

Department of Food and Environmental Hygiene  
Faculty of Veterinary Medicine  
University of Helsinki  
Helsinki, Finland

# HYGIENIC QUALITY OF FOODS SERVED ON AIRCRAFT

Maija Hatakka

ACADEMIC DISSERTATION

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To the memory of my father

Nothing is impossible.  
That what is difficult can be done immediately.  
The impossible takes a little longer.

*Lord Baden-Powell*

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# Abstract

This study set out to evaluate the microbiological hazards associated with the foods served on aircraft. To identify the microbiological quality of hot and cold meals prepared worldwide, samples of aircraft meals were monitored for *Salmonella* between 1989 and 1994, and for indicator bacteria and *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens* between 1991 and 1994. Additionally, a *Salmonella* outbreak among air passengers was investigated to evaluate risk factors for the contamination and growth of *Salmonella* in aircraft meals. In order to find out about the carriage of *S. aureus* among flight catering employees, hand and nasal samples were taken and the carriage was further monitored by characterising the enterotoxicity and the macrorestriction patterns of the bacterial strains.

The monitoring of hot and cold meals revealed *Salmonella* in 7 (0.3%) out of 2 299 hot meal samples, but in only one (0.1%) out of 1 576 cold meal samples. Although *Salmonella* was found in cold meals only once, this finding of *Salmonella enterica* serovar Ohio was subsequently linked to a foodborne outbreak among air passengers on a flight from Bangkok to Helsinki in 1990. There was no evidence that any of the *Salmonella* positive hot meals had caused any outbreaks. Many of the hot and cold meals also exceeded the microbiological standards of the Association of European Airlines (AEA) for *Escherichia coli* (8.2% and 14%, respectively), *S. aureus* (0.6% and 7%, respectively) and *B. cereus* (0.7% and 3%, respectively). The contamination rate by these bacteria was thus considerably higher in cold meals than in hot meals. The final re-heating of hot meals on board is likely to reduce the microbial risk associated with foodborne pathogens because heating food affects the viability of the bacteria. Significant differences were detected in the microbiological quality of the meals depending on the meal preparing country.

A widespread outbreak caused by *Salmonella enterica* serovar Infantis via food prepared in a flight kitchen was investigated. Of those infected, 91 were air passengers on a charter flight from Helsinki to Rhodes, 107 were railway passengers and 28 were flight kitchen employees, including 23 food handlers. The majority of the food handlers (17) were symptom-free carriers and six of them had mild diarrhoea. Widespread contamination of the production of the flight kitchen followed which led to an outbreak of *Salmonella*. The source of infection was traced to the following foods: egg sandwiches served on trains, the aircraft meals served on that particular flight to Rhodes and cold cuts served to the catering staff during breakfast. *S. Infantis* was found in a hot dish that represented the batch served on the flight to Rhodes. The most prominent factor relating to food contamination was that food handlers suffering from mild diarrhoea were not excluded from work, and there was no hygiene education either. A heat wave combined with a shortage of refrigeration facilities and the possible malfunction of the re-heating oven on board contributed to the multiplication of *Salmonella*. After the outbreak, the company employed a food hygienist responsible for food hygiene expertise, such as the implementation of quality assurance system and hygiene education of food handlers.

The study of the carriage of *S. aureus* among flight catering employees showed a remarkable prevalence of enterotoxigenic *S. aureus* in hand and nasal samples, 6% and 12% respectively. Nasal carriers can easily transmit *S. aureus* onto the hands, which means a potential risk of food poisoning if the strain is producing enterotoxin. Nasal sampling was shown to be a better way of detecting *S. aureus* carriers than hand sampling. PFGE macrorestriction profiles revealed a total of 32 different types associated with the 35 employees carrying *S. aureus*, thus indicating great diversity. Molecular characterisation of isolates is of great value, especially if there is a need to trace the contamination source. It also revealed that some food handlers carried more than one clone. Testing food handlers working in high-risk premises such as flight kitchens, provides valuable information about carriers and helps in planning preventive measures.

The production of aircraft meals is a high-risk mass catering operation that has global dimensions. Microbiological hazards are the most prominent risk factors connected with this kind of food production. They arise owing to the complexity of the operation in the flight kitchen, long food production chains and on board service with limited facilities. Therefore, strict quality assurance based on the hazard analysis critical control point (HACCP) system should be applied by the flight caterers and the expertise of the official food control authorities should also meet the requirements of this special catering branch.

# Abbreviations

AEA	the Association of European Airlines
CCP	critical control point
CDC	Centers for Disease Control and Prevention, Atlanta, Georgia
cfu	colony-forming units
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
ETEC	enterotoxigenic <i>Escherichia coli</i>
GHP	good hygiene practice
GMP	good manufacturing practice
HACCP	hazard analysis critical control point
IFCA	International Flight Catering Association
JIT	just in time
KTL	National Public Health Institute, Finland
LSG	Lufthansa Service Gesellschaft
MMWR	Morbidity and Mortality Weekly Report
NCFA	Nordic Committee on Food Analysis
PFGE	pulsed-field gel electrophoresis
PHLS	Public Health Laboratory Service, London, UK
ppm	parts per million
spp.	species
WHO	World Health Organization

# List of Original Publications

The present study is based on the following original articles referred to in the text by the Roman numerals I to V.

- |     |   |     |
|-----|---|-----|
| I   | Hatakka M. Microbiological quality of hot meals served by airlines. <i>J. Food Prot.</i> 1998;61: 1052-1056.  | I   |
| II  | Hatakka M. Microbiological quality of cold meals served by airlines. <i>J. Food Safety</i> 1998; 18: 185-195.   | II  |
| III | Hatakka M. and Asplund K. The occurrence of <i>Salmonella</i> in airline meals. <i>Acta vet. Scand.</i> 1993; 34: 391-396.  | III |
| IV  | Hatakka M. <i>Salmonella</i> outbreak among railway and airline passengers. <i>Acta vet. Scand.</i> 1992; 33: 253-260.  | IV  |
| V   | Hatakka M., Björkroth J., Asplund K., Mäki-Petäys N., and Korkeala H. Genotypes and enterotoxicity of <i>Staphylococcus aureus</i> isolated from hands and nasal cavities of flight catering -employees. <i>J. Food Prot.</i> (in press). | V   |

# 1. INTRODUCTION

The first regular airline passenger service began in 1919 in Europe, between England and France, and food has been served on aircraft since the outset of this operation (Jones and Kipps 1995). Initially the service included sandwiches, tea and coffee, but in the mid-1930s hot meals began to be served.

The advent of the jet aircraft in passenger services in the mid-1960s contributed to the growth of mass tourism. In 1950, there were 25 million international tourist arrivals, in 1960, 69 million, in 1970, 160 million and in the 1990s, 400-600 million tourist arrivals recorded worldwide yearly (Jones and Kipps 1995, World Tourism Organization 2000). This huge increase in air traffic has created a need for a certain type of mass catering. The scope can vary from a small kitchen to a large catering establishment producing up to 40 000 meals per day (Kirk 1995), including provisions for long-haul flights and handling the detailed specifications for many different airlines. A large flight kitchen may have contracts with tens of airlines. The way food is prepared today in large units resembles processing in a food manufacturing plant rather than a catering kitchen.

The provision of meals on aircraft gives rise to many food hygiene problems. Galley space and sanitary facilities on aeroplanes are very limited. Serious problems may arise if a major food poisoning outbreak occurs on board and the aircraft is far away from an airport and from adequate medical services. Foodborne illness during a flight can be extremely serious, especially during prolonged international flights. On a flight from Lima, Peru, to Los Angeles in 1992, 75 passengers went down with cholera (Eberhart-Phillips et al. 1996). Ten of them were hospitalised and one died. These emergencies often present a management dilemma because of the limited medical resources available on board (Godil and Godil 1997). In addition, certain problems specific to air travel complicate the recognition and investigation of outbreaks caused by meals served on aircraft. E.g. if a causative agent has a longer incubation period than the flight takes, passengers become ill after disembarkation. Therefore it may be difficult to recognise a cluster of a foodborne illness among travellers from many different countries and to trace the origin of the outbreak.

It is important to identify the hazards associated with aircraft meals and to develop efficient control methods. Regular microbiological testing of food as a part of the quality assurance system of flight kitchen is necessary to ensure the safety of meals. Controlling the health status of food handling staff and training in food hygiene field is of great importance.

## 2. REVIEW OF LITERATURE

### 2.1 Flight kitchen operation

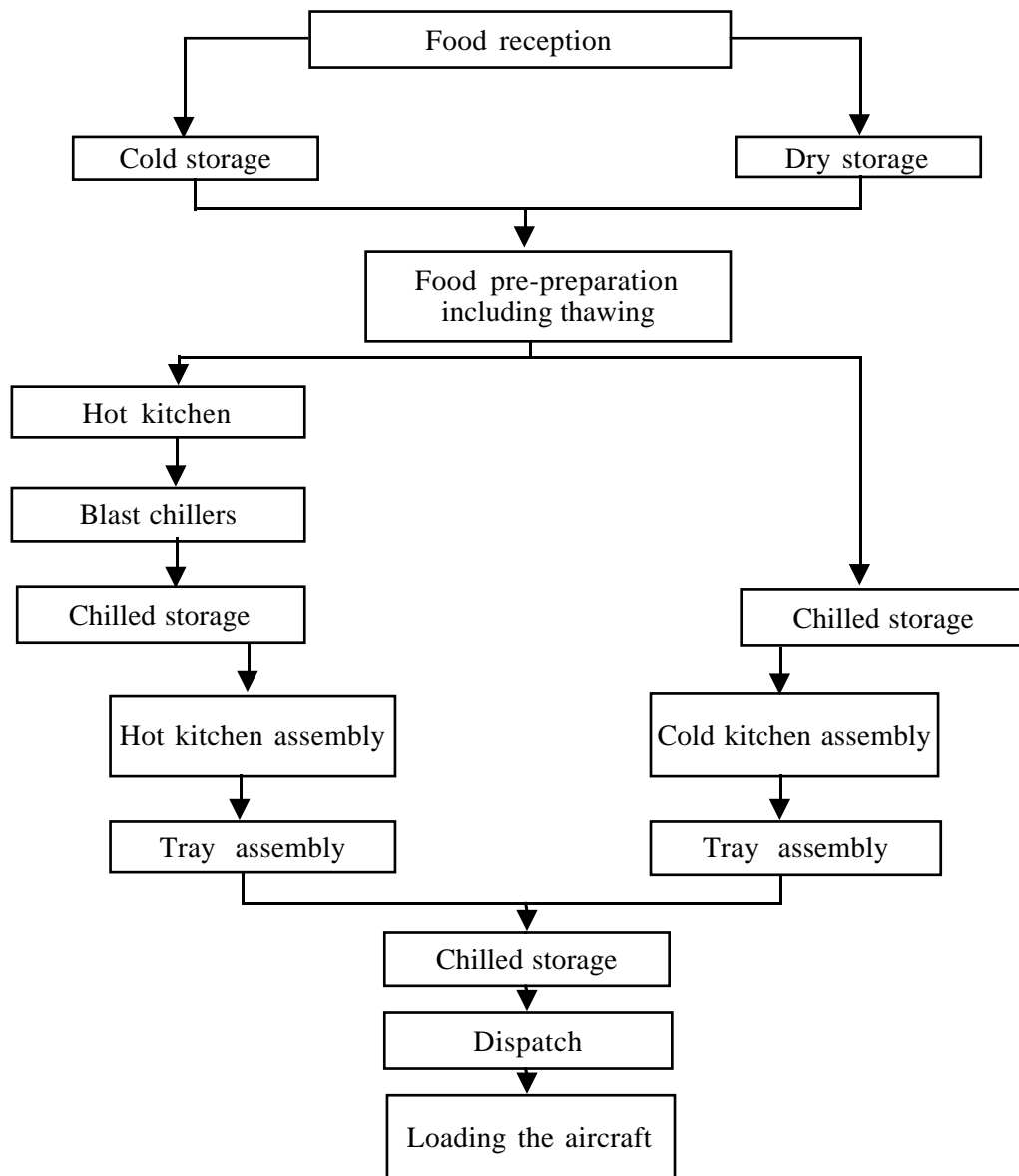
Flight kitchen production is a typical form of mass catering, but has some unique features distinct from food preparation in restaurants and hotels. The time difference between food production in the flight kitchen and finally serving it on board an aircraft with limited kitchen facilities makes flight catering a high-risk food preparation operation. The complexity of the production procedures in the flight kitchen also increases the microbiological hazards associated with this type of food preparation. Major factors affecting the hygienic quality of the food are the size of the operation, the complexity of the in-flight service, the number of airlines catered for, the number of flights serviced during the day and the duration of the flights to be serviced.

