992	373	224	9	4.0
1993	456	227	5	2.2
1994	488	191	15	7.9
1995	834	178	5	2.8
1996	732	161	12	7.5
1997	591	220	3	1.4
1998	609	120	5	4.2
1999	505	87	6	6.9
Total	4588	1408	60	4.3

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
* More that one vehicle may have been reported for any single outbreak. The figures above represent the number of outbreaks in which at least one of the vehicles reported was a salad/vegetable/fruit product.

Causative organisms
The most commonly identified aetiological agent was small round structured virus (SRSV) (Table 2). The causative organism for 41% of outbreaks was reported as unknown although the clinical and epidemiological features of these outbreaks suggest that the majority (64%) were also viral. It is noteworthy that *E. coli* O157 was identified as the causative organism in two of these outbreaks (vide infra). An outbreak of *Shigella sonnei* associated with the consumption of iceberg lettuce illustrates the potential for imported produce to cause illness.

Table 2: Causative organisms for general outbreaks of IID associated with the consumption of salad items, fruit and vegetables, E&W, 1992-1999

Organism	Number of outbreaks	Percent
SRSV	12	20.0
S. enteritidis	10	16.7
Campylobacter	3	5.0
S. typhimurium	3	5.0

E. coli O157	2	3.3
Sh. Sonnei	2	3.3
Sh. Flexneri	1	1.7
S.hindmarsh	1	1.7
Other	1	1.7
Unknown	25	41.7
Total	60	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00

Food Vehicles

A complete listing of the outbreaks and food vehicles, including levels of evidence, by year is given in Appendix 1.1. Food vehicles were implicated in 63% (38/60) of outbreaks using analytical epidemiological techniques, in 5% (3/60) of outbreaks on the basis of microbiological evidence and in the remainder (32%) on the basis of descriptive epidemiology alone.

Outbreak setting

Food had been prepared on commercial catering premises in the majority of outbreaks (Table 3) with meals prepared in hotels and restaurants accounting for 55% of the total.

Table 3: Setting where food was prepared for general outbreaks of IID associated with the consumption of salad items, fruit and vegetables, E&W, 1992-1999

Place	Number outbreaks	of Percent
Restaurant	22	36.7
Hall/caterers	4	6.7
Hotel	11	18.3
Canteen	4	6.7
Other	3	5.0
Armed services	1	1.7
Private	4	6.7
Pub/bar	3	5.0
Shop/retailer	2	3.3
Community	1	1.7
Hospital	3	5.0
University/college	2	3.3
Total	60	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00

Food handling faults thought to have contributed to the outbreaks

The faults identified by investigators as contributing to the outbreaks are outlined in Table 3.

Table 3: Food handling faults thought to have contributed to general outbreaks of IID associated with the consumption of salad items, fruit and vegetables, E&W, 1992-1999

Fault	Number of entries	Percent
Cross contamination	22	33.8
Infected food handler	18	27.7
Inappropriate storage	14	21.5
Inadequate heat treatment	4	6.2
Other fault	7	10.8
Total	65	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
NB For seven outbreaks no fault was recorded

The influence infected food handlers was particularly marked for viral outbreaks or for those where the aetiology was unknown but was thought to be viral (Table 4). Additional evidence supplied with the outbreak forms suggests that, for outbreaks of viral gastro-enteritis, an infected food handler might be the cause rather than the victim. For example, it is recorded in one outbreaks report that a restaurant chef was suddenly taken ill while preparing a meal. He vomited over the salad he was preparing. He rinsed the salad in cold water and it was then served to the customers. Many of these customers subsequently became ill and SRSV was identified in the stools of some of the affected customers. Such reports are not uncommon.

Table 4: Infected food handlers reported in general outbreaks of IID associated with the consumption of salad items, fruit and vegetables, E&W, 1992-1999

Organism	Suspected	Number of instances	Percent
SRSV	-	5	27.8
S. enteritidis pt4	-	2	11.1
S. typhimurium	-	1	5.6
E. coli O157	-	1	5.6
Unknown	Viral	9	50.0
Total		18	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00

Cross contamination was reported as a contributory factor in an outbreak of *E. coli* O157 associated with the consumption of salad (Table 5). Restaurant staff revealed that the salad items were stored in plastic containers prior to serving.

These containers were also used for storing raw beef and were rinsed out before re-using for salad vegetables.

Table 5: Cross contamination reported in general outbreaks of IID associated with the consumption of salad items, fruit and vegetables, E&W, 1992-1999

Organism	Suspected	Number of instances	Percent
SRSV	-	1	4.5
S. enteritidis PT4	-	6	27.3
Campylobacter	-	2	9.1
S. enteritidis	-	3	13.6
S. typhimurium	-	1	4.5
S. hindmarsh		1	4.5
E. coli O157	-	1	4.5
Unknown	Viral	5	22.7
Unknown		2	9.1
Total		22	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00

Discussion

Outbreaks of IID in England and Wales associated with the consumption of salad items, fruit and vegetables formed a small proportion (4.3%) of all foodborne general outbreaks reported to PHLS CDSC between 1992 and 1999. 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 these outbreaks rather than the victim. The food handling faults reported suggest that further food hygiene training is needed in the commercial catering sector. The fact that the food items do not undergo any further cooking is of concern when these products become cross-contaminated with campylobacters, salmonellas or *E. coli* O157. Outbreaks associated with products imported from overseas demonstrate the potential for pathogens to cause illness far removed from the point of origin of the contamination.

These findings require that public health professionals investigating outbreaks consider salad items, fruit and vegetables as possible vehicles of infection.

