OPINION OF THE

Scientific Committee on Veterinary Measures relating to Public Health

On

Salmonellae in Foodstuffs

(adopted on 14-15 April 2003)

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1. EXECUTIVE SUMMARY

Salmonella Enteritidis and *Salmonella* Typhimurium have been reported most frequently as the major causative agents of human salmonellosis. However, other serotypes have caused illness and still others will emerge in the future. Consequently, the finding of any member of *Salmonella* enterica, characterised by serological classification, in a foodstuff indicates public health concern.

While interventions in primary production may reduce or eliminate salmonellae, contamination may occur along the food chain. In particular contamination at slaughter cannot be totally prevented. However, some treatments applied during food processing may have a bactericidal or bacteriostatic effect on salmonellae.

There is a link between the prevalence of various salmonellae serotypes in food categories and the serotypes implicated in human salmonellosis. However, these are often not strongly correlated, due to the various factors that influence the prevalence of salmonellae in food commodities at the time of consumption, including technologies applied in food production and processing, and the final preparation of a meal.

Considering the possibility of hazards to public health posed by food categories, the Committee took account of the reported prevalence of salmonellae, the incidence of human salmonellosis including the serotypes implicated, and the food technologies and/or preparation and handling applied. Food categories possibly posing a greater hazard to public health include raw meat and some products intended to be eaten raw, raw or undercooked products of poultry meat, eggs and products containing raw eggs, unpasteurised milk and some products thereof. Sprouted seeds, unpasteurised fruit juices as well as home-made mayonnaise are also of major concern. Although there have been occasional outbreaks linked to other food commodities, these are considered accidental and not a persistent risk to human health.

However, it should be emphasised that with every food commodity, even those where the risk is normally low, cross-contamination might occur if salmonellae are present in the environment. Thus the possibility of accidental cross-contamination or recontamination should also be considered in all foods.

Microbiological criteria can be applied differently as 'standards' or as 'guidelines'. Each of these applications has a different meaning with regard to the risk management implications. However, the setting of microbiological criteria is only one possible risk management option within the broad range of integrated control strategies.

A microbiological criterion should always include a statement of the micro-organisms of concern (in this case *Salmonella* spp.), the analytical methods for their detection and/or quantification, as well as sampling plans and corrective actions to be taken if the criterion is not met.

Guidelines: Guidelines may be useful in areas where the prevalence of salmonellae is expected to be high, but where the hazard to public health is reduced due to the impact of food processing technology or preparation techniques applied. Implementation of such guidelines together with appropriate corrective actions will, over time, result in a reduction of the prevalence of salmonellae.

The Committee stresses that the use of guidelines is well-established in food chains, where the reduction of the prevalence of food contamination with the agent is the objective. This is especially true at primary production. If there is an immediate concern, due to epidemiological and technical reasons, that the agent is likely to reach the consumer, then microbiological standards could be introduced.

Standards: The application of mandatory criteria (standards) should only be considered where salmonellae are able to reach the consumers, based on their prevalence in the raw materials, the food technologies and processes applied and where a history of cases or outbreaks reflects a recognised hazard to public health. Occasional or accidental recontamination of an otherwise safe product can always occur and should not be considered in the same context. Standards should be implemented where there is a need, and for food commodities where their efficacy and utility has been confirmed or can be expected.

Finally, food commodities most frequently associated with contamination by salmonellae and outbreaks of salmonellosis should be considered for further risk profiling, taking into account the aspects considered in this document and risk profiles and assessments that have already been published.

2. BACKGROUND

The Community legislation on food hygiene is currently under revision. Proposals for this revision have been submitted to the Council and the European Parliament. In this context the Commission has also started a revision of the microbiological criteria in Community legislation.

The Commission is preparing a comprehensive strategy to set these criteria. This strategy would cover for all foodstuffs the whole production and distribution chain (including retail trade) in line with the proposed new hygiene legislation. Criteria would be set for food products on the market, as well as for food products at different stages of the manufacturing process.

The Scientific Committees have already provided several opinions on the subject of microbiological criteria. The opinion on foodborne zoonoses covers the most important foodborne pathogens, including salmonellae and verotoxigenic *Escherichia coli*. These general, and comprehensive, reports indicate the need for more specific information, in order to put into place appropriate measures against the pathogens considered.

3. TERMS OF REFERENCE

The Scientific Committee on Veterinary Measures relating to Public Health is asked to:

- identify categories of foodstuffs where *Salmonella* spp. represents a hazard to public health;

- evaluate the appropriateness of setting microbiological criteria and,

- identify where risk profiles might be useful.

Considering the common field of interest, the Committee is invited to set up a joint working group including experts from both the Scientific Committee on Veterinary Measures relating to Public Health and the Scientific Committee on Food.

4. INTERPRETATION OF THE TERMS OF REFERENCE

The Committee interprets (a) "hazard to public health" as representing a high risk to human health; (b) "appropriateness of setting criteria" as whether the implementation of a criterion will contribute meaningfully to a reduction of the public health risk posed by the particular pathogen-food commodity combination; and (c) "identifying where a risk profile would be useful" as whether the risk evaluation should be continued, including allocating necessary resources. This may, or may not, proceed to a full risk assessment.

5. SALMONELLAE IN RELATION TO PUBLIC HEALTH

5.1. Nomenclature / Taxonomy of Salmonellae

The genus *Salmonella* contains two species (*Salmonella enterica* and *S. bongori*) based on phenotypic criteria. Salmonellae are Gram-negative bacteria belonging to the family Enterobacteriaceae. The species *S. enterica* is divided into 6 subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtanae* and *indica*) (Le Minor and Popoff, 1987). The serology, based on the characterisation of the somatic (O), flagellar (H), and envelope (Vi) antigens, allows classification into serotypes. The greatest number of serotypes belong to the subspecies *S. enterica* ssp. *enterica*. Some of the serotypes belonging to this subspecies are described by a name corresponding to the geographic location of an outbreak (e.g. *S. enterica* subspecies *enterica* serotype Montevideo, referred to as *S.* Montevideo), while others are identified by their antigenic formula, e.g. *S.* Enterica 1, 3, 19:y. All known serotypes (over 2,400) are listed within the Kauffmann-White's scheme (Popoff *et al.*, 1996).

Further characterisation of isolates can be achieved by the use of molecular typing methods, facilitating more detailed epidemiological investigations (Tenover *et al.*, 1995).

5.2. Disease in humans and infectious dose

Humans are particularly vulnerable to *S.* Typhi and *S.* Paratyphi A and B, infections, due to the ability of these strains to invade and multiply within host tissues.

Human salmonellosis comprises several clinical syndromes including enteric (typhoid) fever, localised enterocolitis and systemic infections by non-typhoid microorganisms. Clinical manifestations of enteric fever appear after a period of incubation ranging from 7 to 28 days and may include diarrhoea, prolonged and intermittent fever, abdominal pain and headaches. Enteric fever and septicaemia due to salmonellae are serious human illnesses, but occur relatively rarely in the EU (Mølbak *et al.*, 2002).

Human infections with non-typhoid salmonellae commonly result in enterocolitis that appears 8 to 72 h after ingestion of the invasive pathogen. This clinical condition is generally self-limiting, and remission of the characteristic non-bloody diarrhoeal stools and abdominal pain usually occurs within 5 days of onset of symptoms. Human infections with non-typhoid strains can also progress to systemic infections and result in various chronic conditions such as reactive arthritis, Reiter's syndrome and ankylosing spondylitis (D´Aoust, 1991, 1997, 2000).

In many EU countries the salmonellae that most frequently cause human gastroenteritis are *S.* Typhimurium and, especially in more recent years, *S.* Enteritidis, particularly Phage Type 4 (PT4) (ACMSF, 2001; WHO, 2001a; EC, 2002). The other serotypes involved in human illness vary geographically but frequently include *S.* Agona, *S.* Hadar, *S.* Heidelberg, *S.* Infantis, *S.* Newport, *S.* Panama, *S.* Saint-paul, *S.* Thompson, and *S.* Virchow (WHO, 2001a).

For the period between 1993 and 1998, WHO (2001a) reports identified the most frequently reported *Salmonella* serotypes as *S.* Enteritidis (73.8% of isolates in 1993 and 83.6% in 1998) and *S.* Typhimurium (20.3% in 1993 and 12.0% in 1998). All other serotypes were reported at a far lower percentages. Trend analyses revealed that, as a percentage of overall isolates, the percentage of *S.* Typhimurium isolates decreased, but the number of multi-resistant *S.* Typhimurium Definitive Type (DT) 104 isolates recovered from human patients increased over the same period (WHO, 2001a).

The prevalence of *S.* Typhimurium DT104 in the UK has declined since 1998-1999 (www.phls.co.uk), although isolates showing multi-drug resistance remain a public health concern (Ribot *et al.*, 2002). Another concern is the increasing emergence of quinolone resistance in *S.* Enteritidis (Mølbak *et al.*, 2002).

The infectious dose of salmonellae can vary, depending on the bacterial strain ingested as well as on the immuno-competence of individuals. For serotypes not presenting particular adaptations to an animal host, experimental studies showed that between 10^5 to 10^7 bacteria were required to establish an infection (McCullough and Eisele, 1951). However, data from outbreaks of foodborne diseases indicate that infections can be caused by ingestion of as few as 10-45 cells (D'Aoust *et al.*, 1985; Lehmacher *et al.*, 1995). It has repeatedly been reported that the infectious dose is lower when salmonellae are present in food with a high content of fat or protein, substances which protect bacterial cells against the low pH of gastric juices (D'Aoust *et al.*, 1975; Blaser and Newman, 1982).

Some serotypes have developed a high host adaptation such as *S.* Pullorum and *S.* Gallinarum in poultry, *S.* Dublin in cattle, *S.* Abortus-ovis in sheep and *S.* Cholerae-suis in swine. Animals infected with non-host adapted salmonellae are usually asymptomatic carriers, although some may exhibit clinical signs of low to moderate severity. Carriers that have not been recognised as such present a risk of shedding salmonellae into the environment.

At this stage of knowledge, and in the sense of this document, any serotype that is not host-adapted is considered capable of causing gastrointestinal illness of varying severity in humans.

5.3. Routes of transmission to humans

The principal reservoir for salmonellae is the gastrointestinal tract of mammals, reptiles and birds.