Since each airline has its own specification, the management of multiple contracts increases the complexity of the planning and control. Production planning for flight caterers equates to just in time production techniques (JIT), meaning producing the necessary units, in the necessary quantities, at the necessary time (Briggs and Nevett 1995, Foskett 1995). An airline company has to decide to what extent return catering will be carried out; whether to utilise the flight kitchens of foreign airports and whether to use local suppliers. Frozen meals may be carried if an aircraft is using food from its homeland during the return leg. In general, there is a growing trend in preparing frozen meals for aircraft (Asplund 2000). Economical and production considerations as well as hygienic reasons favour frozen meals. Microbiological examination of a batch can be carried out before it is used, thus ensuring the safety of the food. Using frozen meals reduces the likelihood of the temperature reaching the critical limits within which the bacterial growth may occur.

A typical flow chart for flight kitchens is shown in Fig. 1. Flight kitchens normally use a cook-chill system for the preparation of cooked items (Kirk 1995). Cooked items are then rapidly chilled in blast chillers, according to the Association of European Airlines (AEA 1996) within 4 hours from 65°C to 10°C, and according to LSG-Hygiene Institute (1997) from 60°C to 5°C. A cold kitchen is used for the preparation of snacks, appetisers, salads and desserts. Until portioning and packing, all prepared items are kept chilled. After making up the meal trays, the trays are loaded into a trolley for the flight. If necessary, trolleys are loaded with dry ice in order to minimise the temperature rise in the aircraft galley before the food is served.

### 2.2 Food handling on aircraft

Food storage and preparation for serving takes place in aircraft galleys, which mostly have very limited space and equipment for this purpose. In common with any kitchen, a galley has to provide the following: cold storage areas, regeneration ovens, water boilers and beverage machines and the stowage of waste products. On narrow-bodied aircraft, the meals



*Fig. 1. Flow chart of a flight kitchen.*

are kept chilled by using dry ice located within the trolley. Wide-body aircraft used for long-haul flights are today usually equipped with refrigerators or chiller units for trolleys (Goodwin 1995).

Chilled and frozen meals served hot must be re-heated, so that a core temperature at least 72°C is reached to destroy surviving pathogenic micro-organisms (LSG-Hygiene Institute 1997). In the 1970s, hot meal trays were transported to aircraft in hot ovens for short-haul flights and kept there until serving, the temperature of food being over 63°C (Bailey 1977).

Today, a cook-chill system is mostly used, although hot served foods can still be transported hot to small aircraft if they are not equipped with ovens (Asplund 2000).

## 2.3 Flight kitchen control

### 2.3.1 In-house control

The great need for food hygiene guidelines in flight kitchens was noticed as early as the 1960s (WHO 1960, Bailey 1977). A global survey of 25 flight kitchens showed that 30% had inadequate refrigeration facilities (Mossel and Hoogendoorn 1971). Time-temperature studies of flight kitchens in the United States in 1977 revealed that the equipment used did not always keep food appropriately hot or cold in the flight kitchen or while it was transported to the aircraft (Bryan et al. 1978). In 1984, 20% of American flights were holding food at improper temperatures (Tauxe et al. 1987). A WHO working group issued recommendations for flight catering in consequence of several outbreaks associated with meals served on board (WHO, Regional Office for Europe 1977). Documentation of hygiene training and instructions dealing with good hygiene practice have since been an important part of the quality system of flight kitchens.

With regard to food hygiene risks in airline catering operations microbiological hazards are the most important. Microbiological hazards are associated with the raw ingredients, staff and processes as well as serving on aircraft. Many flight kitchens now use the hazard analysis critical control point (HACCP) system (Gork 1993, Kirk 1995, LSG-Hygiene Institute 1997). In Europe, the European Commission (Council Directive 1993) has set the legal requirements for the food business to adopt a hazard analysis-based approach in food hygiene management. Many flight kitchens use the global quality policy described by LSG-Hygiene Institute (1997). LSG Lufthansa Service Holding AG is the biggest airline catering alliance and provides 390 million meals yearly. Their quality system consists of HACCP combined with quality requirements including standards, good manufacturing practice (GMP) and good hygiene practice (GHP).

While choosing menus for airlines, certain foods that can constitute a health hazard should be avoided as an important preventive measure. Components of aircraft meals can be placed into four risk categories: dangerous, high-risk, medium- and low-risk items (AEA 1996). Products that by nature can constitute a risk as a ready meal, either as such or due to improper heat treatment on board, are classified as dangerous items (Bailey 1977, AEA 1996). These items include dairy products containing raw milk, undercooked poultry and raw or undercooked eggs, raw meat, raw shellfish and raw fish. Neither should raw sprouts be used as components of cold meals due to known *Salmonella* outbreaks (Mahon et al. 1997, O'Mahony et al. 1990, Pönkä et al. 1995, Inami and Moler 1999, van Beneden et al. 1999).

Products which are intensively handled after heat treatment are classified as high-risk items. Such products include poultry and meat de-boned after cooking, stuffed eggs, cold cuts, glazing, cooked shellfish peeled after heat treatment. Medium-risk items have under-

gone a minimum of handling after heat treatment and include fermented and air-dried meats and sausages, stews, rice and pastas. Acidified foods (pH values below 4.6), fresh fruits that can be peeled prior to eating, canned fruits, bread and dry bakery items are considered to be low-risk items.

Food handlers are a potential source of pathogenic micro-organisms, and therefore training and practice for good personal hygiene is needed. Food handlers should have a medical examination prior to employment, and should be kept under regular medical surveillance (Bailey 1977, LSG-Hygiene Institute 1997). A person known or suspected to be suffering from a disease likely to be transmitted through food or any person afflicted with infected wounds, skin infections or sores should not be allowed to work in contact with any unpacked foods.

In order to ensure that food suppliers have implemented and maintain a sufficient control level in their production plant, flight caterers should audit their suppliers (Foskett 1995, LSG- Hygiene Institute 1997).

### **2.3.2 Official control**

The official control of flight kitchens depends on the national legislation of the country where the premises are located. Flight kitchens are subject to different requirements depending on the legislation of the country concerned. The authorities responsible for controlling flight kitchen operations must have good knowledge of the special features of this type of mass catering. The need for closer co-operation between airlines, local airport health authorities and national health administrations became apparent in the 1970s, when large outbreaks were reported in connection with growing mass tourism (WHO, Regional Office for Europe 1977).

### **2.3.3 Hygiene audits made by airline companies**

The last few decades have seen an emphasis on the global feature of flight kitchens serving international airlines. Many airline companies use standardised audit forms to perform regular hygiene audits of their suppliers (AEA 1996). The controlling authority and airline companies alike demand HACCP-based quality assurance. Non-compliance with even a single CCP means a failure to reach the AEA standard. Bacteriological results of food, drinking water and ice cubes are inspected to ensure that the buyer's specifications are being adhered to.

## **2.4 Microbiological control of hygiene in the flight kitchen**

A comparative study of visual inspections and microbiological sampling in high-risk premises showed that neither sampling nor visual assessment monitored the performance of the premises reliably (Tebbut 1989). A combined approach, using selective microbiological examination to support standardised inspections, was suggested for monitoring hygiene in premises preparing high-risk foods. Microbiological testing is needed within a HACCP programme for hazard identification, monitoring CCPs and verification of the HACCP

programme. The microbiological control includes testing of the whole production chain. Samples are taken from food at reception, prepared food items, process lines and environment, water, ice cubes, food handlers and, finally, ready meals.

#### **2.4.1 Surveillance of raw materials and ready meals**

Great emphasis must be placed on purchasing food items for the flight kitchen. Microbiological hazards are linked especially with raw materials. E.g. the prevalence of *Salmonella* and *Campylobacter* may be high in meat and poultry (Uyttendale et al. 1999, Boonmar et al. 1998). Faecal contamination of vegetables may be rather common especially in non-industrialised countries (Monge and Chinchilla 1996). Contaminated raw materials increase the risk of contamination of ready meals. *Listeria monocytogenes* must be taken into consideration particularly when purchasing vacuum-packed ready-to-eat fish products, where the prevalence of *L. monocytogenes* in up to 33% and 50% of cases were found (Lyhs et al. 1998, Johansson et al. 1999). Microbiological testing is needed to prove that the legal requirements as well as the customers specifications are met.

Some flight catering companies take daily traceable counter samples from final meals representing each production batch. These are kept for up to three weeks in a freezer (LSG-Hygiene Institute 1997, Asplund 2000). In case of complaint, the respective frozen samples are tested. In order to assure the safety of meals purchased, airline companies use random sampling from final meals according to a test schedule. These samples are mostly collected on board.