Appendix 1.1 Line listing of outbreaks by year, vehicle of infection and levels of evidence

1992

Organism	Vehicle 1	Vehicle 2	Vehicle 3	Affected	Evidence
S. enteritidis PT4	Prawn fried rice	Crab meat	Beansprout	13	NR*
S. enteritidis PT4	Prawns	Salmon	Salad (pasta)	84	NR
S. enteritidis PT4	Salad			11	S†
S. typhimurium	Coriander			15	NR
Sh. sonnei	Salad (green)			16	S
Unknown	Coleslaw			63	S
Unknown	Lettuce/tomato			12	NR
Unknown	Melon			64	S
Total				278	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
NR*, Not recorded; S†, statistical

1993

Organism	Vehicle 1	Vehicle 2	Vehicle 3	Affected	Evidence
S. enteritidis PT4	Salad (potato)	-	-	18	S*
SRSV	Melon & papaya cockt	-	-	63	S
Unknown	Coleslaw	Beef	-	52	S
Unknown	Pate	Coleslaw	-	55	S
Unknown	Tuna salad	Pork	-	71	S
Total				259	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
S*, statistical

1994

Organism	Vehicle 1	Vehicle 2	Vehicle 3	Affected	Evidence
S. enteritidis PT4	Salad	-	-	65	S*
SRSV	Carrot (raw)	-	-	115	D†
SRSV	Salad (green, pasta)	Coleslaw	Prawn sand	47	S
SRSV	Watercress	-	-	38	D
S. enteritidis	Chicken salad	Salad (pasta)	Coleslaw	61	S
S. typhimurium	Pease pudding	-	-	18	M‡
S. typhimurium	Tuna sandwich	Salmon sandw	Salad (potato)	29	S
Campylobacter	Meat fajitas	Salad	-	8	D
Sh. sonnei	Lettuce (iceberg)	-	-	100	S
Other (E Agg EC)	Salad items	-	-	7	S
Unknown	Coleslaw	Salad	-	8	D‡
Unknown	Pork in red wine sauce	Salad	-	6	D

Unknown	Salad	-	-	69	S
Unknown	Salad (green)	-	-	14	D
Unknown	Salad (green)	-	-	31	S
Total				616	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
S*, Statistical; D†, Descriptive; M‡, Microbiological;

1995

Organism	Vehicle 1	Vehicle 2	Vehicle 3	Affected.	Evidence
SRSV	Salad	-	-	15	NR*
SRSV	Salad (mixed)	-	-	88	S†
SRSV	Salad (raw)	-	-	11	D‡
E. coli O157	Lettuce/tomato	-	-	5	NR
Unknown	Mushrooms (dried)	-	-	23	D
Total				142	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
NR*, Not recorded; S† Statistical; D‡, Descriptive

1996

Organism	Vehicle 1	Vehicle 2	Vehicle 3	Affected	Evidence
SRSV	Cold food	Salad	-	193	S*
SRSV	Potato salad	-	-	42	S, D†
SRSV	Salad (tomato & cucumber)	-	-	18	S
Campylobacter	Lettuce	Tomato	-	16	S
Sh. flexneri	Salad vegetables	-	-	9	D
Unknown	Chicken	Salad	Ice cream	5	D
Unknown	Chicken roll sandwich	Pork pie	Lettuce	9	S
Unknown	Coleslaw	Nuts	-	60	S
Unknown	Coleslaw	-	-	8	D
Unknown	Lettuce	-	-	18	D
Unknown	Potato salad	-	-	23	S
Unknown	Salad (mixed bean)	-	-	4	M‡
Total				405	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
S* Statistical; D†, Descriptive; M‡, Microbiological

1997

Organism	Vehicle 1	Vehicle 2	Vehicle 3	Affected	Evidence
S. enteritidis	Cauliflower	Cheese sauce	-	55	S*
E. coli O157	Salad (mixed)	French fries	-	8	S
Unknown	Orange juice	-	-	29	S
Total				92	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
S* Statistical

1998

Organism	Vehicle 1	Vehicle 2	Vehicle 3	Affected	Evidence
S. enteritidis PT4	Sandwiches	Cold sav. salad	-	29	S*
S. enteritidis PT4	Stuffed pepper	Spanish omelette	-	28	M†, D‡
S. enteritidis	Potato salad	Mayonnaise	-	13	S
Campylobacter	Lettuce	Mayo (garlic)	-	30	S, D
Unknown	Fresh fruit salad / melon	-	-	22	S
Total				122	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
S*, Statistical; M†, Microbiological; D‡, Descriptive

1999

Organism	Vehicle 1	Vehicle 2	Vehicle 3	Affected	Evidence
S. hindmarsh	Lamb Doner Kebab	Salads	Sauces	12	S*, D†
SRSV	Salad	Cheese	-	13	S
	Sandwiches Filling Salad	-	-	165	S
Unknown	Cheese	Beef	Salad	18	S
	Potatoes	-	-	9	S, D
	Salad	-	-	39	S, D
Total				256	

Source: GSURV database, Epidemiology Division, PHLS CDSC – 07/06/00
S*, Statistical; D†, Descriptive

SECTION 2. OUTBREAKS ASSOCIATED WITH READY TO EAT FRUIT AND VEGETABLES: AN INTERNATIONAL PERSPECTIVE.

This section has been reproduced from a discussion paper prepared for the Codex Alimentarius Commission (1998).

Only some foodborne outbreaks are reported to public health officials in countries having a foodborne disease reporting system. This is due in part to problems related to the identification of foodborne disease by the medical community and the identification of a food vehicle. In addition, many countries do not have such a system in place. Therefore, foodborne incidents associated with fresh produce may be under-reported on a world-wide basis and rarely reported in the scientific literature. The following list is not to be considered as exhaustive but rather as examples justifying the need for a Code of Practice.