Salmonellae have been isolated from very different sources and can survive in the environment for prolonged periods and for long periods in foods and in other substrates (ICMSF, 1996). Reported survival times vary greatly e.g. in bovine manure (over 34 months), fish meals (over 24 months), garden soil (over 9 months), poultry litter (over 4 months) or poultry manure (over 1 month) and tap water (over 2 months) (Pietzsch, 1981; Murray, 1991). Salmonellae survived for over 2.5 months in butter stored between -23°C and 25°C (Sims *et al.*, 1969) and for 6 months in milk stored at room temperature or

in an ice-box (Berry, 1927). On a range of vegetables (green beans, beets, cabbage, carrots, celery, cucumbers, lettuce, peppers, radish, spinach, and tomatoes) stored at 2-4°C, salmonellae survived for more than 1 month (Felsenfeld and Young, 1945). Survival in chocolate is prolonged, numbers barely declining over months in milk chocolate. Salmonellae also survive well on surfaces e.g. ceramic, glass, stainless steel (McDade and Hall, 1964) and on human skin (Pether and Gilbert, 1971).

Due to their metabolism and physiology, salmonellae are not restricted to a particular habitat. Most are widely adaptable and thus have the potential for transmission through human, animal and plant habitats, and the environment in general. Consequently, transmission routes vary, complicating tracing and identification of routes of infection in cases of human disease.

6. ROLE OF FOOD TECHNOLOGY FACTORS ON GROWTH, SURVIVAL AND INHIBITION OF SALMONELLAE

6.1. Metabolic / Physiological properties

The general metabolic and physiological properties of salmonellae have been considered in this chapter. Reference is also made to particular serotypes, which differ substantially from the common pattern of other salmonellae in terms of certain characteristics, for example with regard to heat resistance.

Salmonellae are capable of multiplying under aerobic or anaerobic conditions, and over a wide temperature range (5-46°C), with an optimum for growth of between 35°C and 43°C. Growth is markedly slowed at temperatures below 15°C (Table 1). At 8°C doubling times of salmonellae were reported to be between 22 and 35 h (Broughall *et al.*, 1983; Grau, 1987; Gibson *et al.*, 1988). The lowest recorded temperature at which growth occurs is reported as 5.2°C in laboratory medium (Matches and Liston, 1972) and 6.7°C in a food product (Angelotti *et al.*, 1961). Reports of bacterial growth at lower temperatures did not always confirm that the observed growth was due to salmonellae. Although the lowest temperature at which salmonellae may grow is approximately 5°C, most serotypes fail to grow in food stored below 7°C (ICMSF, 1996).

The natural microflora of minced beef was reported to have little effect on the rate of growth of salmonellae (Mackay and Kerridge, 1988, see Table 1), even when numbers of salmonellae were greatly outnumbered by other organisms.

Table 1: The effect of temperature on growth of salmonellae in minced beef (pH 5.4 – 5.7)*.

Temperature (°C)	Generation time (hours)	
	A	**B**
35	0.47	0.43
30	0.65	0.56
25	1.11	0.97
20	1.59	1.43
15	3.03	3.03
10	15.15	10.00

* compiled from ICMSF 1996 (Table 1b, p.231), data of Mackey and Kerridge, 1988

a= high inoculum ratio of salmonellae (10^4 salmonellae and 10^4 total viable count bacteria/gram]

b= low inoculum ratio of salmonellae (40 salmonellae and 10^5 total viable count bacteria /gram]

Values of pH greater than 9 or lower than 4 inhibit the growth of salmonellae. Growth is also inhibited when the water activity (a_w) is lower than 0.94. It is sometimes difficult to distinguish bacteriostatic from bactericidal effects due to the combined influence of a variety of factors.

In principle, the most reliable means of controlling growth of salmonellae are chill storage and heat. Certain strains (e.g. *S.* Senftenberg 775W) are relatively resistant to heat (Anellis *et al.,* 1954; Ng *et al.*, 1969). The response to heat can be quantified by means of the D-value and z-value. D-value is the time in minutes at a given temperature to achieve a 90% reduction in numbers of viable bacteria. The z-value is the temperature change to effect a 10-fold change in the D-value. The D-value varies depending on other factors such as pH and a_w. Representative D-values, and the effects of a_w on heat resistance, are shown in Table 2.

Table 2. Effects of water activity (a_w) and temperature (°C) on heat resistance (D-values) of *S*. Typhimurium and *S*. Senftenberg*.

a_w	D-value (min)					
	S. Senftenberg			*S*. Typhimurium		
	at 60°C	**at 65.5°C**	**at 70°C**	**at 60°C**	**at 65.5°C**	**at 70°C**
0.995	7.2	1.1	0.2	0.18	0.05	<0.05
0.98	7.2	1.1	0.2	0.60	0.066	<0.05
0.94	14.5	3.6	0.5	4.3	0.83	0.3
0.90	11.4	3.8	1.0	4.8	2.66	0.6
0.85	N.R.	4.1	1.1	10	3.53	1.4

*compiled from ICMSF, (1996) (Table 1c, p. 239), and data of Gibson, (1973).

a_w was adjusted using glucose   N.R.: not reported

pH 5.5 – 6.2

Consequently, thermal death rates of salmonellae at different temperatures can be calculated from the D and z values, allowing lethal processes to be calculated for foods where salmonellae are considered a hazard.

Unless the storage temperature is maintained below 7°C, salmonellae can multiply in many foods. It is also important to recognise that salmonellae are able to survive for long periods in some food environments where multiplication cannot occur.

6.2. Food chain

The food chain includes all operations occurring from primary production until the eventual consumption of a food product by the consumer.

Primary production:

Primary production must be regarded as the main reservoir for salmonellae. Some control measures, including Good Agricultural Practices (GAP) have been shown to reduce the prevalence of salmonellae at the farm level and therefore in the primary production environment. Examples of such measures include:

- good animal husbandry practices,
- biosecurity measures,
- standards of buildings and machinery,
- pest control programmes,
- cleaning and disinfection procedures,
- waste management,
- feed quality control,
- detection/ isolation of infected animals and carriers of infections,
- control of salmonellae in the breeding stock used to supply commercial farms (e.g. parent and grandparent poultry flocks),
- Good Veterinary Practices (GVP).

However, even these measures cannot guarantee the absolute absence of salmonellae in primary production.

Transport

Animals may be transported on several occasions where the concept of 'closed farms' cannot be implemented. However, animals originating from closed farms are also transported to reach the abattoir. Transport has to be considered as a phase of significant stress, even where measures are taken to reduce and minimise particularly stressful procedures. Many reports have indicated that during transport otherwise healthy carriers of salmonella can shed the organism, thus contaminating other animals and ultimately the slaughter line.

The slaughter line:

Animals suffering from salmonellosis have to be excluded from the food chain. However, as mentioned above, healthy animals can also be asymptomatic carriers, which cannot be detected by traditional meat

inspection. Animals going for slaughter may carry salmonellae in their intestines and on their external surfaces (hides, fleece, skin or feathers) serving as sources of cross-infection and contamination. Opportunities for cross-contamination include:

- in beef, sheep and goats: skinning (de-hiding cattle, de-fleecing sheep), evisceration (especially upon removal of the rectum or if rupture of the gut occurs),

- in pigs: scalding, de-hairing (scraping), polishing, evisceration,

- in poultry: scalding, de-feathering, evisceration, improperly controlled water chilling.

Some measures related to Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP) in animal production and at the stage of slaughter and dressing can reduce the prevalence of salmonellae. To this end, a HACCP system needs to be applied in abattoirs.

Nevertheless the complete decontamination of raw meat is impossible unless sufficient heating or irradiation processes are used. Other methods, for example hot water rinsing or spraying with decontaminants (such as lactic acid chlorides or trisodium phosphate), may reduce the numbers of bacteria but will not entirely eliminate all contaminating organisms (Anon., 1996; Bacon *et al*., 2000). Cross-contamination may still occur even after such decontamination processes.

<u>Food processing line:</u>

During food processing, foods such as meat, milk, eggs, fruit and vegetables are subjected to various processes, such as cutting, drying, salting, ripening, freezing or heating.

Furthermore, currently available foods and associated preparation techniques frequently involve the combination of products of diverse origins. Many products are produced in 'ready-to-eat' forms (convenience foods) and undergo various manipulations and handling processes before their packaging.

Some technological procedures will impact on microbial contamination of the end product and result in bactericidal or bacteriostatic effects. These preservation techniques include:

✓ heating (various time/temperature combinations), irradiation (ultra-violet and ionising), application of high hydrostatic pressure,

✓ pH modification (acidification, application of organic acids or fermentation involving lactic acid producing bacteria),

✓ a_w modification (drying, salting, addition of sugar),

✓ chilling, deep-freezing,

✓ packaging (e.g. in laminates of different gas permeabilities, storage in modified atmospheres).

Some of these preservation techniques do not have a bactericidal effect, but multiplication of organisms is prevented (e.g. fermented sausage, salami type). However, it needs to be stressed that food can always be re-contaminated prior to consumption.

6.3. Conclusions

Primary production of food animals remains the most important reservoir of salmonellae entering the human food chain, since a salmonellae-free production system cannot be achieved in all animal species. Controls at slaughter and dressing are often not sufficient to prevent salmonellae entering the food chain.

In foods, the main factors affecting microbial growth and survival are pH, a_w and temperature. Other important factors include the competing microflora, the initial number of salmonellae and their physiological state. Technologies applied to foods have a marked influence on the survival of salmonellae in foods.

7. FOOD CATEGORIES WHERE SALMONELLAE MIGHT REPRESENT A HAZARD TO PUBLIC HEALTH

7.1. Procedure and considerations

To answer the first terms of reference, the Committee followed a structured approach, to make the procedure (data and their interpretation) as clear and reproducible as possible.

The following points were considered:

(1)   routes of transmission and prevalence of salmonellae in different food categories throughout the food chain until final consumption.

(2)   food technology applied including final preparation practices prior to consumption.

(3)   incidence of salmonellosis in humans: based on epidemiological data available from the EU Member States and other countries.

Generally, the prevalence of salmonellae in food categories and the incidence of human cases or outbreaks of salmonellosis must be considered separately, since the prevalence of serotypes in foods and their incidence in human cases are not always directly related.

The prevalence of particular serotypes was also considered when relevant data were available.

Salmonellae are relatively robust organisms that are able to survive for long periods in the food-processing environment. That capacity to survive is important when assessing the risk of possible transmission.

The origin of the raw materials needs to be taken into account, with different prevalence data depending on whether the food originates from areas or holdings where salmonellae are 'endemic' or from areas or holdings with a lower prevalence.

Additional factors such as the technology applied to the food categories, as well as the final preparation before consumption, were also taken into account.

However, when considering estimated prevalences, intermittent breakthroughs of salmonellae contamination can occur along the food chain. Such breakthroughs may not be captured by point prevalence estimates and, therefore, those estimates may not always fully reflect the true situation.