#### **2.4.2 Food contact surfaces and utensils**

Microbial biofilms that remain on surfaces after cleaning are of great concern in the food processing industry (Zottola and Sasahara 1994). Methods for measuring the efficiency of cleaning of the production environment are necessary in food premises manufacturing high-risk foods. Agar contact plates and the swabbing method can be used for hygiene control (NCFA 1987, Tebbut 1991). Commercial agar contact plates are also useful for the hygiene control of food premises (Rahkio and Korkeala 1997). They are mainly used for monitoring indicator bacteria. For specific micro-organisms, such as *Listeria* and *Salmonella*, selective enrichment and media must be chosen. The method of measuring adenosine-5'-triphosphate (ATP) bioluminescence gives results in a few minutes, thus making this system very suitable for on-line monitoring in HACCP programmes (Poullis et al. 1993, Vanne et al. 1996, De Boer and Beumer 1999). However, the ATP measured does not originate from bacteria only but the total ATP from all organic material on the surface. A significant opportunity for the future may be the provision of pathogen specificity to the ATP assays (Stewart 1997).

#### **2.4.3 Food handling staff**

The legal requirement in Finland demands that a food handler having travelled outside the Nordic countries must be tested for *Salmonella* (Anonymous 1994). In Finland, flight kitchen food handlers are additionally screened for *Salmonella* once a year (Asplund 2000). Many airline companies have imposed stricter rules than the legal requirements. A

*Salmonella* test from flight kitchen employees after travelling abroad, although not legally required, is demanded by many airlines.

Frequent microbiological tests on hands are useful to control hand hygiene. According to De Witt and Kampelmacher (1988), 8% of food handlers showed high numbers ( $>10^5$  /hand) of *Enterobacteriaceae* and *S. aureus* on their hands. Normal hand washing resulted in a lower number of transient micro-organisms, but however, it did not lower the number of *S. aureus*.

Food handlers harbouring enterotoxigenic strains of *S. aureus* constitute a potential source of contamination of food via the hands. The primary reservoir in people are the anterior nostrils, and nares are the most consistent area from which this organism can be isolated (Williams 1963). The nasal carriage of *S. aureus* results easily in transfer of the bacteria to the hands.

*Table 1. Microbiological limit values for food items used for aircraft meals according to the Association of European Airlines (AEA 1996).*

Food item	Total count cfu/g	Coliforms cfu/g	<i>E. coli</i> cfu/g	<i>S. aureus</i> cfu/g	<i>B. cereus</i> cfu/g	<i>C. perfringens</i> cfu/g	<i>Salmonella</i> spp. /25 g
Bulk items that have not been handled, only portioned after heat treatment (e.g. hot meats)	$5.0 \times 10^5$	$1.0 \times 10^3$	10	$1.0 \times 10^2$	$1.0 \times 10^3$	$1.0 \times 10^3$	D <sup>1</sup>
Items that have been handled after heat treatment (e.g. sandwiches, snacks, all cold)	$1.0 \times 10^6$	$1.0 \times 10^4$	10	$1.0 \times 10^2$	$1.0 \times 10^3$	$1.0 \times 10^3$	D
Undercooked items (e.g. vegetables, deep-frozen blanched vegetables, steaks that will receive no more heat treatment before leaving the flight kitchen)	NA <sup>2</sup>	NA	10	$1.0 \times 10^2$	NA	NA	D
Raw vegetables or raw fruits (examination after wash and/or disinfection)	NA	NA	10	NA	NA	NA	D
Cheeses	NA	NA	10	$1.0 \times 10^2$	NA	NA	D

<sup>1</sup>D Detected

<sup>2</sup>NA No analysis

## 2.5 Microbiological quality of meals served on aircraft

The AEA has issued recommendations for microbiological analyses and limits for aircraft food (1996) (Table 1). Bulk items, such as hot meats, which have been portioned after heat treatment should not exceed the value of  $5.0 \times 10^5$  cfu/g for total count and  $1.0 \times 10^3$  cfu/g for coliforms. For items that have been handled (e.g. slicing, cutting) after heat treatment, higher values of total count and coliforms are permitted. Although the results of the total count and coliforms can be higher than the limit values, the food is not considered to be unsafe, but according to the AEA (1996) an investigation of food production practice is advised. Enumeration of total bacteria and coliforms is not considered necessary for cold meals containing raw vegetables, fruits and garnishes as well as for undercooked items, because they naturally contain high counts of these bacteria. If the AEA limits for *Escherichia coli*, *S. aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Salmonella* spp. (Table 1) are exceeded, the food must be considered to be unsafe. Monitoring meals for indicators may reveal food processing or food handling errors but it is not advisable or valid to predict the safety of food based on these indicators alone (Tompkin 1983, Sofos et al. 1999).

Many airline companies demand stricter microbiological limits than those set by the AEA (1996). The microbiological analyses and limits used by an official food control laboratory in Finland to testing of meals served on aircraft are presented in Table 2.

Table 2. Microbiological limits used for aircraft meals by the Food and Environmental Laboratory of the City of Vantaa\*.

Microbes	Hot meal	Cold meal
Total count	$1.0 \times 10^5$ cfu/g	NA <sup>1</sup>
<i>Enterobacteriaceae</i>	$1.0 \times 10^2$ cfu/g	NA
<i>Escherichia coli</i>	10 cfu/g	10 cfu/g
<i>Staphylococcus aureus</i>	$1.0 \times 10^2$ cfu/g	$1.0 \times 10^2$ cfu/g
<i>Bacillus cereus</i>	$1.0 \times 10^2$ cfu/g	$1.0 \times 10^3$ cfu/g
<i>Clostridium perfringens</i>	$1.0 \times 10^2$ cfu/g	$1.0 \times 10^2$ cfu/g
<i>Salmonella</i> spp.	Detected/25 g	Detected/25 g

<sup>1</sup>NA No analysis

\* Official food control laboratory

Although many flight kitchens and airline companies record the microbiological quality of meals served on aircraft, only a few studies have been published. In a survey made in Bangkok in 1976, a high contamination rate of *Salmonella* (9%), *Vibrio parahaemolyticus* (3%) and *S. aureus* (2%) was found (Steffen et al. 1985). Monitoring *Salmonella* from approximately 6000 samples from flight kitchens in 40 worldwide locations showed a prevalence of 1%, and meals prepared in India and Indonesia were most frequently *Salmonella* positive (Munce 1986).

Between 1984 and 1986 a study was conducted on meals (567) produced by ten flight catering units at Heathrow airport, London (Roberts et al. 1989). Colony counts higher than  $10^6$  cfu/g were found in 24% of hot dishes. The proportion of samples exceeding 10 cfu/g for *E. coli* was 21%. Samples of meat products showed an incidence of *C. perfringens* and *B. cereus* of 0.2% and 3.0% respectively. *Salmonella* was isolated from 0.4% of samples.

The microbiological quality of food items was monitored in a Greek flight kitchen in 1992 (Lambiri et al. 1995). *Salmonella* was found in 1% of hot food items. Of the cold food items and desserts, 24% contained *S. aureus*  $>1.0 \times 10^2$  cfu/g. *E. coli* higher than 10 cfu/g was found in 12% and 7% of hot and cold food items, respectively. The number of *Salmonella* positive raw poultry samples was high (24%). Implementation of the HACCP system in 1993 followed by a new monitoring showed considerable improvement in the microbiological quality of food items (Lambiri et al. 1995).

Enterotoxin formation was tested in 47 *S. aureus* strains, which were isolated from aircraft-ready meals in a four-year survey in Denmark. Immunological testing showed that 51% of the isolates were enterotoxigenic (Ewald and Christensen 1987).

## 2.6 Outbreaks associated with meals served on aircraft

### 2.6.1 Outbreaks

Since tracing of the first foodborne outbreak associated with a meal served on aircraft in 1947, 41 outbreaks have been reported altogether (Table 3). *Salmonella* spp., *S. aureus* and *Vibrio* spp. have been the most commonly reported agents. Thousands of flights have been involved. Approximately 9000 air passengers and crew members have been reported to have suffered from food poisoning. The number of reported deaths was 11. In consequence of a *Salmonella enterica* serovar Typhimurium (hereafter *S. Typhimurium*) outbreak via infected cold salads from Las Palmas served on charter flights, six deaths occurred in 1976 (Table 3, outbreak 21). *Salmonella enterica* serovar Enteritidis (hereafter *S. Enteritidis*) was the reason for two deaths in a major outbreak, where passengers and crew at risk on 3103 flights were reported in 1984 (Table 3, outbreak 30). *Vibrio cholerae* caused two deaths, in 1972 and 1992 (Table 3, outbreaks 15, 39). The causative agent remained unknown in one foodborne outbreak, which was followed by one death in 1971 (Table 3, outbreak 11).

Foodborne outbreaks traced to meals served on aircraft are most probably underreported for several reasons. The incubation period is often longer than the flight time, and passengers may be unaware of each other's illness. Therefore recognising a cluster of foodborne illness may be difficult. When an outbreak is identified, it always gives rise to a bad reputation and great financial losses (Pakkala 1989). Therefore airline companies, just as any companies providing a food service, do not like publishing any data on foodborne outbreaks. The authorities should recognise outbreaks associated with aircraft meals. In order to prevent dissemination or recurrence of outbreaks and the incidence of health hazards, a rapid international exchange of information is also needed.

#### *Salmonella* spp.

*Salmonella* has been the most common pathogen associated with outbreaks traced to aircraft food (Table 3). It has been reported to cause 15 outbreaks and to infect approximately 4000 people. Eight different serotypes have been identified, with *S. Enteritidis* being the most common, causing 6 outbreaks. *Salmonella enterica* serovar Typhi (hereafter *S. Typhi*) was the cause of two outbreaks. Typical for *Salmonella* outbreaks in most cases was that the dissemination of contaminated food continued for several days and many flights were involved.

The first widespread *Salmonella* outbreak connected with airline meals occurred in the early years of mass tourism on intercontinental flights from Sydney to London via Vienna in 1967 (Table 3, outbreak 7). It affected almost 400 people. Contaminated mayonnaise prepared in a Vienna flight kitchen was found to be the source of infection. A great number of shortcomings in hygiene found during the investigation of the kitchen led to it being closed for 6.5 weeks. The largest *Salmonella* outbreak occurred in 1976. Approximately 1800 people from several European countries fell ill as a result of eating airline food served on charter flights (Table 3, outbreak 21). Findings from ill passengers and epidemiological evidence revealed cold salads with mayonnaise prepared in Las Palmas, Spain to be the source of infection. In 1984 a widely spread *Salmonella* outbreak occurred, where the suspected food, appetisers, was served on 3103 flights, and 631 first class and club class passengers and 135 air crew members were affected (Table 3, outbreak 30, Burslem et al. 1990). *S. Enteritidis* was isolated from a great number of cold food items with aspic glaze. Two large outbreaks involving over 400 people in each were reported in the 1990s (Table 3, outbreak 36, 41). Both outbreaks involved several charter flights, the first one catered for by a flight kitchen in the Greek islands and the second one in the Canary Islands.