Salmonella

Several outbreaks of salmonellosis have been associated with the consumption of fresh fruit and vegetables. In 1979 and 1991, Salmonella infections were associated with watermelons (Blostein, 1993; CDC, 1979). In 1990, cantaloupes from Southern Mexico were linked to *S. chester* infections (Ries et al., 1990) and in 1991 pre-sliced cantaloupes originating from Texas were linked to *S. poona* infections (CDC, 1991). Since these outbreaks, the produce industries of some countries have voluntarily implemented a Melon Quality Program.

An outbreak of *S. saint-paul* infection occurred in United Kingdom in 1988. This outbreak was associated with the consumption of bean sprouts obtained from several producers (O'Mahony et al., 1990). A similar outbreak of *S. saint-paul* infections, also associated with the consumption of bean sprouts, was reported in Sweden. Investigation concluded that the outbreak was caused by contamination of mung bean seeds (O'Mahony et al., 1990).

In 1995, *S. stanley* was epidemiologically linked to alfalfa sprouts in the US and Finland (Mahon et al., 1996). In 1996, another outbreak of *S. newport* infection was also associated with alfalfa sprouts inUS and Canada. The cause of these two outbreaks was traced to contaminated seeds distributed by the same shipper (Van Beneden, 1996). According to CDC, alfalfa sprouts were associated with two more outbreaks of salmonellosis in 1996 and 1997 (NACMCF, 1998). More outbreaks of *S. infantis* and *S. anatum* infections occurred in 1997 in the US. These outbreaks were also associated with the consumption of alfalfa sprouts and contaminated seeds were identified as the likely cause (NACMCF, 1998).

In 1997, ninety-two cases of salmonellosis caused by *S. meleagridis* were confirmed in the Canadian provinces British Columbia, Alberta, Saskatchewan, Manitoba and Ontario. Epidemiological investigation pointed to alfalfa sprouts as the likely source of infection. The suspected sprouts were all produced from the same seed lot (personal communication, 1998, Health Canada and Canadian Food Inspection Agency).

In 1990 and 1993, outbreaks of *S. javiana* and *S. montevideo* infections respectively occurred in US and were linked to the consumption of fresh tomatoes (Wood et al., 1991; CDC, 1993). A water bath used by the tomato packer was identified as the most likely source of contamination.

Shigella

Several outbreaks of shigellosis have been attributed to the consumption of contaminated raw vegetables. In 1985, an outbreak caused by *Shigella sonnei* and linked to the consumption of lettuce occurred at university campuses in the US (Martin et al., 1986). It was determined that this outbreak was caused by contaminated lettuce.

In 1994, a number of cases of *S. sonnei* infection occurred in several European countries, including Norway, Sweden and the United Kingdom. Epidemiological evidence indicated that iceberg lettuce from Spain was the likely source of infection (Frost et al., 1995; Kapperud et al., 1995).

In 1994, an outbreak of shigellosis was reported in the US. This outbreak was epidemiologically linked to the consumption of scallions (Cook et al., 1995) and was traced to green onions from US and Mexican farms. Evidence indicated that the contamination of green onions may have occurred at harvest or during packing.

Escherichia coli

In 1995, an outbreak of *Escherichia coli* O157:H7 infections was reported in the US and was epidemiologically linked to the consumption of lettuce (CDC, 1995a). Although the mechanism of contamination was not determined, it was understood that the most likely cause of this outbreak were contamination from irrigation water or unsanitary handling of lettuce. Also, in 1995, another outbreak of *E. coli* O157:H7 infections was linked to the consumption of iceberg lettuce in the US (CDC, 1995b). The probable cause of this outbreak was cross-contamination from meat products during food preparation or storage.

Two additional outbreaks of *E. coli* O157:H7 involving mesclun mix lettuce in US were reported to CDC in 1996. Epidemiological data from these two outbreaks implicated the same grower. *Escherichia coli* O157:H7 was not isolated on the farm of this grower but poor manufacturing practices were observed in the on-farm processing facility. Potential sources of bovine and avian fecal contamination were also present on the farm (NACMCF, 1998).

Other infections due to enterotoxogenic *E. coli* from carrots were reported in 1993 in the US. A total of 168 cases was reported (CDC, 1994). The cause of contamination is unknown.

The world's largest reported outbreak of *E. coli* O157:H7 to date occurred in Japan in 1996. This outbreak affected 6,000 people and involved the death of three school children. This outbreak was linked to the consumption of raw radish sprouts served in school lunches which were prepared in central kitchens. After considerable investigation the potential source of contamination remains unknown (Michino et al., 1996). In two other outbreaks of *E. coli* O157:H7 in Japan in 1997 the O157 O antigen synthesis gene DNA (orf2) and VT1 and VT2 genes were detected in sprouted radish seeds. However, the organism could not be cultured from unsprouted radish seeds (Onuma et al., 1998).

Another recent outbreak of *E. coli* O157 occurred in 1997 in the US and was linked to the consumption of alfalfa sprouts (CDC, 1997b). Microbiological analyses of the seeds used to sprout this produce failed to isolate the organism and the potential cause of this outbreak is still unknown.

Listeria

An outbreak of *Listeria monocytogenes* occurred in 1981 in the Maritime provinces of Canada. A case-control survey of the implicated individuals indicated that coleslaw was the most likely cause of this outbreak. Further investigation revealed that the cabbage used for preparing the coleslaw may have been contaminated with *L. monocytogenes* from sheep manure at the farm (Schlech, et al., 1983).