7.2. Incidence in humans

7.2.1. Reported cases

Epidemiological data from Europe are available from several sources e.g. the WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe (WHO, 2001a), the EU "Zoonoses Reports" (EC, 2002), the journal Eurosurveillance via www.phls.co.uk, and from the USA on Foodnet via www.cdc.gov.

In the year 2000, 150,165 cases of human salmonellosis were reported from 17 regions in Europe, covering 14 EU Member States and Norway (an additional 1,489 cases) (EC, 2002). That total includes countries where salmonellosis is notifiable and countries where notification is not obligatory, with reports based on laboratory isolates.

Throughout the European Union, the reported number of cases of salmonellosis in humans is quite high, in the year 2000 ranging from 79,535 in Germany to 164 domestic cases in Norway (EC, 2002).

However, it should be emphasised that these figures are based upon different monitoring systems and cannot be directly compared. Some countries distinguish between domestically acquired cases and cases acquired abroad. Data from Norway, Sweden and Finland show that about 80% of the reported cases are acquired abroad. Data may also reflect different habits in food preparation and consumption in different countries, as well as differing prevalences of salmonellae in foods. Also, a large part of the observed variation might be accounted for by different diagnostic methods and differences in surveillance systems and ways of reporting.

Even after considerable efforts in the reporting countries to standardise both testing and reporting, differences remain (WHO, 2001a) and comparison of data still proves difficult. From 1999 to 2000, the total number of reported cases of salmonellosis decreased by 9.2 % in the EU (EC, 2002).

Details of a number of major foodborne outbreaks of human salmonellosis are given in the annex (Tables 3 to 8).

7.2.2. Foods and serotypes involved

Relatively few outbreaks are fully investigated epidemiologically, and in many outbreaks a food source for the pathogenic agent is not identified. The 7th WHO report for the period 1993-1998 (WHO, 2001a) identifies some foods implicated in salmonellosis outbreaks.

Of the outbreaks of foodborne illness recorded in the WHO report for 1993 to 1998, salmonellae were most often reported as the causative agent. Salmonellae were involved in 54.6 % of cases. Of these cases, *S.* Enteritidis was the causative agent in 34.7 % (WHO, 2001a). In that report the most important foods, where salmonellae caused outbreaks were:

eggs and egg products: 35%

cakes and ice-cream: 28%

meat and meat products: 8%

meat and eggs: 7%

poultry and poultry products: 4%

salads, dressings and mayonnaise: 4%

Generally *S.* Enteritidis was the predominant serotype in outbreaks involving eggs and egg products. With regard to human outbreaks, 62.8% of *S.* Enteritidis-linked outbreaks were connected with eggs and egg products, while 39% of *S.* Typhimurium cases were linked to eggs and egg products.

Most frequently reported serotypes involved in outbreaks in European countries are *S.* Enteritidis and *S.* Typhimurium, occurring in a ratio of approximately 3:1 in Europe from the years 1993 to 1998 (WHO, 2001a). In the year 2000, the most frequently reported serotypes in data from 9 countries were *S.* Enteritidis (59.14%), *S.* Typhimurium (13.03 %), *S.* Hadar (1.36 %) and *S.* Virchow (1.36%) (EC, 2002).

Over time, variation occurs in the most commonly isolated serotypes, as well as the animal species in which they are commonly isolated, for reasons that are not always clear. For example, in England and Wales *S.* Hadar emerged as a common cause of salmonellosis in the 1970s (D'Aoust, 1994). This was traced to infection in turkey breeding flocks, and preventive measures eliminated it. Consequently, it virtually disappeared as a cause of human salmonellosis in the mid-1980s.

In the USA, *S.* Newport has been the third most commonly isolated serotype. During 1997-2001 the number of laboratory-confirmed *S.* Newport infections reported to the Communicable Diseases Centers rose from 5% to 10% of the total number of cases. In addition, many isolates exhibited multi-drug resistance. During January to April 2002, *S.* Newport was isolated from 47 people in 5 states, with exposure to raw or undercooked ground beef identified as the vehicle of transmission (MMWR, 2002a).

Mayonnaise has been implicated in many outbreaks of salmonellosis. A major outbreak of ca 10,000 cases in Denmark was traced to factory-produced mayonnaise and led to a Danish regulation that the pH of mayonnaise should be <4.5 (ICMSF, 1998 Chapter 11, pp. 390-417, "Oil- and fat-based foods"; Michel and Koning, 2000; Petersen, 1964; Meyer and Oxhøj, 1964).

In 1976, a serious outbreak of salmonellosis occurred in Spain and approximately 500 people became ill, with six fatalities. *S.* Typhimurium phage type 96 was isolated from mayonnaise used (pH not reported) and from a food handler involved in the preparation of the mayonnaise (Davies and Wahba, 1976).

A dramatic increase in salmonellosis occurred in the 1980s due to contamination of hen's eggs with particular phage-types of *S.* Enteritidis. In Spain, homemade mayonnaise was the cause of a significant increase in foodborne illness due to *S.* Enteritidis (Perales and Audicana, 1988). In many countries, e.g. USA, Argentina, there have been outbreaks of salmonellosis from homemade or restaurant-made mayonnaise (St. Louis *et al.*, 1988; Eiguer *et al.*, 1990; Telzak *et al.*, 1990). Worldwide there have been many other reports of salmonellosis linked to mayonnaise (Michels and Koning, 2000).

Chocolate and cocoa powder were not recognised as causes of salmonellosis until two outbreaks occurred in 1970 and 1973. Cocoa powder contaminated with *S.* Durham, and used in confectionery products, was responsible for an outbreak affecting 110 people in Sweden (Gästrin *et al.*, 1972). In Canada and in the United States 200 people, mostly children with an average age of 3 years, were infected by chocolate contaminated with *S.* Eastbourne (Craven *et al.*, 1975; D'Aoust *et al.*, 1975). Contamination was traced to cross-contamination in the factory, due to inadequate separation between clean and unclean zones.

In more recent years various outbreaks from chocolate have been reported (Gill *et al.*, 1983; Hockin *et al.*, 1989; Kapperud *et al.*, 1989a, 1989b, 1990; Torres-Vitela *et al.*, 1995). Cases from contaminated chocolate were also identified in Sweden, Canada, Austria, Belgium, Australia, Finland and Croatia (Anon, 2002).

The infective dose of salmonellae consumed in chocolate is very low: an average infective dose of 1.6 cells/g was determined for *S.* Napoli (Greenwood and Hooper, 1983), of 0.2-1.0 cells/g for *S.* Eastbourne (D'Aoust and Pivnick, 1976) and as low as 0.005-0.025 cells/g for *S.* Nima (Hockin *et al.*, 1989). These very low infective doses are probably a consequence of the short intra-gastric residence time and the protective effect conferred by the fat content of chocolate against inactivation of salmonellae by gastric acids (Tamminga *et al.*, 1976; D'Aoust, 1977, BgVV, 2002).

7.3. Salmonellae and food commodities

Certain foods have been found to be frequently associated with outbreaks of salmonellosis (see Tables 3-8 in the Annex). The EU Report on Trends in

Zoonoses (EC, 2002) is a guide to the distribution and occurrence of salmonellae within the EU.

However, it remains very difficult to compare data originating from different countries because methodologies used are not identical, not only regarding the sample preparation (size of samples, sites of sampling) but also the methods used to detect salmonellae in the food.

Using epidemiological information and the results of surveys (prevalence and incidence), as well as data from scientific literature, the Committee allocated major food commodities to a 'risk' category of human salmonellosis, taking account of technological impacts and metabolic pattern of salmonellae. This chapter refers only to the most important food commodities. Niche products, ethnic foods and particular food combinations are not considered in detail. In general, consideration has been restricted to the major food commodities and principal production lines.

However, it is important to highlight that accidents or cross-contamination cannot be excluded and may cause an increased prevalence of salmonellae.

Many data were summarised by D'Aoust (2000), collecting reports published in various countries (see Annex, Tables 3-8, also D'Aoust, 1994).

Prevalence in such data collected from the literature tends to be higher than in data from 'baseline studies' where sampling and microbiological methods are standardised (Rose *et al.*, 2002), or collected within mandatory surveillance programmes (EC, 2002).

However, prevalence figures cannot always be taken 'at face value' and the epidemiological unit, sampling frame, analytical unit and procedure ought to be compared and shown to be equivalent, before prevalences are compared.

7.3.1. Meat from mammals

There is considerable variation in the reported prevalence of salmonellae in meat animals, in both the live animal and in carcases (chapter 7.3.2).

For example, D'Aoust (2000, Table 45-5) reported figures in pigs, ranging from 5.5 of pigs to 35.7% (3 reports, 16,173 samples tested). Denmark, the Netherlands and Norway reported varying prevalences of salmonellae in pig herds (4.1, 35.1 and 0.6% respectively) (EC, 2002). Rates of isolation of salmonellae from porcine lymph nodes were reported by Norway (0.07%), Finland (0.09%) and Sweden (0.19%) (EC, 2002).

In cattle, herd-based data was reported based on isolation rates of salmonellae from faecal samples, at rates of 2.7% in Denmark and 1% in the Netherlands. Salmonellae were isolated from lymph nodes at rates of 0.12% in Sweden, 0.08% in Norway and 0.03% in Finland.

7.3.1.1. Fresh red meat

Meat: defined as all parts of domestic bovine animals (including the species B*ubalus bubalis* and B*ison bison*) swine, sheep, goats and solipeds that are suitable for human consumption (Directive 64/433/EEC);

Farmed game meat: defined as all parts of wild land mammals and wild birds including the species referred to in Article 2 (1) of Directive 90/539/EEC and ratites (rattae)-bred, reared and slaughtered in captivity which are fit for human consumption.

The prevalence of salmonellae in fresh meat is clearly associated with the prevalence in the animals, as well as being dependent on the processing undergone.

In pork, data from carcase swabs on the prevalence of salmonellae were reported at 17.4% for Belgium, 0% for Norway and Finland, and 0.03% for Sweden (EC, 2002). Data based on pork sampled at the abattoir gave figures of 0.08% for Denmark, 0% for Finland and 0.02% for Sweden (EC 2002). Pork at the retail level was found to be contaminated in 1.12% of samples in Denmark, 0.27% in Norway, 0% in Finland and 3.7% in Germany (EC, 2002). D'Aoust (2000, Table 45-5) indicated prevalences of salmonellae on pork post-slaughter of between 0.8 and 17.5% (11 reports, 57,540 samples tested in Australia, Canada, Denmark, Germany, India (2 reports), Japan (2 reports), Portugal, Romania and the USA).

In beef, the data using carcass swabs gave figures of 0.10% for Finland and 0.06% for Sweden. Beef at the abattoir was contaminated with salmonellae in 0.48% of cases in Denmark and 0.08% of cases in Finland. Data of beef sampled at retail level gave figures of 1.2% for Denmark and 0.34% for Norway (EC, 2002).