#### *Staphylococcus aureus*

Eight outbreaks caused by *S. aureus* have been reported (Table 3). Compared to *Salmonella* outbreaks, only a few flights were involved. In five outbreaks cold desserts were the vehicles of infection, and in three cases hot dishes.

In the 1970s, two major outbreaks occurred. The first one broke out on three flights from Rome to the USA via Lisbon in 1973 (Table 3, outbreak 17). The flights were catered for in Lisbon. Custard dessert, bavarois, was traced to be the source of infection. High counts

( $10^5$  to  $10^8$  cfu/g) of *S. aureus* were detected in the dessert. The evidence was conclusive, because *S. aureus* with the same antibiogram was isolated in patients as in the dessert. The second major outbreak took place on a long-haul flight from Tokyo to Paris via Anchorage and Copenhagen in 1975 (Table 3, outbreak 19). Snacks and breakfasts were loaded onto the plane in Anchorage. Ham included in the breakfast was shown to be contaminated with the same phage type and enterotoxin-producing strain as was isolated in the patients and in inflamed finger lesion of one cook. A great number of passengers (142) and one crew member required hospitalisation during intermediate landing in Copenhagen. The onset of symptoms began very soon, 0.5-2.5 h after eating the meal in both outbreaks. The symptoms were severe, mostly with nausea, vomiting and severe abdominal cramps. A high attack rate, 56% and 57% respectively, was found in connection with both outbreaks (Table 3, outbreaks 17, 19).

Investigations of two smaller outbreaks indicated high levels of *S. aureus*,  $10^9$  and  $10^6$  cfu/g in eclairs and in chocolate cake, respectively (Table 3, outbreaks 23, 37). The same types were found in patients, but the possible role of food handlers being the source of infection was not investigated. Fast exchange of information between the public health agencies in the United Kingdom and the United States facilitated the rapid identification of an international outbreak, its aetiology and the food vehicle responsible for the outbreak in 1991 (Table 3, outbreak 37). It led to the withdrawal of the chocolate cake, and so the prevention of further illnesses.

#### *Vibrio* spp.

*Vibrio* spp., *V. cholerae* O1, *V. cholerae* non O1 and *V. parahaemolyticus* were reported as causing six outbreaks via aircraft food (Table 3). The incubation period varied from 24 to 48 h for *V. cholerae*, but for *V. parahaemolyticus* as short as four hours incubation period was noticed. An outbreak caused by *V. parahaemolyticus* was reported to have broken out already during the flight, and 28 passengers were admitted to hospital (Table 3, outbreak 25).

The endemic occurrence of cholera in some Asian countries since 1961 caused the seventh cholera pandemic. It was apparently linked to *V. cholerae* outbreaks registered during long-haul flights from Europe to Australia in the 1970s. The gastrointestinal illness of passengers was traced to cold food loaded in Bahrain (Table 3, outbreaks 15, 16). Bahrain was experiencing an outbreak of cholera at the time. The outbreaks were caused by *V. cholerae* O1 in 1972 and *V. cholerae* non O1 in 1973 and in 1978 (Table 3, outbreaks 15, 16, 26). Contaminated cold plates were suspected to be the source of infection. Ice might also be a vehicle, because vibrios may survive for long periods in ice water.

Food prepared in a Hong Kong flight kitchen was suspected to be the reason for two outbreaks among two American tour groups to the Orient in summer 1969 (Table 3, outbreaks 8, 9). Multiple pathogenic bacteria were isolated in patients, but the gastrointestinal illness was best correlated with the isolation of non-cholera vibrios.

The largest airline-associated-outbreak of cholera occurred in 1992 (Table 3, outbreak 39). Seventy-five of the 336 passengers who had flown from Lima, Peru, to Los Angeles became infected and one died. Epidemiological study indicated a strong association between eating a cold seafood salad and illness (Eberhart-Phillips et al. 1996). This outbreak demonstrated the potential of airline-food-associated spread of cholera from endemic areas, such as South America. The outbreak highlighted the risk associated with eating cold foods prepared in cholera-infected area. Epidemic cholera appeared in South America for the first time in the 20th century during January 1991. In 1992, the epidemic had spread to 20 countries in Latin America, and more than 600 000 cases and 5 000 deaths were reported (CDC 1991, CDC 1992). The year 1998 was marked by increase of nearly 100% in cholera cases on all continents (WHO 1999 b).

*Vibrio parahaemolyticus* caused two aircraft-meal-associated outbreaks in the 1970s (Table 3, outbreaks 14, 25). Seafood appetiser from Bangkok and a seafood cocktail from Bombay were connected with the outbreaks. *V. parahaemolyticus* is widely distributed in inshore marine waters throughout the world and is a well-documented and among the most common food poisoning bacteria in Japan, India and South-East Asia as well as in the United States, and is responsible for the summer peak of gastroenteritis (Zen-Yoji et al. 1965, WHO 1999 a).

#### *Shigella* spp.

Four outbreaks caused by *Shigella* via aircraft meals have been reported (Table 3). The first one, in 1971, was traced back to food served to charter passengers on several flights from the Canary Islands to Sweden (Table 3, outbreak 12). The food was prepared in Las Palmas, and was reported as having infected 219 passengers. The seafood cocktail served on flights was epidemiologically connected with the illness of 19 persons (Table 3, outbreak 13). A wide *Shigella* outbreak associated with aircraft meals on 219 flights to 24 states in the United States and to England, Germany, Japan, and Mexico in 1988 (Table 3, outbreak 33). Illness was due to the consumption of cold food items prepared in the kitchen in Twin Cities, Minnesota. Relatively low attack rates (4%) on scheduled flights, a long incubation period (1-4 days), and the dispersion of ill individuals demonstrated the difficulties in detecting a foodborne outbreak among airline passengers who live in widely scattered geographic areas. The outbreak was identified because it also involved a professional football team travelling together (Hedberg et al. 1992). *Shigella* has also caused several outbreaks linked to food served on other traffic vehicles, such as cruise ships (Gikas et al. 1996, Koo et al. 1996).

#### *Clostridium perfringens*

*Clostridium perfringens* has been reported to cause one outbreak, the vehicle being a hot meal (Table 3, outbreak 10). A hot dish containing turkey was epidemiologically shown to be the source of this outbreak. A total of 394 persons on eight flights had been at risk. A great number of crew members (22/62) and a few passengers suffered from gastrointestinal illness with diarrhoea as the main symptom, with the mean incubation period of 11 h, characteristic to *C. perfringens*.

Table 3. Foodborne outbreaks associated with meals served on aircraft in 1947-1999.

No <sup>1</sup>	Year	Food origin	Agent	Food type	Vehicle	No of flights	Infected passengers	References
1	1947	Anchorage	<i>Salmonella</i> Typhi	C <sup>2</sup>	Sandwiches	1	4	Williams et al. 1950
2	1961	Vancouver	<i>Staphylococcus aureus</i>	H <sup>3</sup>	Chicken	1	13	CDC 1961
3	1965	Adelaide	<i>Staphylococcus aureus</i>	H	Roast turkey	2	4*	Munce 1978
4	1966	Adelaide	<i>Salmonella, Staphylococcus</i>	NK <sup>4</sup>	Roast chicken	1	3*	Munce 1978
5	1966	New Delhi	<i>Staphylococcus aureus</i>	C	Trifle dessert	1	15*	Munce 1978
6	1967	London	<i>Eschericia coli</i>	C	Oysters	6	1	Preston 1968
7	1967	Vienna	<i>Salmonella</i> Enteritidis	C	Mayonnaise	several	380	Munce 1978
8	1969	Hong Kong	Multiple	NK	Unknown	1	21	Tauxe et al. 1987
9	1969	Hong Kong	Multiple	NK	Unknown	1	24	CDC 1969
10	1970	Atlanta	<i>Clostridium perfringens</i>	H	Turkey	8	3	CDC 1971
11	1971	Bangkok	Unknown	C	Shrimp and crab salad	1	23	Mossel and Hoogendoorn 1971
12	1971	Gran Canaria	<i>Shigella sonnei</i>	NK	Unknown	several	219	Oden-Johanson and Böttiger 1972
13	1971	Bermuda	<i>Shigella sonnei</i>	C	Seafood cocktail	1	78	CDC 1971
14	1972	Bangkok	<i>Vibrio parahaemolyticus</i>	C	Appetiser, seafood	1	9	Peffer et al. 1973, Tauxe et al. 1987
15	1972	Bahrain	<i>Vibrio cholerae</i> O1	C	Appetiser	2	47	Sutton 1974
16	1973	Bahrain	<i>Vibrio cholerae</i> non O1	C	Appetiser with chopped egg	1	64	Dakin et al. 1974
17	1973	Lisbon	<i>Staphylococcus aureus</i>	C	Custard	3	247	CDC 1973
18	1973	Denver	<i>Salmonella</i> Thompson	NK	Breakfast	1	17*	WHO, Regional Office for Europe 1977, Tauxe et al. 1987
19	1975	Anchorage	<i>Staphylococcus aureus</i>	H	Ham	1	196	Eisenberg et al. 1975
20	1975	Rome	<i>Salmonella</i> Oranienburg	NK	Unknown	1	23	Munce 1978