Cyclospora

In the summer of 1996, a large outbreak of *Cyclospora cayetanensis* infections occurred in Canada and US (Herwaldt and Ackers, 1997). After several epidemiological investigations, this outbreak was linked to Guatemalan raspberries. Although the growing areas of some of these raspberries were identified, no oocysts of *C. cayetanensis* were detected in berries or environmental samples obtained from these growing areas. The lack of a conclusive link between the outbreak of *C. cayetanensis* and raspberries may be due to the limited methods available for detecting oocysts of *C. cayetanensis* from food or water, the non-availability of implicated berries, and the lack of understanding of the risk factors during production, picking and packaging.

More recently, in the summer of 1997 raspberries, mesclun, lettuce, basil and basil-containing products were associated with numerous outbreaks of *C. cayetanensis* in the US (CDC, 1997a). These recent outbreaks of *C. cayetanensis* implicated three different types of fresh produce and therefore emphasised the importance of strengthening prevention and control measures to promote the safety of such produce.

Viruses
Hepatitis A and Small Round Structured Viruses (SRSVs) are the most commonly documented viral contaminants in food. In 1998, an outbreak involving 202 cases of hepatitis A infection in Kentucky was linked to the consumption of commercially distributed lettuce (Rosenblum et al., 1990). The investigation suggested that contamination most likely occurred before local distribution.

Outbreaks of viral gastroenteritis have resulted from the handling of implicated food by ill food handlers. Salads were the implicated vehicle in some reported outbreaks (Griffin et al., 1982; Hedberg and Osterholm, 1993).

Helminths
Diseases due to helinths have been associated with consumption of fresh produce such as *Fasciola hepatica* infections associated with watercress (Facey and Marsden, 1960; Coudert and Triozon, 1957). One of these outbreaks was associated with contaminated irrigation water.

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SECTION 3. PRODUCTS OF PARTICULAR CONCERN

Tables 3.1 to 3.4 summarise the information currently available on outbreaks for which raw fruit and vegetables, or their products were strongly suspected as being the source. The data supplied is not intended to be fully comprehensive but rather is meant to illustrate the types of products and organisms of concern.

Intact Product

It was assumed that the product was intact unless available information indicated otherwise. Table 3.1 lists the products that fall into this category.

As one might suspect, given that it forms the largest potential group of product, this category comprises the majority of known outbreaks. Not surprisingly, the generic term "salad" is the most common product type but almost any fruit or vegetable can be implicated. As would be expected from the ecology of these products, almost any pathogen, bacterial, viral or protozoan can be associated with them. However, the overall frequency with which Enterovirulent *E. coli* and related organisms appears indicates cause for concern, particularly given the serious morbidity and mortality that can be associated with such organisms.

Cut Product

For the purposes of this report "Cut Product" is defined as "Any product where the intact protective surfaces of the plant have been breached or removed". The process of cutting can have four potential consequences:

1. It can remove the pathogen if it is present on the outside of the plant but only if the process is carried out in a proper hygienic manner.

2. It can give the pathogen access to the nutrients available on and from the inside of the plant. This can lead to multiplication of certain pathogens during storage but does not apply to pathogens that are incapable of growth, e.g. viruses.

3. It can spread the pathogen from contaminated to uncontaminated product as a result of inappropriate hygiene of large batches of the product during processing.

4. Any combination of the above.

Cut/sliced/skinned/shredded product
There are eight reports that can be assigned to this category (Table 3.2) As is perhaps to be expected the range of products and pathogens is broadly similar to that for Intact Products (Table 3.1).

Sprouted seeds
These are products where the germination stage breaches the barrier of the seed coat allowing pathogens access to the nutrients from the growing plant. Further, some seeds undergo a process known as "scarification" whereby the seed coat is mechanically scratched to allow ingress of water to improve germination. Inappropriate hygiene and temperature control during germination and subsequent storage and transport can provide conditions that will allow certain pathogens to multiply. For these reasons, sprouted seeds are considered to be a "special problem" particularly in the USA (Reference 1).

There are twelve reports listed for these products (Table 3.3). The organisms involved are primarily *Salmonella spp.* and Enterovirulent *E. coli*. Outbreaks can occur throughout the world and can cover more than one country, reflecting the international distribution of these products. In some countries, the perception that these are "healthy" products appears to be responsible for an increase in the amount and types of sprouted seeds that are consumed.

Fruit Juices
As with sprouted seeds, these products can potentially allow growth of the pathogen once contaminated. It is notable that products once thought to be "safer" because their acidic properties might restrict the growth of pathogens figure prominently on the list (Table 3.4. The well-documented acid tolerance of Enterovirulent *E. coli*, allied to its low infective dose, is likely to play a major role in this instance.

Products of Potential Concern
Changes in dietary habits and in the technologies available for processing food could raise new concerns not experienced to date. Two important factors to bear in mind are:

1. New products.
This would include the spread of new products in a country where they have hitherto been absent or restricted in use. An example would be the growth of the sprouted seeds market in the UK both in terms of new varieties and an increased volume of product.

2. New technologies.
A range of new technologies for minimally processing raw fruit and vegetables are becoming available. Examples would include Modified Atmosphere Packaging, new barrier films, ultrasonic decontamination and high pressure processing.