D'Aoust (2000, Table 45-5) reported prevalence in post-slaughter beef between 0.6-20.3% (5 reports, 23,045 samples tested in Denmark, Germany, Nigeria, Portugal and the USA); and in sheep/lamb ranging from 1.6-10% (5 reports, 1,155 samples tested in Germany, India, Iran, Portugal and Spain). D'Aoust (2000, Table 45-7) reported prevalence of salmonellae in meats at retail level of raw beef of 1.3-21.5% (6 reports, 3,743 samples tested in Denmark, Japan (2 reports), Mexico, Thailand and The Netherlands). The prevalences on raw pork at retail level were 0.8-21.5% (6 reports, 19,065 samples tested in Denmark, Italy, Japan (3 reports) and Thailand), while there were 2 reports of exceptionally high prevalences of 76% and 91.8%, both from Mexico.

Edible offals: Heart, lungs, liver, kidney may also be used for human consumption. Arroyo and Arroyo (1995) gave the following data on the prevalence of salmonellae in various offals from lambs: liver 50%, heart 33.3%, oesophagus 27.3% and lungs 9%. In total 31.4% of 264 samples from chicken and lamb were contaminated.

However, edible offals are generally consumed only after heating, which lowers the risk of transmission of salmonellae and may also be lethal for heat-sensitive salmonellae.

7.3.1.2. Minced meat, meat preparations and meat products

Minced meat: defined as meat that has been minced into fragments or passed through a spiral-screw mincer (Directive 94/65/EEC);

Meat preparations: defined as meat within the meaning of Article 2 of Directives 64/433/EEC (fresh meat), 71/118/EEC (poultry meat) and 92/45/EEC (game) and satisfying the requirements of Articles 3, 6 and 8 of Directive 91/495/EEC (rabbits and farmed game) which has had seasonings or additives added to it or which has undergone a treatment insufficient to modify the internal cellular structure of the meat and thus to cause insufficient to modify the internal cellular structure of the meat and thus cause the characteristics of fresh meat to disappear (Directive 94/65/EEC);

Meat products: defined as products prepared from or with meat which have undergone treatment such that the cut surface shows that the product no longer has the characteristics of fresh meat (Directive 77/99/EEC).

Its related treatment are chemical or physical process such as heating, smoking, salting, marinating, curing or drying, intended to lengthen the preservation of meat or animal products whether or not associated with other foodstuffs, or a combination of these various processes (Directive 77/99/EEC);

Prepared meat meals: defined as wrapped meat products corresponding to culinary preparations, cooked or pre-cooked and preserved by cold (Directive 77/99/EEC)

Salmonellae were detected at various levels in the Member States in minced meat intended for human consumption. In Germany, results ranged from 2.5 to 4.3% (EC, 2002). Recent data from the US suggest raw or undercooked ground beef as a vehicle of transmission for multi-resistant *S.* Newport, presently an increasingly reported serotype in the USA of emerging importance (MMWR, 2002a).

The reported prevalence of salmonellae in raw minced (ground) meat was 3.2-57.1% (5 reports, 3,237 samples tested in Mexico (2 reports), The Netherlands (2 reports) and the USA) (D'Aoust, 2000, Table 45-7). In raw sausages prevalences of 2.4-46% were found (9 reports, 4,054 samples tested in Brazil, Iraq, Italy, The Lebanon, Mexico (2 reports), Nigeria, Scotland and the USA).

In recent years meat preparations have been marketed in some countries, consisting of raw ground (minced) beef and other meat (pork, chicken, turkey) with added compounds (e.g. salts, spices). Although such meat preparations may be cooked, they are commonly eaten raw. They do not belong to the usual category of meat products, because they are not preserved by means of reduced a_W/pH. The probability of salmonellae occurring in "meat preparations" is high, especially in products stemming from raw material with a high prevalence of salmonellae, such as poultry. If the product is eaten raw, the risk of contracting salmonellosis is consequently higher.

Fermented meat products: There is a very wide range of fermented meat products and many are cured using nitrites or nitrates, although the levels used may not prevent multiplication of salmonellae and some time is required for the product to reach its ultimate pH. Fermented sausages rely for microbiological control upon rapid development of lactic acid to reduce the pH below ca. 4.6-5.0 and a reduced a_w during a period of maturation and drying.

Some, for example "Frische Mettwurst", are marketed within 3 to 5 days of production as a fresh fermented sausage. That environment allows salmonellae to survive. However, data show that the prevalence of salmonellae in fresh fermented sausages is within the range of that found in dry fermented sausages: Schmidt (1985) recovered salmonellae from fresh fermented sausages in 4.3% of samples (655 samples), while in dry fermented sausages prevalences of 0.4-11% were found (3 reports, 655 samples tested in Italy and The Netherlands (2 reports)) (D'Aoust, 2000, Table 45-7). The prevalence of salmonellae in retail raw sausages was 2.4-46% (9 reports, 4,054 samples tested) (D'Aoust, 2000, Table 45-7).

7.3.2. *Poultry, poultry meat and poultry meat products*

Poultry meat: defined as all parts fit for human consumption from domestic birds of the following species: domestic fowl, turkeys, guinea-fowl, ducks and geese (Directive 71/118/EC);

Fresh poultry meat: defined as poultry meat, including meat which is vacuum-wrapped or wrapped in a controlled atmosphere, which has not undergone any preserving process other than chilling or freezing;

Meat preparations: defined as meat within the meaning of Article 2 of Directives 64/433/EEC (fresh meat), 71/118/EEC (poultry meat) and 92/45/EEC (game) and satisfying the requirements of Articles 3, 6 and 8 of Directive 91/495/EEC (rabbits and farmed game) which has had foodstuffs, seasonings or additives added too it or which has undergone a treatment insufficient to modify the internal cellular structure of the meat and thus to cause insufficient to modify the internal cellular structure of the meat and thus cause the characteristics of fresh meat to disappear (Directive 94/65/EEC).

In live birds, the reported prevalence in turkeys is 18.5% (1 report, 173 samples tested and in chicken (broilers) 5.0-27% (5 reports, 5,067 samples tested in Denmark, Japan (2 reports), Switzerland and The Netherlands) (D'Aoust, 2000, Table 45-5). From 1995 to 1998, turkey flocks (more than 10,000 birds) were tested for salmonellae. In the first quarter of 1996, more than 40% of the flocks tested positive with a declining tendency from the 3rd quarter of 1997 (Hansen *et al.*, 2000).

Data from various regions in the EU or other countries are difficult to compare due to the variety of schemes employed. Some Member States operate an approved control programme according to Directive 92/117/EC (Denmark, Finland, Ireland, Sweden, Norway, Austria, France), while others such as Germany, Spain and the UK use a sampling scheme according to Directive 92/117/EC or operate similar schemes (Greece, Italy, Portugal).

Consequently interpretation of the data must take account of these differences. Examples of data of salmonellae prevalence at flock level were 2.1% positive in Denmark, 0.1% in Sweden, 3.5% in Austria and 8.9% in Spain (EC, 2002). In Scandinavia in particular, it has been demonstrated that application of control programmes can markedly contribute to a reduction in the prevalence of salmonellae in poultry, through measures such as Good Husbandry Practices and biosecurity, especially at poultry and egg production.

Fresh poultry meat: The following data were provided on the prevalence of salmonellae in fresh meat from poultry at abattoir level: Austria 17.4%, Spain 14.9%, Belgium 12.2%, the Netherlands 8.8%; Greece 14.2%, Italy 3.6% and Portugal 8.5% (EC, 2002). However, as always, the results are influenced by differences in sampling and methods used, and the differences between results when sampling for salmonellae on poultry skin as opposed to poultry muscles.

D'Aoust reported prevalences in carcases of chickens of 10.5-55% (10 reports, 15,445 samples tested in Cuba, Denmark (2 reports), Italy, Japan (2 reports), Portugal, Thailand, and the USA (2 reports)). In the UK, 33% of chilled carcases sampled were found to be contaminated with salmonellae in 1994 (ACMSF, 1996), while in a survey of samples taken at retail level in 2001, 4.2% of fresh and 9.8% of frozen poultry were found to be contaminated (Corry et al., 2002; http://www.food.gov.uk).

A report regarding salmonellae in turkeys found the prevalence of salmonellae to be up to 41% in carcasses after chilling (Salvat et al., 1995) and even up to 40% in negative monitored herds (Hafez et al., 1997). In a survey performed at two turkey processing plants with varying facilities, different numbers of carcasses (n=168) tested positive for Salmonella (respectively 58% and 24% positive). The variation was attributed to different types of de-feathering systems used (Fries et al., 2003).

The high reported prevalence of salmonellae in poultry also applies to edible poultry offals, which are frequently contaminated with salmonellae (Fries, 2002). However, during preparation in the kitchen, most poultry offal will be heated. If heating is insufficient to kill salmonellae, surviving viable cells on the surface may be ingested and cross-contamination might also occur. A much greater risk of cross-contamination is posed by handling raw chicken, or the raw giblets, failing to wash the hands thoroughly, and contaminating working surfaces or other food items than from eating cooked poultry. Only in rare cases is poultry meat consumed raw, which would increase the risk considerably.

Regarding poultry meat at the retail level, data indicated prevalence of 17.4% in Austria, 8.4% in Spain, 2.4% in Greece and 7.7% in Italy (EC, 2002).

D'Aoust (2000, Table 45-8) reported the following prevalences of salmonellae in retail poultry: chicken (carcasses/cut-up) 6.9-81.5% (13 reports, 3,583 samples tested in Denmark, France, Germany, India, Italy, Japan, Malaysia, Mexico, Northern Ireland, Thailand, The Netherlands,

Turkey and the UK); minced chicken 42% (1 report, 162 samples tested in the USA); minced turkey 31.7% (1 report, 165 samples tested in the USA); chicken liver 11.1-90.2% (3 reports, 167 samples tested in Malaysia, Mexico and Thailand); chicken gizzards 44-88% (2 reports, 63 samples tested in Malaysia and Thailand).

Poultry meat products: These commodities originally stemmed mainly from turkey, but now increasingly originate from other types of meat (from broilers, ducks). Products such as sausages (frankfurter type) can be considered safe because of their reproducible technological record (core temperature approximately 71°C). For fermented poultry products many processing steps are similar to those applied to fermented red meat products (see chapter 6.3.3).

7.3.3. Eggs and eggs products

The presence of salmonellae in eggs and eggs products, and more particularly of *S.* Enteritidis, is strongly correlated to the prevalence in poultry breeding stocks (Guard-Peter, 2001).