21	1976	Las Palmas	<i>Salmonella</i> Typhimurium	C	Cold salads, mayonnaise	11*	1800	WHO Weekly Epid. Rec.1976 a, Svensson 1998
22	1976	Paris	<i>Salmonella</i> Brandenburg	C	Cold dishes	45	232	WHO Weekly Epid. Rec.1976 b, Böttiger and Romanus 1977
23	1976	Rio de Janeiro	<i>Staphylococcus aureus</i>	C	Eclair	1	28	CDC 1976
24	1976	New Delhi	<i>Salmonella</i> Typhi	NK	Tourist class menu	1	13	Anonymous 1977, Tauxe et al.1987
25	1976	Bombay	<i>Vibrio parahaemolyticus</i>	C	Unknown	1	28	Desmarchelier 1978
26	1978	Dubai	<i>Vibrio cholerae</i> non O1	C	Chicken sandwiches	1	61	Desmarchelier 1978
27	1982	Lisbon	<i>Staphylococcus aureus</i>	C	Custard	2	6	Tauxe et al. 1987, Svensson 1998
28	1983	New York	<i>Salmonella</i> Enteritidis	NK	Swiss steak	2	12	Tauxe et al. 1987, Svensson 1998
29	1983	Acapulco	<i>Shigella</i>	NK	Unknown	1	42	Tauxe et al. 1987, Svensson 1998
30	1984	London	<i>Salmonella</i> Enteritidis	C	Appetiser with aspic glaze	3103	631	Anonymous 1984, Tauxe et al. 1987, Burslem et al. 1990
31	1985	Faro	<i>Salmonella</i> Enteritidis	C	Mousse with cream	1	30*	WHO Weekly Epid. Rec. 1989
32	1986	Vantaa FIN	<i>Salmonella</i> Infantis	NK	Multiple	1	91	Hatakka 1992 (IV)
33	1988	Twin Cities	<i>Shigella sonnei</i>	C	Cold dishes	219	240	Hedberg et al. 1992
34	1989	Palma de Mallorca	<i>Salmonella</i> Enteritidis	NK	Unknown	1	80	Jahkola 1989
35	1990	Bangkok	<i>Salmonella</i> Ohio	C	Ham	1	5	Jahkola 1992
36	1991	Greece (islands)	<i>Salmonella</i>	NK	Unknown	several	415	Lambiri et al. 1995
37	1991	Los Angeles	<i>Staphylococcus aureus</i>	C	Chocolate cake	3	25	PHLS Communicable Dis. Rep. 1991, Sockett et al. 1993
38	1991	Melbourne	Norwalk-like agent	C	Orange juice	several	3053	Lester et al. 1991
39	1992	Lima	<i>Vibrio cholerae</i> O1	C	Seafood salad	1	80	Eberhart-Phillips et al. 1996
40	1993	Charlotte, USA	ETEC	C	Unknown	40	56	CDC 1994
41	1997	Canary Islands	<i>Salmonella</i> Enteritidis FT1	C	Chocolate eclair	several	455	De Jong 1998

\* at least

1 No

2 C

3 H

4 NK

Outbreak number

Cold dish

Hot dish

Not known

### *Escherichia coli*

Oysters contaminated by *E. coli* incapacitated 22 crew members over a period of four days in 1967 (Table 3 and 4, outbreak 6). The oysters were served on six international flights from London. *E. coli* showed faecal contamination, but viruses might have been involved, too. The incubation period and symptoms were similar to Norwalk-like viruses, but there was no method for virus detection at that time. The development of methods for the recovery of viruses from bivalve molluscs has proved that raw or cooked shellfish contaminated by Norwalk-like viruses was documented as being the reason for numerous outbreaks in 1990s (Chalmers and McMillan 1995, Dowell et al. 1995, Leeds et al. 1995).

One outbreak caused by enterotoxigenic *E. coli* (ETEC) was described in the United States in 1993 (Table 3, outbreak 40). The illness of 47 air passengers was most strongly associated with eating salad. In addition, nine passengers reported gastrointestinal illness from a different flight where the same meal was served. Investigation of a local outbreak at the same time revealed an ETEC outbreak, too. Epidemiological investigation showed the salad to be the source of infection. Carrots were found to be the common ingredient in these salads.

### Norwalk-like viruses

More than 3000 persons were affected on several flights from Melbourne (Table 3, outbreak 38). It was established that catering arrangements were independent, apart from a common supplier of orange juice. Surveys revealed attack rates of illness of up to 100% among orange juice drinkers, and 0% among non-orange juice drinkers. A sudden onset of severe vomiting and diarrhoea developed in between 1 and 3 days. The clinical picture was typical for a viral disease, and the presence of Norwalk-like agent was detected from faecal samples. In this case strong epidemiological evidence between gastrointestinal illness and drinking orange juice was found, although detection of the agents from the orange juice failed.

## **2.6.2 Special considerations of outbreaks involving air crew**

Air crews have been involved in 11 outbreaks associated with aircraft meals (Table 4). Gastrointestinal illness resulting from food poisoning is the leading cause of airline pilot incapacitation and causes an in-flight safety hazard (Beers and Mohler 1985).

Food contaminated by *S. aureus* served to crew during a flight led to dangerous situations in the air due to a short incubation period in connection with three outbreaks (Table 4). On the flight from Lisbon to Boston in 1982 all crew members became ill, but fortunately, the crew was still able to operate the aircraft and the plane landed safely.

Crew members eating left over food, oysters from passengers, led to foodborne illness (Table 4). A long incubation period meant that none of the crew members became ill whilst air-borne, but the crew's illness seriously hampered the operation of scheduled services.

Table 4. Aircraft-meal-associated outbreaks among air crew members in 1947-1999<sup>1</sup>.

Year	Food origin	Agent	Vehicle	Crew		Incubation period h
				At risk	Infected	
1967	London	<i>Escherichia coli</i>	Oysters	NR <sup>2</sup>	22	30
1967	Vienna	<i>Salmonella</i> Enteritidis	Mayonnaise	NR	8	8
1970	Atlanta	<i>Clostridium perfringens</i>	Turkey	62	22	11
1972	Bangkok	<i>Vibrio parahaemolyticus</i>	Appetisers	6	3	9
1973	Bahrain	<i>Vibrio cholerae</i> non O1	Appetisers	NR	2	12
1975	Anchorage	<i>Staphylococcus aureus</i>	Ham	20	1	0.5-2.5
1976	Paris	<i>Salmonella</i> Brandenburg	Cold dishes	NR	58	12-48
1982	Lisbon	<i>Staphylococcus aureus</i>	Custard	NR	10	NR
1984	London	<i>Salmonella</i> Enteritidis	Appetisers	NR	135	7
1988	Twin Cities	<i>Shigella sonnei</i>	Cold dishes	NR	9	12-96
1991	Los Angeles	<i>Staphylococcus aureus</i>	Chocolate cake	NR	1	2-4
				271		

<sup>1</sup>Data compiled from: Anonymous 1984, Burslem et al. 1990, CDC 1971, Dakin et al. 1974, Eisenberg et al. 1975, Hedberg et al. 1992, Munce 1978, Peffers et al. 1973, Preston 1968, Socket et al. 1993, Tauxe et al. 1987, WHO Weekly Epid. Rec. 1976 a.

<sup>2</sup>NR Not reported

*Salmonella* infected a great number of crew (135) via appetisers with aspic glaze on (Table 4). The investigation showed that some flight crew members had been eating the same appetisers that were being served to the passengers. The airline company warned their staff of the food incident and reminded them that under airline policies crew members are expected to eat from menus that differ from those served to passengers and to each other. Crew members are also expected to eat at different times (Anonymous 1984). Incapacity of crew members emphasises the importance of providing separately produced meals for the flight crew. Airline crews should be advised of the dangers associated with food and should ensure the safest possible eating habits, especially in developing countries (Masterton and Green 1991).

### 2.6.3 Contributing factors to the outbreaks associated with aircraft meals

The most frequent factor leading to the foodborne outbreak via airline food was insufficient refrigeration (Table 5). The next was contamination of the food by an infected food handler. Similar reasons have been shown to be important errors generally leading to foodborne outbreaks (WHO 1995).

*Salmonella* spp.

The most prominent contributing factors reported in connection with *Salmonella* outbreaks were infected food handlers and inadequate refrigeration. These non-conformances often

appeared together (Table 5). In four outbreaks, food handlers suffering from gastrointestinal symptoms were working in the kitchen either from ignorance or negligence. Investigation of the first outbreak of *S. Typhi* in 1947 revealed that the itinerant cook was a symptomless carrier and that she was found to have a previous history of typhoid fever 16 years earlier. Inadequate refrigeration with rough errors was detected in five outbreaks. The reasons were: the use of an ice box as the only means of refrigeration, mayonnaise with a pH of 5.3 stored for several days at room temperature, bulk aspic kept in an ambient temperature for three days and still used for glazing cold meals, shortcomings in the cold facilities due to flight delay and the lacking cold storage facilities on a long-haul flight. The misuse of high-risk food items such as mayonnaise and aspic glaze resulted in three outbreaks. Inadequate hygiene standards in the kitchen, such as a lack of hand washing facilities or grossly inadequate toilet facilities, were detected in three cases. Cross-contamination was discovered in two cases.

#### *Staphylococcus aureus*

Investigation of *S. aureus* outbreaks has only once been traced to an infected food handler (Table 5). A cook with an inflamed finger lesion was the source of infection. The storage of contaminated ham at 10°C in the flight kitchen for 14.5 h, then at room temperature in the galley oven on an aircraft for seven hours resulted in multiplication of *S. aureus* and the formation of enterotoxin.

#### *Vibrio* spp.

A cold dish was the vehicle in all *Vibrio* outbreaks. Common for the *Vibrio cholerae* outbreaks was that the food was purchased and prepared in cholera-affected areas. There is an inherent great risk of contamination of cold food and therefore this items should be avoided on aircraft menus in endemic areas (Table 5).

#### *Shigella* spp.

The preparation of cold food, which was also stored at an inadequate temperature by eight food handlers sick with diarrhoea (10% of staff), caused an outbreak. It was also found that the sanitation was unsatisfactory and not according to HACCP (Table 5).

#### *Clostridium perfringens*

A cook-chill system was not used in 1970 and a foodborne illness of passengers and crew members (Table 3) occurred on eight flights because of inadequate heat treatment and maintenance at 55°C.

#### Norwalk-like viruses

Several problem areas where potential contamination could have occurred were identified in the factory producing juice. Plumbing connections were suspected (Lester et al. 1991).

Table 5. Number of aircraft-meal-associated outbreaks where contributing factors have been detected or suspected in 1947-1999<sup>1</sup>.

Contributing factor	<i>Salmonella</i> spp.	<i>S. aureus</i>	<i>Vibrio</i> spp.	<i>Shigella</i> spp.
Contaminated raw material	1	NR <sup>2</sup>	1	NR
Cross-contamination	2	NR	2	NR
Inadequate refrigeration	5	4	1	1
Inadequate heating	1	1	NR	NR
Infected food handler	5	1	2	1
Flight kitchen in endemic area	1	NR	2	NR
Risk items in menu	3	NR	2	1
Inadequate personal hygiene	1	NR	NR	NR
Inadequate hygiene level in kitchen	3	1	NR	1

<sup>1</sup> Data compiled from: CDC 1973, CDC 1976, Dakin et al. 1974, De Jong 1998, Desmarchelier 1978, Eberhardt-Phillips et al. 1996, Eisenberg et al. 1975, Hatakka 1992 (IV), Hedberg et al. 1992, Jahkola 1992, Lambiri et al. 1995, Munce 1978, Peffers et al. 1973, Preston 1968, Tauxe et al. 1987, Socket et al. 1993, Sutton 1974, WHO Weekly Epid. Rec. 1976, Williams et al. 1950.