Tables 3.1 – 3.4. Outbreaks associated with products of particular concern

Table 3.1. Intact Product

Product	Origin	Country	Organism	Ref
Salad	Not reported	UK	S. enteritidis PT4	5
Potato salad	Not reported	UK	S. enteritidis PT4	5
Coriander	Not reported	UK	S. typhimurium	5
Melon	Not reported	USA	Salmonella javiana	2
Cantaloupe	“imported”	USA	Salmonella chester	2
Tomato	Not reported	USA	Salmonella javiana	2
Meney	Guatemala	USA	Salmonella typhi	3
Mango	Not reported	USA	Salmonella spp.	3
Lettuce Tomato	Not reported	UK	E.coli O157	5
Salad (mixed)	Not reported	UK	E.coli O157	5
Lettuce	Not reported	USA?	E.coli O157:H7	2
Salads	Not reported	USA	E.coli O157	4
Lettuce	Not reported	USA?	E.coli O157:H7	2
Lettuce	Not reported	UK	Campylobacter	5
Salad vegetables	Not reported	UK	Shigella flexneri	5
Salad	Not reported	UK	Shigella sonnei	5
Iceberg Lettuce	Spain	UK Sweden Germany	Shigella sonnei	6
Baby Corn	Thailand	Denmark	Shigella spp.	4
Parsley	Mexico	USA	Shigella spp.	3
Lettuce	Not reported	USA?	Hepatitis A virus	2
Frozen Raspberries	UK	UK	Hepatitis A virus	2
Frozen Strawberries	Not reported	USA	Hepatitis A virus	2
Tomato	Not reported	USA	Hepatitis A virus	2
Carrot	Not reported	UK	SRSV	5
Salad (mixed)	Not reported	UK	SRSV	5
Salad (green)	Not reported	UK	SRSV	5

Salad(raw)	Not reported	UK	SRSV	5
Salad(raw)	Not reported	UK	SRSV	5
Tomato & Cucumber	Not reported	UK	SRSV	5
Watercress	Not reported	UK	SRSV	5
Celery	? Non potable water	USA	Norwalk virus	2

Cut Product				
Table 3.2. Cut/sliced/skinned/shredded product				
Product	**Origin**	**Country**	**Organism**	**Ref**
Shredded coconut	Malaysia	UK	Salmonella java PT Dundee	6
Cantaloupe fruit salad	Mexico?	USA Canada	Salmonella poona	6
Watermelon	Not reported	USA?	Salmonella Oranienburg	2
Shredded lettuce	Not reported	USA	Shigella spp.	2
Coleslaw	Not reported	USA	E.coli O157	4
Cabbage?	Canada	Canada	Listeria monocytogenes	2
Sliced Raw Vegetables	Not reported	USA	Giardia lambia	4
Fruit Salad	Not reported	USA	Giardia lambia	4
Table 3.3 Sprouted seeds				
Product	**Origin**	**Country**	**Organism**	**Ref**
Alfalfa	Netherlands?	Denmark Canada	Salmonella Newport	1
Alfalfa	Not reported	Sweden Finland	Salmonella Bovismorbidificans	1
Cress	Not reported	UK	Salmonella Gold-Coast	1
Mung Bean	Australia Thailand	UK Europe	Salmonella St Paul	7
Alfalfa	Various origins	Finland USA	Salmonella stanley	6
Radish	Not reported	Japan	E.coli O157:H7	7
Alfalfa	USA?	USA	E.coli O157:H7	7
Alfalfa / clover	USA	USA	Salmonella senftenberg	1, 7
Soy, cress, mustard	Not reported	USA	Bacillus cereus	7

Alfalfa	Not reported	USA Canada	Salmonella Newport	1
Alfalfa	USA (Chicken & Horse manure?)	USA Canada	Salmonella Montevideo Salmonella Meleagridis	7
Alfalfa, radish, snow pea, rose	USA	USA	Salmonella Infantis Salmonella Anatum	1

Table 3.4. Fruit Juices

Product	Origin	Country	Organism	Ref
Melon & Papaya	Not reported	UK	SRSV	5
Apple cider	USA	USA	E.coli O157:H7	2
Apple cider	USA	USA	Salmonella typhimurium	2
Apple cider	USA	USA	Cryptosporidium spp.	2
Frozen coconut milk	"imported"	USA	Vibrio cholerae O1	
Orange Juice	Not reported	USA	Salmonella Hartford	2
Orange Juice	Not reported	USA	Salmonella Anatum	3
Orange Juice	Not reported	USA	Salmonella Typhi	3
Orange Juice	Not reported	USA Canada	Salmonella Muenchen	3
Orange Juice	Not reported	USA	Salmonella Anatum	3
Orange Juice	Not reported	USA	Bacillus cereus	3

Section 3 References

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SECTION 4. THE MICROBIOLOGICAL STATUS OF READY TO EAT FRUIT AND VEGETABLES.

Two studies of the microbiological status of ready to eat fruit and vegetables have been conducted in England and Wales in recent years.

ECCFCP Study of Refrigerated Salads and Crudités

The European Community Coordinated Food Control Programme (ECCFCP) fulfils the requirement for Member States to carry out a coordinated programme of inspection and sampling of foods for microbiological parameters. In the UK it has been implemented through the Department of Health. In 1995 the PHLS, along with some non-PHLS laboratories, contributed to by testing the microbiological quality of 2552 samples of refrigerated salads and crudités (Little *et al*., 1997). Samples were examined for *Listeria spp.*, *L. monocytogenes*, *E.coli* and *Aeromonas spp.* following the protocol required by EC Recommendation 95/77/EEC. Five (0.2%) samples were considered to be of unacceptable quality when judged against the PHLS Microbiological Guidelines for Ready to Eat Foods (Gilbert et al., 1996). One per cent of salads and 2% of crudités had *E. coli* counts of 10^2 cfu or more per gram and 0.2% of salads and 0.4% of crudités had *E. coli* counts of 10^4 cfu or more per gram. *Listeria spp.* and *L. monocytogenes* were present in low numbers in 3% of salads and in less than 1% of crudités. *Aeromonas spp.* were detected in 54% of salads and in 55% of crudités.

As far as is known, the EC has not produced a formal report of the EC wide study other than a two page summary, which is reproduced in Appendix 4.1. It is available from
http://europe.eu.int/comm/dg24/health/afh/afh03_en.html.