Shell eggs: In some countries the prevalence of salmonellae in egg products is lower due to the implementation of *Salmonella* spp. control programmes, including the maintenance of parent and grandparent stock free of salmonellae.

The reported prevalence of salmonellae in egg-layer flocks and shell eggs is: chicken flocks 5.5-86.5% (8 reports, 3,776 samples tested in Canada (2 reports), Denmark, Germany, Greece and the USA (3 reports)); chicken eggs <0.1-13.2% (14 reports, 691,451 samples tested in Canada, Denmark, France, Germany (2 reports), Hawaii, India (2 reports), Spain (2 reports), Thailand (2 reports) and the USA (2 reports)); duck eggs 11-12.4% (2 reports, 1,128 samples tested in Thailand (2 reports)) (D'Aoust, 2000, Table 45.9). Data provided from the Member States show different patterns (EC, 2002): Sample-based data for 2000 give a range for eggs of 0.11 % (Italy) to 3.85 % (Spain), egg products (raw material) showed 0 to 1.36 % in Austria or 7.6% in Ireland. Egg products (final products) showed 0% (Ireland, Italy, the Netherlands) to 0.99 % in Germany.

The serotype involved in layers is primarily *S.* Enteritidis, which became endemic in laying flocks in many countries. In the UK the prevalence of *S.* Enteritidis in eggs has fallen each year for the last 3 years, and this is believed to be due to improved biosecurity and the implementation of vaccination in most laying flocks (ACMSF, 2001).

Similar measures applied in other Member States in line with the Zoonoses Directive 92/117/EC, include control and destruction of infected flocks and possible vaccination and other preventive measures. Following these measures a decrease of the contamination of laying flocks with *S.* Enteritidis is observed.

Egg products: The wide range of egg products and the different pasteurisation processes applied are described in detail in ICMSF (1998) for liquid egg (pp. 495-501) and dried eggs (pp. 507-509). In all egg products,

one objective is to eliminate any salmonellae by heating. Such pasteurisation processes have been used for many years, are well-understood, and can be controlled. In all egg products there is the opportunity for recontamination with salmonellae, commonly from the processing environment. However, it should be noted that some of these treatments are not sufficient to result in the total destruction of strains of salmonellae with induced thermal tolerance (Shah *et al*, 1991).

The involvement of eggs and eggs products in human outbreaks between 1993 and 1998 has been reported by WHO (2001a), accounting for 35% of cases.

7.3.4. *Fish and shellfish*

Fish and fishery products: defined as all seawater or freshwater animals or parts thereof, including their roes, excluding aquatic mammals, frogs and aquatic animals covered by other Community acts;

Aquaculture products: defined as all fishery products born and raised in controlled conditions until placed on the market as a foodstuff. However seawater or freshwater fish or crustaceans caught in their natural environment when juvenile and kept until they reach the desired commercial size for human consumption are also considered to be aquaculture products. Fish and crustaceans of commercial size caught in their natural environment and kept alive to be sold at a later date are not considered to be aquaculture products if they are merely kept alive without any attempt being made to increase their size or weight.

Fresh fish: In marine fish the prevalence of salmonellae is considered to be low. However, fish from aquaculture can sometimes be contaminated with salmonellae, depending on the geographical region and farming practices (Heinitz *et al.*, 2000; Murray, 2000).

Fish products: In principle, the technologies applied to fish products are well-established and can be controlled e.g. drying, fermentation, "pickling" and heating. Nevertheless, occasional outbreaks of salmonellosis have been reported. For example, in 1998 14 persons in Germany were infected by *S. Blockley* traced to contaminated smoked eels from a smokehouse where the hot-smoking procedure was inadequate (Fell *et al.*, 2000).

However, some consumption habits, including consumption of raw or low-salted products, such as sushi and carpaccio, might increase the risk of ingesting salmonellae, although no outbreaks have yet been reported.

Bivalve Molluscs: Although areas where molluscs are harvested are monitored for water quality, the water may sometimes become contaminated. Under EC Directive 91/492/EEC, harvesting areas are assigned to one of three categories depending on levels of *E. coli* and faecal coliforms per 100g of shellfish flesh sampled. Bivalve molluscs from the cleanest waters may go directly for human consumption. Some bivalve molluscs (e.g. oysters, clams) are eaten raw. However, according to the WHO (2001a), fish and shellfish were implicated in only 1% of outbreaks of salmonellosis.

The reported prevalence of salmonellae in fish and shellfish are: fish 0.4-15.8% (6 reports, 2,481 samples tested in India (3 reports), Mexico (2 reports) and Thailand), and shellfish 0.1-16% (11 reports, 7,399 samples tested in Australia, Cuba, India (3 reports), Italy, Malaysia, Northern Ireland, The Philippines and Thailand (2 reports)), and a higher prevalence in 5 reports 25-86% (69 samples tested in India (3 reports) and Malaysia (2 reports)) (D'Aoust, 2000, Table 45-11). According to the Zoonoses Report (EC, 2002), the contamination rate differs in the Member States: Italy and Spain up to 2.3%, whereas in Germany and Ireland the reported data were much lower (0.3 and 0.4% respectively).

In general, the prevalence of salmonellae in fish is considered to be low. However fish and shellfish can be contaminated if the water becomes contaminated. This may be a problem in aquaculture if the water is contaminated due to the run-off of effluent from agricultural land or contamination of shallow estuaries or aquaculture harvested areas with untreated sewage.

7.3.5. Milk and milk products

Raw milk: defined as milk produced by secretion of the mammary glands of one or more cows, ewes, goats, buffaloes, which has not been heated beyond 40°C or undergone any treatment that has an equivalent effect (Directive 92/46/EEC).

Milk for the manufacture of milk-based products: defined as either raw milk for processing or liquid or frozen milk obtained from raw milk, whether or not it has undergone an authorised physical treatment, such as heat treatment or thermisation, or is modified in its composition, provided that these modifications are restricted to the addition and/or removal of natural milk constituents

Heat-treated drinking milk: defined as either drinking milk intended for sale to the final consumer and to institutions, obtained by heat treatment and presented in the forms defined in Annex C, Chapter I.A.4 (a), (b), (c) and (d) or milk treated by pasteurisation for sale in bulk at the request of the individual consumer

Milk-based products: defined as milk products, namely products exclusively derived from milk, it being accepted that substances necessary for their manufacture may be added, provided that these substances are not used to replace in part or in whole any milk constituent, and composite milk products, namely products of which no part replaces or is intended to replace any milk constituent and of which milk or a milk product is an essential part either in terms of quantity or for characterisation of the product.

Heat treatment: any treatment involving heating that causes, immediately after it has been applied, a negative reaction on the phosphatase test

Thermisation: the heating of raw milk for at least 15 seconds at a temperature between 57°C and 68°C such that after treatment the milk shows a positive reaction to the phosphatase test.

Other definitions (ICMSF, 1998):

Cream: the fat-rich part of milk that is separated by skimming or by other techniques.

Cultured or fermented milks: milk products intended for consumption after fermentation by lactic acid bacteria.

Cheese: the product of coagulation of casein coagulation in the milk, followed by separation and removal of the whey from the curd. Apart from certain fresh cheese, curd is then textured, salted, formed, pressed and finally ripened. Cheese varieties include fresh, soft, semi-soft, hard and blended cheeses.

Ice cream and ice milk: formulated milk products intended for consumption in the frozen or partially frozen state.

Pasteurisation is the major safety factor in milk and milk products provided that process-control measures are rigidly applied, while other technologies, such as microfiltration, are under development. Several outbreaks of salmonellosis have been linked to milk improperly pasteurised on the farm or recontaminated after processing. Thermised milk involves heating to between 57°C and 68°C and is a less severe heat treatment than pasteurisation. With regard to consumption of unpasteurised milk, there is no record regarding the heating patterns applied by consumers at home having bought unpasteurised milk.

Historically, consumption of unpasteurised milk has led to large outbreaks of salmonellosis (e.g. in the UK), and resulted in the sale of unpasteurised milk being banned in Scotland in 1983 (Sharp *et al.*, 1985), but not in England and Wales and other Member States. Salmonellosis from raw milk is not common but large outbreaks have occurred (Potter *et al.*, 1984). Raw milk, even that from healthy animals, occasionally contains salmonellae. The reported prevalence of salmonellae in raw milk is 0.16-8.9% (11 reports, 9,172 samples tested in Canada (3 reports), England, England and Wales (1 joint report), France, India, Ireland and the USA (3 reports)) (D'Aoust, 2000, Table 45-12). In principle, products derived from unpasteurised milk may also be contaminated with salmonellae. Although outbreaks involving raw milk products have been reported they are relatively rare (see Table 4 of the Annex) considering the quantities consumed daily worldwide.

According to the Zoonoses Report (EC, 2002), investigations of raw milk, treated milk and milk products usually showed low contamination levels.

In principle, using unpasteurised milk in milk products increases the likelihood of salmonellae being present in the products.

During the various steps in cheese production, a variety of physical or chemical parameters of the products (pH, a_w) as well as the competitive microflora may influence survival of microorganisms. When considering raw milk cheeses, growth of salmonellae is not observed in some of them (i.e. hard cheeses) because the a_w is between 0.885 and 0.95 (Bauchman and Spahr, 1995). Some salmonellae are able to survive the fermentation of milk by

lactobacilli and the ripening/maturation process of some soft cheeses. In summary, salmonellae can survive in some cheeses, depending on the physical and chemical parameters of the product. Concerning raw milk cheeses, and especially soft cheeses and whey cheeses, hygienic control steps, following EU Directive 92/46, can be used to avoid contamination and multiplication of pathogenic bacteria during product processing and storage.

7.3.6. Fruits and vegetables

Fruit: The fleshy edible part of a perennial plant associated with the development of a flower.

Vegetable: The fresh edible portion of an herbaceous plant consumed either raw or cooked. The edible part may be a root, tuber, stem, bud, bulb, petiole or leafstalk, leaf and a mature or immature fruit.

Sprouts: Germinated (sprouted) seeds of alfalfa, mung beans and other seeds that are usually eaten raw.

Sprouted seeds: Several large outbreaks of salmonellosis have been traced to contaminated sprouted seeds (Beuchat, 1996; and Annex, Tables 5, 6, 7 and 8). In March 2001 the California Dept. of Health Services identified a cluster of *S.* Kottbus isolates with indistinguishable PFGE patterns in 23 patients. A matched case-control study revealed a significant association with eating alfalfa sprouts produced at a single facility, and from a single seed lot. A total of 32 patients infected with the outbreak strain of *S.* Kottbus were identified in California (24), Arizona (6), Colorado (1) and New Mexico (1) (MMWR, 2002b).