<sup>2</sup> NR Not reported

### 3. AIMS OF THE STUDY

This study was conducted in order to:

1. examine the microbiological quality of hot meals served on aircraft with regard to indicator bacteria and *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, and to evaluate the results according to the microbiological criteria set by the AEA (I).
2. examine the microbiological quality of cold meals served on aircraft with regard to indicator bacteria and *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, and to evaluate the results according to the microbiological criteria set by the AEA (II).
3. examine the occurrence of *Salmonella* in meals served on aircraft (I-III).
4. investigate a *Salmonella* outbreak among airline and railway passengers and to evaluate the contributing factors leading to the outbreak (IV).
5. monitor the carriage of *Staphylococcus aureus* among flight catering employees, and to characterise the isolated strains by determining enterotoxicity and pulsed-field gel electrophoresis (PFGE) types (V).

## 4. MATERIALS AND METHODS

### 4.1 Sampling

#### 4.1.1 Food samples (I-IV)

##### *Microbiological quality (I-III)*

Meals served on aircraft were collected for a survey of microbiological quality including *Salmonella* analyses between 1991 and 1994 and for the specific *Salmonella* survey between 1989 and 1992. Samples were collected in accordance with the control programme of an airline company.

Altogether 1 687 dishes were sampled for microbiological quality. This material consisted of 1 012 hot and 675 cold dishes. The hot dishes comprised 112 breakfasts and 900 main dishes, and the cold dishes 273 appetisers, 168 salads and 234 desserts. Approximately half of the dishes 791 (47%) were prepared in Finland and the remaining 896 (53%) in 32 other countries.

The material for the microbiological survey, including *Salmonella*, consisted of 1 011 hot meals and 653 cold meals. The material for *Salmonella* examinations consisted of two surveys. During the specific *Salmonella* survey, 1 288 hot dishes and 923 cold dishes, such as 400 appetisers, 278 salads, 19 cheese dishes and 226 desserts, 2 211 samples in all, were collected. Of the samples 1 477 (67%) were of Finnish origin. The remaining 734 (33%) were prepared worldwide by flight kitchens located in 28 countries. Altogether 2 299 hot meals and 1 576 cold meals were tested for *Salmonella* in the studies.

Onboard sampling took place before serving. Hot aircraft dishes of pre-cooked food were sampled before the final re-heating on board. The samples were stored using dry ice and frozen at -20°C immediately after the flight. They were carried frozen within 1 to 3 days to the laboratory. Samples taken from Finnish flight kitchens for the *Salmonella* study were chilled to 4°C and transported chilled to the laboratory. For the microbiological survey between 1991 and 1994, the samples were delivered frozen to the laboratory.

##### *Salmonella outbreak (IV)*

During inspection of the *Salmonella* outbreak 153 food samples were taken. They consisted of 148 food samples of cooked egg products, raw chicken products, cold cuts and Swiss rolls sliced in the cold kitchen from the catering establishment. Two samples represented a batch with 1 200 portions of Viennese goulash prepared on the 30th July for charter flights. Raw materials to simulate the preparation of the dishes such as Viennese goulash, fresh salad and Swiss roll served on the Rhodes flight were sampled.

#### 4.1.2 Faecal samples (IV)

Human faecal samples for *Salmonella* were collected in connection with investigation of the *Salmonella* outbreak. In the catering establishment all the staff were sampled: 118 food

handlers and 44 persons from other departments. In order to be tested for *Salmonella*, railway and airline passengers at risk, 600 and 350, respectively, were informed by two nationwide announcements to contact their local health authorities. The total number of railway and airline passengers tested is not known, only the numbers of the positive *Salmonella* findings 107 and 91, respectively, have been recorded.

#### **4.1.3 Hand and nasal samples (V)**

Hand and nasal samples of Finnish flight catering employees were collected between January 1995 and May 1997. Altogether 153 hand samples from 117 persons and 136 nose samples from 111 persons were taken. In most cases sampling was done once.

Nasal samples were taken by applying a sterile cotton-tipped swab 1-2 cm inside the anterior nares. To sample the hands, the right hand was rinsed for 10 seconds in a sterile plastic bag containing a 20 ml physiological saline solution with 1% peptone (Bacto Peptone, Difco Laboratories, Detroit, Mich.).

## **4.2 Methods**

### **4.2.1 Microbiological methods (I-V)**

The methods used for the microbiological analyses of food samples are included in the collection of the Nordic Committee on Food Analysis (NCFA) and they are presented in Table 1 (I) and Table 1 (II). These methods are in common use for official control of food in the Nordic countries. The *Salmonella* method used was NCFA 1991 (I-III) and NCFA 1986 (IV). Before analysis, all the ingredients of the sample were homogenised. For the quantitative methods 10 g and for the qualitative methods 25 g of homogenate were used.

Faecal samples for *Salmonella* were examined by the method of Kelly et al. (1985). Subtyping of the *Salmonella* species was carried out by the National Salmonella Centre of Finland, National Public Health Institute (KTL) using the method of Kauffman (1966).

Blood agar (Blood agar base, Difco) containing 5% sheep blood and Baird-Parker agar plates (Lab M, Burry, UK) was used to isolate *S. aureus* from nasal swabs. For the hand samples, Baird-Parker agar was used. Wherever possible, at least five typical colonies were picked from both plates and further subjected to Gram-stain, catalase reaction and coagulase test performed with swine plasma. Gram positive, catalase positive cocci that were coagulase positive and had shown typical reaction on Baird-Parker agar plates were regarded as *S. aureus*.

### **4.2.2 Measurement of pH and $a_w$ (IV)**

The pH value was measured from the homogenate of 10 g of food mixed with 10 ml of distilled water (Digital-pH-Meter E 632, Metrohm, Switzerland). The water activity ( $a_w$ ) was measured according to the method of the NCFA (1984) using a hygrometer (Lufft GmbH, Stuttgart, F.R.G.).

#### **4.2.3 *In situ* DNA isolation and PFGE (V)**

All isolated *S. aureus* strains (42) were characterised using DNA macrorestriction analysis. Cells were harvested from 2 ml of BHI broth (Oxoid, Basingstoke, UK) and cultures grown overnight at 37°C. *In situ* DNA isolation was performed as described by Maslow et al. (1993) with the modifications described by Björkroth et al. (1996). *SacI* and *SmaI* were used for the cleavage of DNA. The samples were electrophoresed through 1% (w/v) agarose gel (SeaKem Gold, FMC BioProducts, Rockland, Maine) in 0.5 x TBE (45mM Tris, 4.5 mM boric acid, pH 8.3, and 1 mM sodium EDTA) at 14°C in a Gene Navigator system with the hexagonal electrode (Pharmacia, Uppsala, Sweden). Interpolation ramped from 0.5 s to 15 s for 18 h at 200 V was used for *SacI* and from 0.5 s to 25 s for *SmaI*.

#### **4.2.4 Enterotoxin analysis (V)**

Enterotoxin determination was performed on the basis of the PFGE types. From each of the 32 different PFGE pattern groups, one to three isolates were tested for enterotoxin production. From the biggest type-specific groups containing isolates from different persons more than one isolate was tested. In these cases the isolates chosen were from different persons. Toxin detection was performed using the ELISA test for staphylococcal enterotoxins A, B, C and D with the test kit SET-EIA from Dr. Bommeli AG (Liebefeld-Bern, Switzerland) according to the manufacturer's instructions.

#### **4.2.5 Epidemiological investigation (IV)**

Standardised questionnaires were sent to all 162 employees of the catering establishment and to all 350 air passengers after the flight on 1st August from Helsinki to Rhodes. Thirteen railway passengers who had suffered gastroenteritis after travelling by train on the 1st, 2nd and 3rd of August and were living in the Helsinki area were interviewed by telephone. They were asked about the time of the onset of the symptoms, the symptoms and the duration of the illness, and the food they had eaten. A wider questionnaire study could not be carried out because the names of the other railway passengers were not known.

#### **4.2.6 Inspection of the catering establishment (IV)**

The local health authorities inspected the catering; storage of raw materials, food preparation areas, refrigeration equipment, general cleanliness and repair of the equipment and establishment. The food preparation methods of egg sandwiches for trains and the meal served on the Rhodes flight were especially checked.

#### **4.2.7 Statistical methods (I, II, IV)**

For the statistical analyses of hot and cold meals, Student's t-test and Chi<sup>2</sup>-test were carried out using Statistica for Macintosh™ (Tulsa, Oklahoma). In connection with the investigation of *Salmonella* outbreak statistical testing of questionnaires was done by using the Chi<sup>2</sup>-test.

## 5. RESULTS

### 5.1 Microbiological quality of hot meals served on aircraft (I)

The aerobic colony counts, coliforms and *E. coli* counts were significantly higher ( $p < 0.05$ ) in the main dishes than in the breakfasts (I, Table 2). Although the means of total counts and coliforms or *Enterobacteriaceae* were rather low in the hot meals, high maximum values were detected. Total counts higher than  $10^6$  cfu/g, which is the AEA limit for food items handled after heat treatment, were found in 9.2% of hot meal samples. Many of the hot meals 33 (8.2%) also exceeded the microbiological standards given by the AEA (1996) for *E. coli*. The number of the hot meal samples not meeting the standard were for *S. aureus* 6 (0.6%), *B. cereus* 7 (0.7%) and *C. perfringens* 2 (0.7%) (I, Table 4). The maximum counts were  $4.0 \times 10^3$  cfu/g for *S. aureus*,  $3.0 \times 10^4$  cfu/g for *B. cereus* and  $1.0 \times 10^3$  cfu/g for *C. perfringens*.

There were statistically significant differences between preparing countries regarding the means of total count and *E. coli* in hot meals (I, Table 5). The two countries showing the highest means of total bacteria also showed highest means of *E. coli*.

### 5.2 Microbiological quality of cold meals served on aircraft (II)

Appetisers and salads showed significantly higher aerobic colony counts and *E. coli* counts ( $p < 0.05$ ) than desserts (II, Table 2). *E. coli* counts were over 10 cfu/g in 33 (18%) of the appetizers, in 23 (16%) of the salads and in eight (6%) of the desserts, and totally in 64 (14%) of the cold meals (II, Table 3). *S. aureus*, *B. cereus* or *C. perfringens* were found in 50 (7%) of the cold meal samples (II, Table 4). The requirements set for *S. aureus* and *B. cereus* by the AEA standard were not met in 23 (7%) and 13 (3%) of the samples, respectively. In two poultry appetisers and one fresh salad prepared in the same country *E. coli* counts as high as  $1.0 \times 10^6$  cfu/g were found (II, Table 2). The maximum value of *S. aureus*,  $3.3 \times 10^3$  cfu/g, was found in an appetiser containing meat as the main ingredient. For *B. cereus*, the highest value was  $5.0 \times 10^4$  cfu/g, which was found in an appetiser containing pâté.