PHLS Study of Retail Unprepared Whole Lettuces

In 1998 the PHLS undertook a small study of the microbiological quality of retail imported unprepared whole lettuces in England and Wales (Little et al.,1999). No *Salmonella spp., Shigella spp., Campylobacter spp., E. coli* O157:H7, *E. coli, Vibrio cholerae* or *Listeria monocytogenes* were recovered from any of the 151 samples examined.

LACOTS/PHLS Study of Ready to Eat Organic Fruit and Vegetables

LACOTS/PHLS studies are designed to maximise the gains from routine EHO sampling by ensuring that samples are taken and examined in a coordinated fashion according to a predetermined protocol. These studies are not formal structured surveys in that Local Authorities can choose whether or not to participate and the sampling is not random. Nevertheless they are a useful adjunct to formal structured surveys in that they are easy and quick to set up, use resources that are already in place, are published quickly in peer reviewed journals and can provide useful pointers for the design of more structured formal surveys. Two such surveys are normally carried out each year.

In late 1999 the PHLS received repeated requests from the media for information on the microbial pathogens carried by organic foods. No such information existed. In view of this, and in response to a number of requests from LACOTS/PHLS Food Liaison Groups around the country, a study was designed in consultation with EHOs and the Soil Association**.**

The objectives of the study are:

- To identify the microbiological quality of uncooked ready to eat organic vegetables from a range of retail outlets.

- To use this information in support of the PHLS Microbiological Guidelines for Ready to Eat Foods.

- To use the information and experience gained in order to determine the requirements for further studies into this product area.

In many ways the study is an initial fact-finding exercise. It is specifically not designed to compare, for example, domestic versus imported product or, organic versus non-organic product. Such studies would require the resources available only to agencies such as the Food Standards Agency. However, it would be a useful starting point in determining the approach that would need to be taken for such a survey.

The study will run from 1st May to 30th June 2000.

Proposed MAFF/DH Surveys.

The MAFF/DH JFSSG Microbiological Safety of Food Surveillance Group has proposed surveys of Prepared Pre-Packed Salads and of Sprouted Seeds. The status of these proposals is not known.

Other studies

Table 4.2 is derived from a WHO review of fruits and vegetables eaten raw (WHO, 1998). It can be seen that there is surprisingly little information available regarding the microbiological status of RTE fruit and vegetables in the international arena resulting from formal structured surveys. Less than half of the studies reported are based on a sample size of more than 100. Only three studies appear to have involved more than 250 samples.

Table 4.2 Bacterial Pathogens Isolated From Raw Vegetables

Vegetable	Country	Pathogen	Prevalence
Alfalfa	United States	*Aeromonas*	Not given
Artichoke	Spain	*Salmonella*	3 / 25 (12.0%)
Asparagus	United States	*Aeromonas*	Not given
Bean Sprouts	Malaysia	*L.monocytogenes*	6 / 7 (85%)
	Malaysia	*Salmonella*	2 / 10 (20%)
	Sweden	*Salmonella*	Not given
	Thailand	*Salmonella*	30 / 344 (8.7%)
Beet Leaves	Spain	*Salmonella*	4 / 52 (7.7%)
Broccoli	Canada	*L.monocytogenes*	2 / 15 (13.3%)
	United States	*Aeromonas*	Not given
	United States	*Aeromonas*	5 / 16 (31.3%)
Cabbage	Canada	*L.monocytogenes*	2 / 92 (2.2%)
	Canada	*L.monocytogenes*	1 / 15 (6.7%)
	Mexico	*E.coli* O157:H7	1 / 4 (25.0%)
	Peru	*V.cholerae*	Not given
	Saudi Arabia	*L.monocytogenes*	Not given
	Saudi Arabia	*Y.enterocolitica*	Not given
	Spain	*Salmonella*	7 / 41 (17.1%)
	United States	*C.botulinum*	1 / 337 (0.3%)
	United States	*L.monocytogenes*	1 / 92 (1.1%)
Carrot	Lebanon	*Staphylococcus*	(14.3%)
	Saudi Arabia	*L.monocytogenes*	Not given
	Saudi Arabia	*Y.enterocolitica*	Not given
Cauliflower	Netherlands	*Salmonella*	1 / 13 (7.7%)
	Spain	*Salmonella*	1 / 23 (4.5%)
	United States	*Aeromonas*	Not given
Celery	Mexico	*E.coli* O157:H7	6 / 34 (17.6%)
	Spain	*Salmonella*	2 / 26 (7.7%)
Chili	Surinam	*Salmonella*	5 / 16 (31.3%)
Cilantro	Mexico	*E.coli* O157:H7	8 / 41 (19.5%)
Coriander	Mexico	*E.coli* O157:H7	2 / 10 (20.0%)
Cress Sprouts	United States	*B.cereus*	Not given
Cucumber	Malaysia	*L.monocytogenes*	4 / 5 (80%)
	Pakistan	*L.monocytogenes*	1 / 15 (6.7%)
	Saudi Arabia	*L.monocytogenes*	Not given
	Saudi Arabia	*Y.enterocolitica*	Not given
Vegetable	**Country**	**Pathogen**	**Prevalence**
	United States	*L.monocytogenes*	Not given
Egg Plant	Netherlands	*Salmonella*	2 / 13 (1.5%)