The technology and environment of sprouting (high humidity, warm temperatures, no preservation step) enable salmonellae, if present, to proliferate (Steward *et al.*, 2002). Therefore, due to an increasingly high consumption by consumers of sprouted seeds, and consequent increased production, sprouted seeds were considered as an important food category with regard to risk of salmonellosis (SCF, 2002).

Fruits: In the case of poor agricultural practices such as collection of "windfalls", and particularly the use of contaminated irrigation water, fruit, vegetables and crops can be contaminated with salmonellae (SCF, 2002). In the case of melons and cantaloupes, the processing steps of cutting and handling can contaminate freshly cut surfaces (Tamplin, 1997). Moreover, contact with soil cannot be prevented. (see Annex Tables 5, 6 and 7).

Unpasteurised fruit juices have been associated with several large outbreaks of salmonellosis (Beuchat, 1996) and must be regarded as a high risk commodity (For details see SCF opinion, 2002).

Vegetables: The reported prevalence of salmonellae in domestic fresh and processed vegetables is: bean sprouts 8.7-20.0% (2 reports, 354 samples tested in Malaysia and Thailand); cabbages 17.1% (1 report, 41 samples tested in Spain); carrots 1.1-7.1% (2 reports, 137 samples tested in Spain and the USA); celery 2.1-7.7% (2 reports, 74 samples tested in Spain and the USA); leafy vegetables 1.9-7.7% (7 reports, 745 samples tested in Greece,

Iraq, Malaysia, Spain (2 reports), The Netherlands and the USA and 1 report of 69.9% in 209 samples tested in Italy); onions 4.4% (1 report, 45 samples tested in Spain); parsley 4.3% (1 report, 23 samples tested in Spain); radishes 1.1% (1 report, 95 samples tested in the USA); sesame seeds 10-48.2% (4 reports, 132 samples tested in Canada, Egypt and Saudi Arabia); tofu 12.5% (1 report, 8 samples tested in Malaysia) (D'Aoust, 2000, Table 45-13).

Kaneko *et al*. (1999) examined raw vegetables from 27 retail shops in Tokyo and detected coliforms, although most were of non-faecal origin. Some recent outbreaks of salmonellosis from fruits and vegetables are listed in Table 5 of the Annex.

7.3.7. *Nuts, coconuts, chocolate*

Before processing, cocoa beans are cleaned by screening, air currents and magnets to remove extraneous materials. Sound, undamaged beans have few, if any, microorganisms inside the cotyledons. Roasting (treatments of 15 min to 2 h at 105-150°C) develops chocolate flavours and is the only processing step in chocolate production capable of destroying salmonellae. Subsequent processing steps of the roasted beans, nibs or liquor, such as milling and refining, mixing, conching, tempering or moulding, have little influence on the microflora of chocolate.

A particular characteristic of salmonellae found in chocolate products (ICMSF, 1998; Chapter 10, pp.379-389, "Cocoa, chocolate and confectionery) is their capacity for survival over very long periods of time, up to several years (Dockstader and Groomes, 1971; Rieschel and Schenkel, 1971; Tamminga, 1979). Furthermore, salmonellae show a very high heat resistance in chocolate, due to the low a_w and the protective effect of fat. *S.* Anatum was the most heat-resistant species isolated from chocolate (Barrile *et al*., 1970). Temperatures of 70-80°C reached during milling, refining or conching do not effectively destroy salmonellae, which are protected by the low water activity and the high fat content (Goepfert and Biggie, 1968; Mattick *et al*., 2000). Even overheating (>100°C) failed to inactivate low numbers of *S.* Senftenberg (Rieschel and Schenkel, 1971). If 2% of water was added, salmonellae were inactivated at 71°C (Barrile and Cone, 1970).

Considering the huge quantities of chocolate consumed daily, the low number of reported outbreaks and the industrial application of HACCP and GHP (IOCCC, 1991, 1993), chocolate must be considered a low risk commodity. However, there is no general pattern of contamination and prevalence of salmonellae in chocolate and if outbreaks do occur, they can be of a large magnitude (Torres-Vitela *et al*., 1995; Annex 1, Tables 6, 7 and 8).

7.3.8. *Oil / fat-based foods*

Salads with a mayonnaise base: the risk depends on the ingredients (whether eggs used to produce the mayonnaise are raw or pasteurised) and the microbiological state of the raw vegetables. A high proportion of the reported outbreaks have been linked to the consumption of home-made products.

In principle, oils do not constitute a suitable environment for bacterial metabolism and multiplication (Baumgart, 1997). However, salmonellae survive well in oils, and their heat resistance is higher in these products.

Reported prevalence: unfortunately, the 7th WHO report combines mayonnaise, salads and dressings in a single category and this food group was involved in 4% of outbreaks of salmonellosis (WHO, 2001a).

In a review, Radford and Board (1993) reported *S.* Enteritidis and *S.* Typhimurium as causative agents in outbreaks between 1955 and 1988, where home-made mayonnaise was involved. Mayonnaise has frequently been implicated in salmonellosis, often due to *S.* Enteritidis from raw eggs (Perales and Garcia, 1990; Doherty *et al.*, 1997; Hernandez *et al.*, 1998; D'Argenio *et al.*, 1999; Hayes *et al.*, 1999; Smittle, 2000).

Following the first documented outbreak of salmonellosis associated with the consumption of peanut butter, the survival of salmonellae in peanut butter was assessed experimentally (Burnett *et al.*, 2000). Survival at low temperatures was found to be greater than at higher temperatures, and survival was also associated with the size and stability of the water droplets in the colloid matrix.

An overview on major outbreaks of salmonellosis from mayonnaise-based salad is shown in Table 10 of Annex I.

7.3.9. Other foods

Spices: Industrial experience (Michels and Koning, 2000) is that the incidence rate of salmonellae in raw spices and herbs is about 1%. In Sweden, 10% of spices and vegetables (Oriental) were contaminated with salmonellae (EC, 2002). Black pepper and paprika have been the source of outbreaks of salmonellosis. Although such events are rare, when outbreaks occur the number of cases can be high (see Annex, Table 6 paprika chips; Table 7, black pepper).

Cereals: Due to the technical characteristics of the products and the absence of reported outbreaks, the risk of salmonellosis is considered to be low. Only very limited data regarding the contamination of cereals with salmonellae are available.

Bakery products: Of particular concern are bakery products where ingredients containing egg, such as egg custards, are added after the baking procedure, and which might introduce salmonellae if the eggs are not pasteurised. Unfortunately, the WHO report (WHO, 2001a) also includes ice-cream in this food category (cakes and ice-creams). Thus, despite the general low risk categorisation, the risk should be recognised (Harvey *et al.*, 1961).

Confectionery products: Sweden and Australia reported outbreaks-cases associated with consumption of helva (WHO, 2001a). Helva is a low-moisture confectionary made from honey, sesame seeds, nuts, rose-water and is often coloured with saffron. The survival of *S.* Enteritidis was

investigated and the organism was found to be capable of surviving up to 8 months' storage in the product (Kotzekidou, 1998).

A national outbreak of salmonellosis in the UK in 1989 was traced to savoury snacks. Autolysed yeast powder and flavourings were contaminated with *S*. Manchester, a rare serotype in the UK (Joseph *et al*., 1991).

7.3.10. Ready to eat foods

In industrial nations food preparation is increasingly focussing on 'ready-to-eat food' requiring minimum preparation before consumption. These products can arise from a single raw material, but in many cases result from an assembly of raw materials of various origins and characteristics. A range of technologies are used for the preparation of these products: some undergo, in their packaging, a thermal treatment (pasteurisation) destroying the pathogenic vegetative flora; while others are marketed without treatment and thus present a risk due to the possibilities of recontamination during their preparation (Mosupye and Von Holy, 1999; Levine *et al.*, 2001). The risk of salmonellosis is not limited to ready-to-eat products containing meats. A recent outbreak in the UK, caused by *S*. Newport PT33, was traced to salad vegetables (Sagoo *et al*., 2003).

7.3.11. Potable water

The EU Directive on potable water (98/83/EC) does not refer specifically to salmonellae as a parameter to be monitored. However, Member States could apply it if deemed necessary, and different criteria apply to bottled water. It follows that with respect to pathogens, the occurrence of salmonellae in potable water is not considered likely. Water from bore-holes and wells is associated with a higher risk since contamination from the environment is possible.

7.4. Summary of the reviewed food commodities

Domestic mammals, fresh red meat and meat preparations from domestic mammals: The likelihood of salmonellae being present in meat preparations is high, especially when originating from raw materials with a high prevalence of salmonellae. The risk of salmonellosis is high when meat is eaten raw, and lower when meat has been cooked. This applies also to edible offal from mammals (and poultry), where the prevalence of salmonellae is higher than on the carcass. However, edible offals are generally cooked, which reduces the risk.

With fermented meats (salami type), the risk is generally lower due to the lower pH and a_W. Although the product is preserved/ stabilised, salmonellae can survive. However, freshly fermented meat products have a relatively high a_W and salmonellae could survive more easily. Such fresh meat specialities are sold within 3-5 days of being produced and rely on being stored under refrigeration for their safety.

Poultry, poultry meat and poultry meat products: Regarding poultry meat products, the risk is related to the high prevalence of salmonellae in poultry species after slaughtering. However, outbreaks linked to poultry

products have also been recorded, due to undercooking and/ or cross-contamination during kitchen preparation. Poultry meat is generally cooked before consumption.

Fermented poultry products: because of a relatively high a_W, salmonellae may survive. The risks are as described for fermented red meat products.

Breaded poultry products: being flash fried at production, the product appears cooked, but may be almost raw. Salmonellosis has resulted after undercooking by the consumer.

Poultry meat preparations are not preserved by pH or a_W, and are reliant on refrigeration for their safety. They may be heated before consumption but are also consumed raw. Due to the high prevalence in poultry and product characteristics (pH and a_W) the risk may be consequently higher.

Eggs and egg products: Many outbreaks of salmonellosis have been attributed to eggs and egg products. For eggs, the prevalence has decreased steadily due to control measures applied (e.g., Good Agricultural Practices, biosecurity, hygiene measures, vaccination at primary production level on farm of hens producing table eggs). For products containing raw eggs, the risk is considered to be high, but depends on technologies applied (controlled process, avoidance of recontamination) and the history of the egg producer. Even following pasteurisation the risk could remain high if insufficient thermal treatment is applied or if there is the possibility of re-contamination. Outbreaks have been reported linked to the use of unpasteurised eggs in cakes, ice-cream and home-made mayonnaise (WHO, 2001a).

Milk and milk products: Pasteurised milk and pasteurised milk products at retail level are considered to pose a low risk because of the initially low prevalence of salmonellae in milk and the application of pasteurisation, which reliably eliminates salmonellae.