The means of total counts and *E. coli* counts showed significant differences between preparing countries in cold meals as well as in hot meals. In addition, the means of *B. cereus* also revealed differences (II, Table 5).

### 5.3 *Salmonella* in meals served on aircraft (I-III)

In the microbiological survey including *Salmonella*, *Salmonella* was detected in 3 (0.3%) out of 1 011 hot dishes (I, Table 3), but not in any of the 653 cold dishes (II, Table 4). In the specific *Salmonella* survey, out of the 1 288 hot dishes five (0.4%) and out of the 923 cold dishes one (0.1%) were found to be *Salmonella* positive (III, Table 1). Altogether,

*Salmonella* was detected in seven (0.3%) out of 2 299 hot meals and in one (0.1%) out of 1 576 cold meals.

The serotypes found in hot dishes were, *Salmonella enterica* serovar Manchester (hereafter *S. Manchester*), *Salmonella enterica* serovar Morbificans (hereafter *S. Morbificans*), *Salmonella enterica* serovar Hadar (hereafter *S. Hadar*) and *Salmonella enterica* serovar Braenderup (hereafter *S. Braenderup*). *S. Manchester* was isolated in a main dish containing beef, potatoes and cooked vegetables prepared in Kenya, *S. Morbificans* in a main dish containing chicken and boiled vegetable, *S. Hadar* in a breakfast containing omelette and cheese. Four hot dishes prepared in China, which contained chicken, potatoes and beans, beef, potatoes and cooked vegetable, fish, rice and cooked vegetable and snack crepes, and which were sampled during the same week, were shown to be contaminated by the same serotype, *S. Braenderup*.

The serotype found in the cold dish was *Salmonella enterica* serovar Ohio (hereafter *S. Ohio*), and it was isolated in an appetiser prepared in Thailand. It contained ham, Edam-type cheese, boiled egg and cooked and marinated vegetables.

#### 5.4 *Salmonella* outbreak (IV)

An outbreak by *Salmonella enterica* serovar Infantis (hereafter *S. Infantis*), infecting a total of 226 people, occurred in Finland at the beginning of August 1986. It was found that three clusters of people were infected; railway passengers, charter flight passengers and employees of a Finnish catering establishment. The source of infection was traced to foods prepared in the same catering establishment. In spite of operating as a flight kitchen, the catering had other food business, such as supplying sandwiches for trains. The number of culture-confirmed persons was 226. The number of persons at risk and those infected among railway and airline passengers and catering establishment staff was 107/600 (18%), 91/350 (26%) and 28/162 (17%), respectively. The test results of catering staff (162) revealed that the infection had spread to almost every group of employees. Out of the 118 food handlers, 23 (19%) became infected. Of those infected, 17 (74%) were symptom-free carriers and only 6 (26%) had symptoms (IV, Table 3).

Through questionnaires and other investigations, the source of infection was traced to the following foods: egg sandwiches served on trains, the meal served on aircraft consisting of Viennese goulash, fresh salad and Swiss roll, and cold cuts served to the catering establishment staff during breakfast. A statistical significant association was shown between the infection of catering staff and the cold cuts from the cold kitchen that were served during breakfast ( $p < 0.05$ ), whereas there was no statistical association between eating lunch and illness. No single dish served to air passengers revealed a statistically significant association with illness.

*S. Infantis* was detected in the routine control from one hot meal sample taken from a batch of 1 200 Viennese goulash portions, from which 350 portions had been sent to a charter

flight to Rhodes. *Salmonella* was not detected in any other food samples (152) tested during investigation of the outbreak.

Inspection of the catering establishment revealed several structural and functional shortcomings. The transport routes of raw and cooked foods were not separated, thus causing a risk of cross-contamination. There was no facility for fast chilling hot food. A lack of cold storage was also reported. Of great importance was that the food handlers suffering from diarrhoea were not excluded from work. Neither was any food hygiene training included in their education. The spread of the outbreak was further influenced by a heat wave at that time in Finland.

### 5.5 *Staphylococcus aureus* among flight catering employees (V)

The prevalence of *S. aureus* was much higher on the basis of nasal sampling compared with hand sampling, 32 of 111 (29%) and 10 of 117 (9%) respectively. Seven persons showed growth both in nasal and hand samples, and 20 persons only revealed *S. aureus* from nasal culture. Almost all hand carriers showed growth also in nasal samples (V, Table 1). Enterotoxigenic *S. aureus* types were found in 13 out of 111 (12%) and 7 out of 117 (6%) food handlers according to nasal and hand sampling, respectively.

PFGE macrorestriction profiles revealed a total of 32 different types associated with the 35 employees carrying *S. aureus*. Eight PFGE types were obtained from the hands and 30 types from nasal samples. In 4 cases out of 7 the same type colonised both hand and nose. PFGE type 6 was the most common type, colonising 5 persons. PFGE types tested for enterotoxin production showed that 12 of 32 (38%) types produced enterotoxin. The most common PFGE type 6 produced enterotoxin B.

## 6. DISCUSSION

### 6.1 Microbiological quality of hot meals served on aircraft (I, III)

Many of the hot meal samples exceeded the limit of the AEA standard for *E. coli* counts (8.2%). The number of samples having a higher total count than  $1.0 \times 10^6$  cfu/g was 9.2%, which is the limit set by the AEA for food items that have been handled after heat treatment. A previous study reported an even higher proportion of hot meals exceeding these values, for total counts (24%) and *E. coli* counts (25%) (Roberts et al. 1989). The following reasons may result in high bacterial counts in hot meals. Critical aspects controlling the bacterial level in hot meals are chilling, the time-temperature combination during portioning and packing, the temperature during storage in the flight kitchen, transport to the aircraft and the storage on board before serving. Considerable differences in the means of total bacteria and *E. coli* counts may indicate differences in the hygienic levels between countries. However, undercooked food items as deep-frozen blanched vegetables and steaks are commonly used in hot meals. They may be one important factor contributing to the high counts of total bacteria, *E. coli*, coliforms and *Enterobacteriaceae*, too.

The frequency of *S. aureus*, *B. cereus* and *C. perfringens* was lower than reported in previous studies (Roberts et al. 1989, Lambiri et al. 1995). *B. cereus* was the most common pathogen in this study, as was the case in a previous study, too (Roberts et al. 1989). However, quite few samples of hot dishes (0.7%) exceeded the AEA standard limit ( $10^3$  cfu/g). Present knowledge suggests that *B. cereus* is highly underreported, and that any food containing more than  $10^3$  cfu/g can not be considered completely safe for consumption (Granum 1996). *B. cereus* is widely distributed in nature. The organism is present in most raw materials used in food manufacture and its ability to form spores ensures its survival through all stages of food processing. Cooking food items which are then used as ingredients for hot meals leaves a residual flora of spores of *B. cereus* as well as *C. perfringens*. Temperature abuse and inefficient heat treatment on board may lead to food poisoning.

The occurrence of *S. aureus* was rather low in this study (0.6%). However, the occurrence in processed foods shows contamination via the hands, indicating inadequate personal hygiene among food handlers during the preparation of food. Cooked food can be contaminated by a colonised person during handling and portioning in the flight kitchen. The storage of contaminated foods at an inappropriate temperature (7 to 46°C) could possibly have led to the multiplication of *S. aureus* and the formation of enterotoxins, which are very resistant to heat and will survive cooking, even some sterilisation processes (Mossel and van Netten 1990). Because *S. aureus* is a poor competitor, it seldom causes problems with raw products. Heat-treated proteinaceous food items are good media for their growth. *S. aureus* cells are salt-tolerant and may be selected for in salt-containing products or products with lowered water activities (Genigeorgis 1989). Ham included in a hot breakfast was

traced to be the vehicle of one large aircraft-meal-associated outbreak (Eisenberg et al. 1975).

The occurrence of *Salmonella* in hot meals during the present surveys (0.3%) was similar to that of a previous study dealing with hot meals (Roberts et al. 1989), but lower than that reported by a Greek study (1%) (Lambiri et al. 1995). The source of contamination can be raw material, cross-contamination via raw materials, surfaces, utensils and infected food handlers. Although the meals undergo a final re-heating procedure on board before serving, the risk of foodborne disease is associated with contaminated hot meals. Proper heat treatment is sufficient to destroy surface contamination, but a malfunctioning oven used on an aircraft has been suspected as being the reason for an outbreak of *S. Infantis* (IV). Tauxe (1987) has also reported that one *S. Enteritidis* outbreak was associated with a hot meal served on an aircraft. Several hot meals contaminated by the same serotype during a period of one week in a Beijing flight kitchen may indicate that there was a carrier among the food handlers or that the surfaces and facilities of the kitchen were contaminated. However, no foodborne illnesses were reported after these findings.

## 6.2 Microbiological quality of cold meals served on aircraft (II, III)

There are no limits for aerobic colony counts in the AEA standards for cold dishes (1996). Total bacteria counts examined showed the proportion of cold meals as having higher counts than  $10^6$  cfu/g to be 10-41%. This was similar to a previous study (Roberts et al. 1989). A high total bacteria count fails to reflect the microbiological quality of cold meals, because appetisers, salads and desserts often include raw items such as fresh vegetables, fruits or garnishes, and they normally contain a high count of total bacteria. The use of sausages and cheeses produced using starter cultures as items in appetisers increases bacterial count, too. Many of the cold dishes (6-18%) in this study had higher *E. coli* counts than the AEA standard permits (1996). However, the results showed a better level than in the previous study, where 19-35% of the cold dishes exceeded the AEA limit of 10 cfu/g for *E. coli*. The occurrence of *E. coli*, especially in such high values as  $1.0 \times 10^6$  cfu/g detected, indicates contamination and poor microbiological quality. Raw items are commonly used for appetiser and salad dishes. The highest contamination rates were found in these dishes.

The frequency of *S. aureus* (7%) and *B. cereus* (5%) in this study was higher compared to the previous study (Roberts et al. 1989), where it was 0.3% and 3% respectively. A considerably higher frequency of *S. aureus* (24%) was reported by Lambiri et al. (1995). Cold meals need a lot of manual handling and contamination via the hands is therefore possible. Contamination of cooked items may occur during handling and portioning. The storage of contaminated food items that are inadequately refrigerated permits the multiplication of *S. aureus* and enterotoxin formation. In respect to cold dishes, desserts such as custards and chocolate cakes have been implicated with aircraft outbreaks. Flight delays and subsequent temperature abuse was proved to be the final reason for two *S. aureus* outbreaks via desserts served on board (Munce 1978, CDC 1973). The frequency of *B. cereus* detected

in this study indicates that it is difficult to avoid this microbe in cold meals. The source of contamination is raw materials, where low counts of *B. cereus* are commonly found (Kramer and Gilbert 1989). Inadequate refrigeration and storage at too high a temperature leads to a growth of bacteria and enterotoxin formation.