Endive	Netherlands	*Salmonella*	2 / 26 (7.7%)
Fennel	Italy	*Salmonella*	4 / 89 (71.9%)
Green Onion	Canada	*Campylobacter*	1 / 40 (2.5%)
Leafy Vegetables	Malaysia	*Salmonella*	1 / 24 (4%)
	Malaysia	*L.monocytogenes*	5 / 22 (22.7%)
Leeks	Spain	*L.monocytogenes*	1 / 5 (20%)
Lettuce	Italy	*Salmonella*	82 / 120 (68%)
	Canada	*Campylobacter*	2 / 67 (3.1%)
	Canada	*L.monocytogenes*	3 / 15 (20%)
	Lebanon	*Staphylococcus*	(14.3%)
	Netherlands	*Salmonella*	2 / 28 (7.1%)
	Saudi Arabia	*L.monocytogenes*	Not given
	Saudi Arabia	*Y.enterocolitica*	Not given
	Spain	*Salmonella*	Not given
	United States	*Aeromonas*	Not given
Mungbean Sprouts	United States	*Salmonella*	Not given
Mushrooms	United States	*C.jejuni*	3 / 200 (1.5%)
Mustard Cress	United Kingdom	*Salmonella*	Not given
Mustard Sprouts	United States	*B.cereus*	Not given
	Canada	*Campylobacter*	1 / 42 (2.4%)
Parsley	Egypt	*Shigella*	1.250 (0.4%)
	Lebanon	*Staphylococcus*	(7.7%)
	Spain	*Salmonella*	1 / 23 (4.3%)
Pepper	Canada	*L.monocytogenes*	1 / 15 (6.7%)
	Sweden	*Salmonella*	Not given
	United States	*C.botulinum*	1 / 201 (0.5%)
	United Staes	*Aeromonas*	Not given
Potatoes	Saudi Arabia	*L.monocytogenes*	Not given
	Saudi Arabia	*Y.enterocolitica*	Not given
	Spain	*L.monocytogenes*	2 / 12 (16.7%)
	United States	*L.monocytogenes*	19 / 70 (27.1%)
	United States	*L.monocytogenes*	28 / 132 (21.1%)
	Canada	*Campylobacter*	1 / 63 (1.6%)
Prepacked Salads	Northern Ireland	*L.moncytogenes*	3 / 21 (14.3%)
	United Kingdom	*L.monocytogenes*	4 / 60 (13.3%)
	United Kingdom	*L.monocytogenes*	Not given
Radish	Lebanon	*Staphylococcus*	(6.3%)
	Saudi Arabia	*L.monocytogenes*	Not given
Vegetable	**Country**	**Pathogen**	**Prevalence**
	Saudi Arabia	*Y.enterocolitica*	Not given
	United States	*L.monocytogenes*	25 / 68 (36.8%)
	Canada	*Campylobacter*	2 / 74 (2.7%)

	United States	*L.monocytogenes*	19 / 132 (14.4%)
Salad Greens	Egypt	*Salmonella*	1 / 250 (0.4%)
	United Kingdom	*S.aureus*	13 / 256 (5.1%)
Salad Vegetables	Canada	*L.monocytogenes*	6.15 (40%)
	Egypt	*Shigella*	3 / 250 (1.2%)
	Egypt	*S.aureus*	3 / 36 (8.3%)
	Spain	*Aeromonas*	2 / 33 (6.1%)
Salad Vegetables	Spain	*L.monocytogenes*	21 / 70 (30%)
	United States	*Staphylococcus*	Not given
	Germany	*L.monocytogenes*	6 / 263 (2.3%)
	Northern Ireland	*L.monocytogenes*	4 / 16 (25%)
	United States	*C.botulinum*	2 / 82 (2.4%)
	United Kingdom	*Y.enterocolitica*	Not given
Seed Sprouts	Canada	*Staphylococcus*	13 / 54 (24%)
Soybean Sprouts	United States	*B.cereus*	Not given
Spinach	Canada	*Campylobacter*	Not given
	Spain	*Salmonella*	2 / 60 (3.3%)
	United States	*Aeromonas*	2 / 38 (5.2%)
Sprouting Seeds	United States	*B.cereus*	56 / 98 (57%)
Tomato	Pakistan	*L.monocytogenes*	2 / 15 (13.3%)
Vegetables	Egypt	*Salmonella*	2 / 250 (0.8%)
	France	*Y.enterocolitica*	4 / 58 (7%)
	France	*Y.enterocolitica*	15 / 30 (50%)
	Iraq	*Salmonella*	3 / 43 (7%)
	Italy	*L.monocytogenes*	7 / 102 (6.9%)
	Italy	*Y.enterocolitica*	1 / 102 (1.0%)
	Spain	*L.monocytogenes*	8 / 103 (7.8%)
	Spain	*Salmonella*	46 / 849 (5.4%)
	Taiwan	*L.monocytogenes*	6 / 49 (12.2%)
	United Kingdom	*L.monocytogenes*	4 / 64 (6.2%)
	United States	*Salmonella*	4 / 50 (8.0%)

Appendix 4.1. Report on the ECCFCP Study of Refrigerated Salads and Crudités
Source: http://europe.eu.int/comm/dg24/health/afh/afh03_en.html

Listeria monocytogenes, Escherichia coli **and** ***Aeromonas*** **in refrigerated salads and seasoned crudités**

This element had been done by 13 Member States. Next table shows the summary of the results per gram of products.