However, major outbreaks of salmonellosis have occurred from consumption of raw milk and products thereof. Milk "pasteurised" on farms has been implicated in several outbreaks, suggesting that the process is sometimes not fully under control.

Cheeses: The risk depends on the ripening procedure applied. Concerning soft/fresh cheeses made with raw or thermised milk, occasional outbreaks of salmonellosis occur. The risk might be considered low for hard cheeses, even when made with raw milk (due to the ripening time and technology applied).

Post process contamination of milk products has occasionally resulted in outbreaks of salmonellosis e.g. from dried infant formula contaminated at production during cooling, and from ice cream contaminated during distribution and sale.

Fish and shellfish: Salmonellae in fish are rare. In fish from aquaculture the risk may be higher than in marine captured fish, due to possible exposure to untreated sewage, but overall, and in comparison with other food

commodities, both remain low risk. The history of low prevalence of salmonellae in raw fish, and the technologies applied to fish products, result in fish products also being classified as of low risk.

Crustaceans: There is some concern, especially with imported cooked products derived from aquaculture that are eaten without further cooking/ heating.

Bivalve molluscs: The environment is controlled and monitored but may be contaminated. Oysters and clams are sometimes eaten raw, but. No outbreaks of salmonellosis have been reported.

Raw or under-salted fish (e.g. sushi): Although there is no process to eliminate salmonellae, salmonellosis has not been recorded.

Fruits and vegetables: Unpasteurised fruit juice and sprouted seeds were considered as foods of most concern (SCF, 2002). Major outbreaks occurred due to sprouted seeds, melons (cantaloupes) and juice. In the US, outbreaks have also been linked to "cider" (unfermented apple juice) (SCF, 2002). Contamination is possible from poor agricultural practices, contaminated irrigation water, and during cutting of melons. With respect to sprouted seeds, which are eaten raw, no salmonellae-reduction step exists before consumption and the environment allows salmonellae, if present, to multiply during the sprouting process.

Retail sale of pre-cut fruit presents a risk because salmonellae can multiply on the cut surfaces (unless refrigerated).

In cereals the risk is considered low. With some bakery products, a risk of possible recontamination following baking must be flagged, as must contamination from ingredients added, such as meats or eggs.

Melons, cantaloupes: Because of contact with soil, the importance of GHP at irrigation and harvesting should be stressed. Outbreaks of salmonellosis have been traced to contamination during cutting and slicing.

Spices: Black pepper, white pepper, paprika. Outbreaks of salmonellosis are rare, but may be widespread.

Oil/ fat-based foods: For mayonnaise the risk depends on the ingredients, especially whether the eggs are raw or pasteurised. When used in salads, the microbiological state of the raw vegetables and the manufacturing process applied to the mayonnaise must also be considered. For commercial mayonnaise products the risk is considered to be low, due to the measures taken during manufacture. If prepared at home or in a restaurant, and especially if the pH is not controlled, the risk of salmonellosis from mayonnaise made with raw eggs can be high.

Nuts, coconut, chocolate: Considering the huge quantities consumed daily, the systems applied during production, and the low number of reported outbreaks, chocolate must be considered as a low-risk commodity. However, when outbreaks occur they tend to be larger than those from other foods, due to very low infective dose of salmonellae in chocolate. Desiccated coconut

has been implicated in salmonellosis, but raw coconut meat can be immersed in water at 80°C to eliminate salmonellae.

Ready to eat foods: It is impossible to allocate these foods to a single risk category due to the wide range of ingredients, products and processes involved.

Potable water: The risk from potable water is considered low. However, water in bore-holes can be contaminated via agricultural practices or flooding, and the associated risk must be recognised.

7.5. Foods where *Salmonella* spp. might represent a hazard to public health

Using epidemiological information and the results of surveys (prevalence and incidence), as well as the technology applied to the particular food commodities, the Committee allocated the greatest risk of human salmonellosis to some major food categories. In this document a food was associated with a potential hazard to public health, if the following conditions applied:

✓ high prevalence of salmonellae in the food commodity;

✓ a food process / technology applied without the capacity to kill salmonellae, or no process applied, intensive handling and/or no final heating prior to consumption (i.e. would allow salmonellae in the food to reach the consumer);

✓ a history of reported cases or outbreaks of human salmonellosis, not as a result of occasional accidental contamination, but because of the nature of the production process;

✓ the serotypes involved in human cases are also found in the foods.

Groups at special risk and consumption data were not specifically addressed in this report, although such aspects need to be considered in any risk profile. Also food combinations/prepared meals were not specifically considered due to the great variety of meals.

Food categories possibly posing a greater hazard to public health include raw meat and some products intended to be eaten raw, raw or undercooked products of poultry meat, eggs and products containing raw eggs, unpasteurised milk and some products thereof. Sprouted seeds, unpasteurised fruit juices and home-made mayonnaise are also of major concern.

These foods generally originate from primary production, without undergoing a processing step able to destroy salmonellae, or where contamination due to inappropriate handling is possible. Consequently, the risk of salmonellosis depends on the prevalence of salmonellae in live animals and on plants, on controls at harvesting or slaughtering, and processes applied.

Such a classification can only be interpreted as a guide, based on available data, and the possibility of recontamination (e.g. cross-contamination, regional and temporal variations, human carriers) due to inappropriate handling must always be acknowledged. Moreover, different food formulations or preparations might have varying effects on the survival of salmonellae.

8. **EVALUATE THE APPROPRIATENESS OF SETTING MICROBIOLOGICAL CRITERIA**

8.1. General considerations

Whether the implementation of a microbiological criterion might contribute meaningfully to the reduction of public health risks, should be judged for each pathogen food commodity combination.

It might be difficult to show a uniform reduction in associated public health risks following the introduction of a criterion since the initial prevalence in a food lot or process will vary. For example, production and processing practices, heterogeneity of pathogen distributions, and regional or seasonal variations all might affect the prevalence of contamination and consequently the efficacy of a criterion. The risk reduction afforded by the implementation of microbiological criteria is correlated with the prevalence of contaminated food items, the number of samples taken and testing negative, and the efficiency of the analytical method used. If the diagnostic sensitivity of methods is less than 100%, the risk reduction afforded by microbiological criteria will be reduced accordingly. On the other hand if the diagnostic specificity is less than 100%, there is a risk of false positives and a need for confirmatory testing procedures (see Annexes II and III).

End-product testing using microbiological criteria may have limited usefulness for food safety for a number of reasons, including low prevalence of the pathogen, or low diagnostic sensitivity of the testing procedure applied. While the finding of a pathogen in a foodstuff may indicate a problem for public health, necessitating appropriate risk management action; the failure on the other hand to detect a pathogen in a food product does not necessarily mean that the pathogen is absent from that food product, process or food lot. The performance of some common sampling plans is illustrated in Annex I, Table 9.

Nevertheless, microbiological testing can be used in monitoring programmes along the food chain, for documentation purposes, HACCP, as an indicator of adherence to Good Hygienic Practices (GHP), on-the spot checks, monitoring the suitability of raw materials or food ingredients, and the hygienic status of the processing environment, all of which play an important role in maintaining food safety. Even if the application of a microbiological criterion does not result in a marked change in average prevalence of the pathogen, its implementation might facilitate official surveillance and inspection, and imposition of corrective action in the case of any unfavourable findings. Moreover, the use of a criterion can yield very useful results when collated and analysed on a national or regional scale, i.e., baseline prevalence studies that can be helpful in assessing risks associated

with a particular pathogen (see Annex I, Table 11). The use of equivalent methodologies is crucial for yielding comparable results.

If there are situations where a high prevalence is suspected e.g., indicated by trace back investigations, microbiological criteria may be useful in reducing the risk (Annex II). However, even the taking of a large number of samples cannot guarantee the absence of a pathogen, merely that the presence (prevalence or concentration) is less than a certain limit with for example 95% confidence (Annex III).

8.2.   Food safety concepts

A short outline of current food safety concepts is presented before discussing the usefulness of microbiological criteria. In considering food safety issues, the answers to the following questions are helpful:

-        what is the microbiological concern associated with the food (hazard)?

-        what is the frequency of its occurrence and consequences?

-        are there appropriate control options available?

-        how can such control options be implemented and what is the expected efficacy?

-        how may control measure(s) be put into operation?

The setting of microbiological criteria is one risk management option available for managers to control a hazard. Microbiological criteria and taking of appropriate corrective action of food found to be contaminated can contribute to improve food safety. However, it appears that in many cases microbiological criteria are not sufficient as a solitary control option, and there is a need for an integrated control strategy i.e., process controls of hazard analysis critical control points (HACCP) is one example. The process of managing a food safety problem is described in the Codex Committee for Food Hygiene (CCFH) document on risk management (CX/FH, 2001). Microbiological criteria should thus be implemented in the context of a risk analysis to clarify the benefits to public health and the cost effectiveness of the criteria.

Hazard analysis critical control points (HACCP) - The recognition that food safety cannot be assured by end-product testing alone led to the development of the concepts of HACCP, to supplement Good Agricultural Practice (GAP), Good Hygiene Practice (GHP), and Good Manufacturing Practice (GMP). The HACCP system was conceived by the Pillsbury Company, together with the National Aeronautics and Space Administration (NASA) and the U.S. Army Laboratories at Natick, who developed this system to ensure the safety of astronauts' food. In the thirty years since then, the HACCP system has become the generally accepted method for food safety assurance. The recent growing worldwide concern about food safety by public health authorities, consumers and other concerned parties and the continuous reports of foodborne outbreaks have given a further impetus to

the application of the HACCP system. The HACCP system achieves process control by identifying hazards and critical control points in the process and establishing critical limits at these control points for the identified hazards (i.e. microbiological criteria), establishing systems for monitoring the critical control points and indicating suitable corrective actions if the critical limits are exceeded, and establishing suitable verification and documentation procedures (WHO, 1998).

Risk profile – Elaboration of a risk profile is the initial step in the risk management of a food safety problem. The risk profile should provide as much information as possible to the risk managers to guide further actions and it should be carried out in collaboration between risk assessors and risk managers. The outcome of the risk profile should guide the risk managers either to develop a control strategy or to commission a formal risk assessment.

Risk assessment - The purpose of the risk assessment is to enable the risk managers to make informed decisions on management options to be taken. The risk profile will assist the managers in defining specific questions that should be addressed. The outcome of a risk assessment is a risk estimate i.e. the likelihood and severity of adverse effects that occur in given population with associated uncertainties (CAC/GL-30, 1999).

Appropriate Level of Protection (ALOP) is a quantification of the disease burden within a country linked to the implementation of food safety systems. ALOP is derived from a risk assessment and is expressed as e.g. the likelihood to suffer a food related illness from a food serving, or the number of cases per 100,000 consumer years. The setting of an ALOP is a risk management decision.