*Salmonella* was found in only one (0.1%) of the 1576 cold meals examined in present studies. It was lower than in the previous survey by Roberts et al. (1989), where it was 0.5% in cold meals. The *Salmonella* positive finding in one cold meal was subsequently found to be connected with an outbreak among air passengers. An appetiser prepared in Bangkok was shown to be the source of *S. Ohio* infection of five Finnish passengers in 1990 (Jahkola 1992). The contamination of a cold meal containing ham, together with the meals being transported unchilled during a long-haul flight, was considered to have been the reason for this outbreak. Vehicles of previous *Salmonella* outbreaks traced to meals served on board were mostly cold meals (Tauxe et al. 1987). In this study *Salmonella* cases indicated a higher risk of foodborne illness associated with cold than with hot meals. In order to avoid faecal contamination via raw vegetables, they are disinfected by soaking in a solution containing chlorine 60-100 ppm in the flight kitchen (Asplund 2000).

The results of this study show differences in the microbiological quality of meals between the countries where the food was prepared. Especially the means of *E. coli* and *B. cereus* refers to differences between hygienic level in production.

### 6.3 *Salmonella* outbreak (IV)

The infection probably spread to the catering establishment staff via contaminated cold cuts sliced in the cold kitchen. The number of infected food handlers (23/118, 19%) was considerable. The fact that most of the infected food handlers were symptom-free carriers (17/23, 74%) and that the rest had mild symptoms, but worked normally, must have led to widespread contamination of the kitchen's production, causing the infection to spread to railway and airline passengers, too. If the medical service of the airline company had immediately started to investigate the cause of the gastrointestinal illness of the food handlers in the beginning of August and excluded them from work, the spread of contamination of the products could possibly have been prevented. An exceptional heat wave in Finland at that time and shortcomings in the cold chain were contributing factors. There was neither any in-house control system in the kitchen nor any food hygiene education for employees at that time. As a consequence of the outbreak the airline company recruited a food hygienist a few years later. The results of a recent study dealing with food handlers' (411) knowledge about foodborne diseases still strongly emphasised the need for educational hygiene courses (Angelillo et al. 2000).

Raw materials used in the catering establishment were widely tested, but they were *Salmonella* negative. Because none of the food handlers had recently been abroad, it was considered possible that one of them became infected via Finnish food during July in 1986. *Salmonella* bacteria were seldom found in Finnish foodstuffs at that time (Nurmi and

Schildt 1987), nor are they today (Ministry of Agriculture and Forestry 1999, 2000). However, the source of the *Salmonella* outbreak remained unclear.

Train passengers on several routes became infected, whereas the gastrointestinal illness of air passengers was traced to one flight, only. Egg sandwiches for trains as well as hot aircraft ready meals were prepared at the same time in the flight kitchen as the infection had spread among kitchen staff. The batch of hot aircraft meals, from where *Salmonella* was isolated, had been delivered to other charter flights, too. The malfunction of the regeneration oven used to re-heat the dishes served hot on board may be the reason for the spread of infection on this particular flight.

In many national epidemiological registries, non-typhoid *Salmonella* spp. continue to figure prominently as the leading cause of bacterial foodborne disease (D'Aust 1994, Notermans and Borgdorff 1997). The growing importance of the international food trade between countries that maintain widely different levels of hygiene in their agricultural and food processing industries presents a health concern also for flight kitchens supplying airline companies. The ubiquitous distribution of *Salmonella* in the natural environment and its prevalence in the global food chain predicate the need for stringent controls at all levels of the food production process. From the 2500 *Salmonella* serovars currently known, only 10 to 15 are of epidemic importance, in the first place *S. Typhimurium* and *S. Enteritidis*. *S. Enteritidis* became the predominant serotype in western Europe, North America and South America in the late 1980s and early 1990s (Rodrique et al. 1990, Bean and Griffin 1990, Sockett et al. 1993). Eggs and raw meat and meat products were of prime importance.

#### 6.4 *Staphylococcus aureus* among flight catering employees (V)

The prevalence of *S. aureus* among Finnish flight catering employees (29% and 9% according to nasal and hand sampling, respectively) corresponded to many previous studies of healthy humans (Williams 1963, McBride et al. 1975, Oteri and Ekanem 1989, Caruso et al. 1992, Namura et al. 1995, Al Bustan et al. 1996). The present study and previous studies (Bergdoll 1989, Röder et al. 1995) showed that half of the strains from human carriers are enterotoxigenic. A person harbouring *S. aureus* must be considered as a potential source of enterotoxigenic strains. In addition, coagulase negative staphylococci isolated on the hands of food handlers may produce staphylococcal enterotoxins and be a potential cause of food poisoning (Danielsson and Hellberg 1984, Udo et al. 1999).

The majority of the food handlers studied had actively used hand disinfectants. This might reflect in the lower detection rate of *S. aureus* in the hands (9%) compared to nasal samples (29%). These results indicate that examining *S. aureus* from hand samples only is not always a reliable way to detect the carriage of *S. aureus*. Preparing food for aircraft is a highly vulnerable operation, and therefore testing carriage among food handlers is of valuable assistance in planning preventive measures. A potential risk of foodborne disease was shown by the results of the present study, which found *S. aureus* in both hot and cold air-

craft meals, 0.6% and 7% respectively (I, II), as well as the study by Ewald and Christensen (1987) dealing with the occurrence of enterotoxin producing *S. aureus* strains in aircraft meals. This should be taken into account in hygiene training. Extra training, such as the proper use of disposable gloves, should focus on food handlers who are *S. aureus* carriers.

PFGE typing revealed a wide diversity in genomic types, showing 32 different types among 35 food handlers. In the present study one clone mainly colonised one person, but three persons were also found to carry 2 different types in the samples taken on the same day. In this study, in 4 cases of the 7 showing *S. aureus* on both the hands and in the nose, the strain was also found on the hands, clearly indicating transmission of the strain from the nose to the hand. As regards the types which were found in hand samples only, a person's hands may naturally become contaminated with strains from a source other than the person him/herself. When the carriage of *S. aureus* in the nares was monitored in Japan (Hu et al. 1995), only one clone colonised one person and it persisted for a long period. This gives rise to major questions: is a persistently colonised individual always inhabited by the same strain, or can strain exchange occur. A recent study following *S. aureus* nasal carriage over eight years identified 47% non-carriers, 17% intermittent carriers, and 36% persistent carriers (Van den Bergh et al. 1999). Further characterisation of *S. aureus* strains by PFGE is very useful in case of tracing the source of contamination.

## 7. CONCLUSIONS

1. Some of the hot meals exceeded the microbiological standards accepted by the AEA for *E. coli* (8.2%), *S. aureus* (0.6%), *B. cereus* (0.7%) and for *C. perfringens* (0.7%). Total counts higher than  $10^6$  cfu/g, which is the AEA limit for food items that have been handled after heat treatment, were found in 9.2% of hot meal samples. Total counts above this limit indicates shortcomings in food preparing practice. Although the frequency of food poisoning bacteria was rather low, their occurrence may indicate a risk of food poisoning via hot aircraft meals. However, hot meals undergo final re-heating on board, which decreases the microbial count of hot meals and thus the risk of food poisoning. There were significant differences between preparing countries regarding the microbiological quality of hot meals reflecting the level of production hygiene.
2. Many of the cold meals failed to meet the AEA standard for *E. coli* (14%), *S. aureus* (7%) and for *B. cereus* (3%). The contamination rate in respect of these bacteria was higher in cold meals than in hot meals. Such a high level as the  $10^6$  cfu/g for *E. coli* found indicated poor microbiological quality of meals. The maximum levels of *S. aureus* and *B. cereus* found,  $10^3$  cfu/g and  $10^4$  cfu/g, respectively, mean shortcomings in microbiological quality and indicate a risk of food poisoning. In the case of cold meals, the level of hygiene of the preparing country seemed to reflect to the microbiological quality of the meals, too. Stricter control measures should be focused particularly on the production of cold aircraft meals, which seemed to be even riskier than the hot ones.
3. The prevalence of *Salmonella* was low in cold meals (0.1%), but the only positive finding detected was connected with an outbreak among air passengers. The prevalence of *Salmonella* in hot meals was higher (0.3%). However, none of them were reported to be connected with outbreaks. If final re-heating on board is properly carried out, it should destroy *Salmonella* contamination. This means a higher risk of *Salmonella* associated with cold served dishes than with hot served ones.
4. Ninetyone airline passengers and 107 railway passengers became infected with *S. Infantis* via food prepared in a flight kitchen. A high number of the flight catering employees 28/162 (17%), also became infected via breakfast prepared in the flight kitchen and served in their canteen. This figure included many 23/118 (19%) of the catering establishment's food handlers. It was impossible to establish the origin of this *Salmonella* outbreak. The employees' breakfast had probably been contaminated by a symptom-free *S. Infantis* carrier in the flight kitchen. Many of the food handlers became infected and this subsequently led to widespread contamination of food products of the flight kitchen. Air passengers became infected via contaminated meals served on a charter flight and railway passengers via contaminated egg sandwiches served on several train routes. *S. Infantis* was isolated from one hot meal sample representing the batch served on the particular charter flight. The most prominent contributing factors

were found to be that food handlers suffering from mild diarrhoea were not excluded from work and that there was no hygiene education or supervision for food handlers. A heat wave combined with a shortage of refrigeration facilities and possible malfunction of the re-heating oven on board were regarded as contributing factors, too. The results of the investigation showed that preparing meals for aircraft is a high-risk operation, which calls for strict hygiene requirements and a thorough knowledge of food hygiene.

5. Hand and nasal sampling showed a substantial prevalence, 6% and 12% respectively, of the carriage of enterotoxigenic *S. aureus* among flight catering food handlers. Nasal carriers can easily transmit *S. aureus* into the hands and this means a potential risk of food poisoning. Because *S. aureus* colonises primarily in the human nose, nasal sampling is a better way of detecting *S. aureus* carriers than hand sampling. Testing food handlers working in high-risk premises such as in flight catering provides valuable information about carriers. It helps in planning preventive measures, such as special hygiene instructions for carriers to avoid contamination of food. Characterisation of isolated strains by pulsed-field gel electrophoresis is very useful especially by tracing the contamination source. It also revealed that more than one clone can be harboured by one employee.

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