	NUMBER OF SAMPLES	NOT DETECTED <10	>10-10^2	>10^2
Listeria m.	7046	3656	561	10
Escherichia c.	7059	5537	459	177
Aeromonas h.	6050	3663	653	86

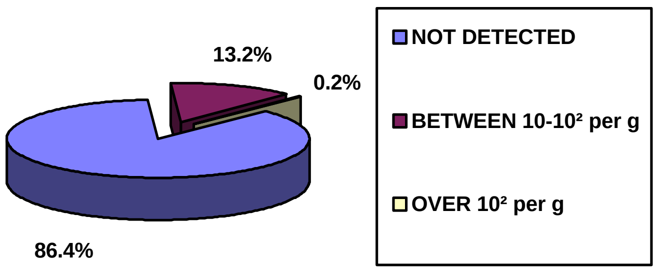
The statutary limit or guide value varies for each microorganism as follows:

Listeria monocytogenes : from absence to 10^4
Escherichia coli : from 10^2 to 10^3
Aeromonas hydrophila : from 10^2 to 10^7

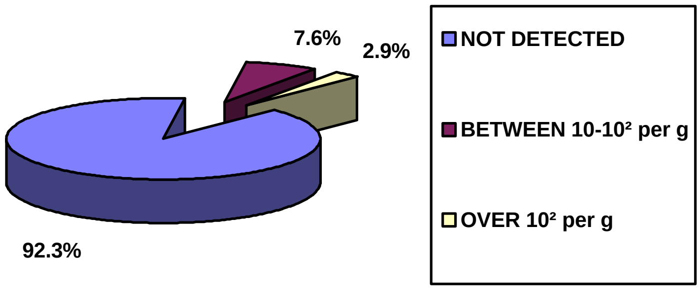
Several Members States did not indicate any limit or guide value.From the results transmitted, one can assume that many samples were not analysed.

If one uses 10^2 as limit or guide value for all the germs, the following charts can be drawn:

Listeria monocytogenes



Escherichia coli



Aeromonas hydrophila

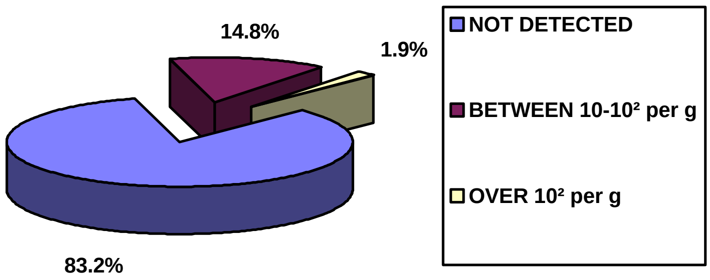


Table 2.1: Listeria monocytogenes in refrigerated salads and seasoned crudités per gram of product.

MEMBER STATE	NUMBER OF SAMPLES	NOT DETECTED -10	$10-10^2$	10^2-10^3	10^3-10^4	10^4-10^5	10^5-10^6	$>10^6$	LIMIT OR GUIDE VALUE FOR REJECTION
AT	251		251						
B	106	104	2						>100
DK	40	38	1						>10
D	2246	2097	143	2	1				$>=10^2-10^4$
EL	23	22	1						
E	658	634	24	0	0	0	0	0	10^2
FI	147	147							0
I	61	61							
L	24	24							Presence in 1g
NL	279	209	70						10^2
P	14	14							
S	35	35							
UK	3162	271	69	5	1	1			
TOTAL	7046	3656	561	7	2	1			

Table 2.2: Escherichia coli in refrigerated salads and seasoned crudités per gram of product.

MEMBER STATE	NUMBER OF SAMPLES	NOT DETECTED	10-10^2	10^2-10^3	10^3-10^4	10^4 -10^5	10^5 -10^6	>10^6	LIMIT OR GUIDE VALUE FOR REJECTION
AT	251		250	1					
B	107	103	1	1	1	1			>100
DK	138	125	3	10					
D	2200	1955	142	57	36	6	1	1	>=10^2-10^3
EL	23	21	1	1					
E	687	648	20	14	3	2	0	0	Absence/g
FI	147	143	4						
I	61	57	4						
L	24	14	6	4					
NL	258	228	28	1	1				
P	16	16							
S	35	35							10^2
UK	3112	2192	144	19	12	2	3		
TOTAL	7059	5537	459	108	53	11	4	1	

Table 2.3: Aeromonas hydrophila in refrigerated salads and seasoned crudités per gram of product.

MEMBER STATE	NUMBER OF SAMPLES	NOT DETECTED	10-10^2	10^2-10^3	10^3-10^4	10^4 -10^5	10^5 -10^6	>10^6	LIMIT OR GUIDE VALUE FOR REJECTION
AT	251		250			1			
B	101	100					1		>100
DK	28		27	1					
D	1492	1346	118	9	5	2	9	2	10^4 -10^7
EL	23	21		2					
E	624	601	14	6	1	1	1	0	10^2-10^3 ufc/g
FI	147	144			1	1	1		
I	60	52		2	1	5			
L	24	7	6	2	5	4			
NL	256		237	5	6	5	3		
P	14	14							
S	20	15	1	3		1			
UK	3010	1363							
TOTAL	6050	3663	653	30	19	20	15	2	

Section 4. References

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SECTION 5. CONCLUSIONS AND AREAS REQUIRING FURTHER WORK

1. Available information suggests that RTE fruit and vegetables account for a very small proportion (4.3%) of outbreaks of infectious intestinal disease in England and Wales.

2. Guidance is required outlining when and how to include consideration of these products in investigations of foodborne infectious intestinal disease.

3. Information on factors contributing to outbreaks suggests the need for food hygiene training for people handling food.

4. Information on the microbiological status of RTE fruit and vegetables is sparse. There is a need to gather further information on the microbiological status of a wide range of RTE fruit and vegetables, particularly with respect to pathogens such as Enterovirulent *E.coli*.

5. The microbiological status of RTE organic fruit and vegetables needs to be examined but it needs to be stressed that the "safety" of organic versus non-organic products will require a detailed Microbiological Risk Assessment – a comparative survey is not enough.

6. Evidence from abroad suggests that Sprouted Seeds and Fruit Juices can be pose specific problems, particularly with respect to Enterovirulent *E. coli.* Although these products do not appear to yet cause significant outbreaks in the UK, preventative measures should be reviewed to ensure that this remains the case.