Food Safety Objectives (FSO) may be an important element in guidance on options to be taken for the future safety of foods. The concept is still evolving and no definition has yet been agreed upon. The proposed definition in the Codex document (CX/FH, 2001) is as follows: "the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of health protection (ALOP)". The FSO does not guide an operator on the control options, such as microbiological criteria, to be taken.

8.3. Microbiological testing and criteria

8.3.1. *Microbiological testing*

Many different types of microbiological testing can be used to assure the safety of foods. Comparisons are easier if sampling procedures and microbiological methods are equivalent.

Microbiological testing in monitoring and surveillance can be used for:

- identifying trends in human illness caused by foodborne pathogens e.g. sentinel studies,

- establishing baseline prevalences in primary production and in later stages of the food chain, i.e. testing foods in distribution or at retail,

- estimating the load of bacterial pathogens in foods reaching the consumer (e.g. when assessing exposures of a pathogen),

- measuring compliance with good hygienic practices, and

- measuring the effect of intervention measures such as control programs.

When sampling procedures and microbiological methods are standardised, monitoring allows inferences to be made about the safety of food derived from more than one batch (lot), as occurs with animals from different farms at a slaughterhouse, or with large consignments of food at port-of-entry.

With similarly standardised sampling procedures and microbiological methods, monitoring can establish the baseline prevalences of bacterial pathogens in foods, e.g. as in the US baseline studies in meat and poultry, and assist risk analyses.

Investigational sampling – is both intensive and focussed. It is mainly used by the food industry to investigate foods when a process is suspected of failure, or when foods have been stored accidentally under inappropriate conditions. The results of investigational sampling are therefore not comparable with the results from baseline studies.

8.3.2. Microbiological criteria

The Codex document on Principles for the establishment of Microbiological Criteria (CAC, 1997a) used the following definition for **microbiological criteria** – *"a microbiological criterion for foodstuffs defines the acceptability of a product or food lot based on the absence or presence, or number of microorganisms including parasites and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot"*.

The Scientific Committee on Veterinary Measure relating to Public Health also gave a definition for a microbiological criterion in its opinion of 1999 (SCVPH, 1999). The definition differs from the Codex definition in that the word "*process*," is included before the word *"product"*, extending the use of microbiological criteria to the whole food chain.

The SCVPH already stressed in a previous Opinion that the mere existence of microbiological criteria does not protect consumer health. The use of Good Hygienic Practice (GHP) and Hazard Analysis Critical Control Point (HACCP) systems will be more important in ensuring that pathogens are eliminated, or minimised to the extent that they cannot cause illness (SCVPH Opinion, 1999).

The intention of microbiological criteria is to ensure the health of the consumer by providing safe, wholesome food products, and to meet the requirements of fair practices in trade. Thus, the introduction and implementation of a criterion should not be an *ad-hoc* measure, but rather the outcome of a deliberate process. Hence, a *"microbiological criterion*

should be established and applied only where there is a definitive need for it and where it can be shown to be effective and practical" (EC, 1997).

A microbiological criterion should include (CAC, 1997b) a statement of the micro-organisms of concern (e.g., *Salmonella* or VTEC O157); the analytical methods for their detection and/or quantification; a plan defining the number of field samples to be taken and the size of the analytical unit; the microbial limits acceptable at that particular point in the food chain; and the number of analytical units samples that should conform to these limits. Moreover, the criterion should state the foodstuff to which criterion applies, the point(s) in the food chain where the criterion applies, and any actions foreseen if the criterion is not met.

When applying criteria for assessing products, it is essential that only appropriate tests are applied to those foods and at those points in the food chain that offer maximum consumer benefits in terms of food safety (CAC, 1997b).

It appears that there is a consensus that microbiological criteria should not be applied arbitrarily, but rather as the outcome of a deliberate process to achieve optimal food safety.

Microbiological criteria can be applied differently, as:

- Microbiological standards,

- Microbiological guidelines, or

- Specifications.

Microbiological standards – are mandatory criteria based on legal requirements, where failure to comply results appropriate actions e.g. reprocessing, rejection or destruction of the food.

Microbiological guidelines – may be established during production and processing, or on the end-products, and should be based on best practices. Manufacturers and food inspectors use guidelines for the verification of safe and hygienic production, and corrective actions in the process are taken when the guidelines are exceeded. Such guidelines should be established to detect deviations from the food process representing a danger for human health or hygiene failures.

Specifications - microbiological criteria used for contractual purposes by food businesses must not be confused with legal requirements of official control purposes (EC, 1997). Specifications are not discussed in this document.

8.3.3. Considerations of sampling and laboratory techniques

Having decided upon the need for a microbiological criterion for a particular food, aspects of the sampling and microbiological techniques are considered.

If the prevalence of a pathogen in the food lot or the diagnostic sensitivity of the procedure applied is low, and/or its distribution in the food is heterogeneous, the probability of detecting the pathogen will be low (WHO, 1988; ICMSF, 2002).

A lot - A lot (batch) is a quantity of food or food units produced and handled under uniform conditions. This implies that the pathogens are homogeneously distributed within a lot, as occurs with liquid foods. However, regarding levels of microbial contamination and distribution, this rarely occurs with most solid foods. This heterogeneity is magnified when a lot is not well defined, as occurs with animals at a slaughterhouse or a large consignment of food. If a consignment is, in fact, made up of several different lots, the stringency of a given sampling plan and its ability to discriminate between acceptable and non-acceptable production may be reduced. Consequently, a poorly defined lot will reduce the efficacy of microbiological criteria.

Stage of processing - other considerations might include stage of processing and where in the food chain the samples are taken. The risk reduction from the application of the criteria will be correlated with the prevalence of pathogens in the foodstuff at the particular sampling point. However, application of microbiological criteria might be of limited relevance to public health if the foodstuff undergoes, for example, heat treatment *after* sampling but *before* consumption.

Pooling of samples – enables reduction of laboratory effort while maintaining the stringency of sampling plans where a single positive results in rejection of the consignment. Considerable cost reductions of analyses can be achieved by pooling analytical units. Alternatively, pooling allows examination of large numbers of analytical units, increasing the stringency of examination, without increasing laboratory effort. This approach is suitable for dried foods and foods of high moisture content including eggs, poultry meat, meat and meat products (Silliker and Gabis, 1973; Gabis and Silliker, 1974). However, Christensen and Gardener (2000) noted that the advantages of pooling were greatest when the prevalence was low (<5%), and that this advantage decreases as the prevalence increases. If the samples from a food lot are pooled into one, it is not possible to assess the 'within lot' prevalence of the pathogen, only the qualitative question of absence or presence of the pathogen. Moreover, the sensitivity of pooled sampling may also be influenced by the detection limits of the analytical procedure and possible dilution effects due the pooling procedure. The effect of pooling of samples will depend on factors such as true prevalence of the pathogen, pool size, amount of specimen to be tested, number of pooled tests and the comparative performance of pooled and individual tests. Therefore, the appropriateness of pooling procedures should be judged on a case-by-case basis for each pathogen commodity combination having regard to all these factors.

Test characteristics - if the tests used have a perfect diagnostic sensitivity and specificity (100%) the measured apparent prevalence will equal the true prevalence. See Annexes II and III for more detailed discussions of

predictive values and test characteristics and the calculated risk reductions by application of microbiological criteria.

If the prevalence of a pathogen in a batch is low, or the diagnostic sensitivity of the procedure applied is low, and/or its distribution in the food is heterogeneous, the consequent probability of detecting the pathogen is also low (WHO, 1998; ICMSF, 2002).

8.4. Appropriateness of setting criteria for salmonellae

Salmonella Enteritidis and *Salmonella* Typhimurium have been reported most frequently as major causative agents of human salmonellosis. However, other serotypes have caused illness and still others will emerge in the future. Consequently, the finding of any member of *Salmonella* enterica, characterised by serological classification, in a foodstuff indicates public health concern.

The presence of salmonellae in the food chain is derived mainly from primary production (plants and animals). Accordingly most foods representing a risk to public health are those eaten raw or undercooked. However, the possibility of recontamination, accidental cross-contamination or poor handling remains in every food. There have been occasional large outbreaks of salmonellosis, e.g. from chocolate or paprika. Normally no salmonellae would be found in these foodstuffs. Consequently, the probability that these outbreaks would have been prevented by microbiological criteria (standards or guidelines) is minuscule.

In considering the possible contribution of microbiological criteria in reducing public health risks posed by a particular food commodity, account should be taken of the history of involvement of that food commodity in human salmonellosis, the prevalence of salmonellae in the original material and the record of safety of the processing technology applied. It should be considered also, whether the application of microbiological criteria and the taking of appropriate actions can contribute to a meaningful reduction in risk to consumers.

The performance of sampling plans and the usefulness of microbiological criteria depends on several factors including the prevalence, concentration and distribution of the target organism, the size and number of samples taken, and the diagnostic sensitivity of the method applied (ICMSF, 2002).

Microbiological criteria can be used differently, as guidelines and as standards:

Microbiological guidelines: if there is a high prevalence of salmonellae in the original source, but a reliable and safe technology during processing, the agent will not be able to reach the consumer, and it might be sufficient to take measures *that are not mandatory*, i.e. which might be able to reduce the prevalence of salmonellae at the source. Microbiological guidelines have been effective in reducing the prevalence of salmonellae along the food chain, and the application of this model can improve control of salmonellae in primary production.

Concluding, it is deemed useful to implement guidelines where, salmonellae are a recognised problem, be it the animals, plants or products thereof. Guidelines should be used in order to decrease the general burden of a particular food chain, e.g. in primary production including biosecurity measures and other management options such as GAP, GHP, GMP, HACCP etc..

Microbiological standards: In general, standards should be considered only when there is a history of human salmonellosis from consumption of a particular food commodity. Efforts should be focused on those foods where there is a relatively high probability that salmonellae are able to reach the consumers, taking account of the technologies and processing steps. Application of such criteria has been shown to reduce effectively the prevalence of salmonellae in primary production and in other stages of the food chain. For some serotypes, for poultry breeders as well as for some regions in Nordic countries, mandatory criteria have been established.

Concluding, if there is evidence from testing that, despite progress achieved with guidelines, the agent is still present in the food at the point of consumption, mandatory measures could be considered. However, the Committee stresses that the application of standards will only be a partial solution to such a food safety problem and that in these cases an integrated approach to control microbiological contamination is required (e.g. control measures at primary production, prevention of cross contamination).

The Committee identified foods that pose a hazard to public health representing a high risk of human salmonellosis. The implementation of microbiological criteria, be they standards or guidelines, should be reflected against the background of the particular food commodity and considering the points mentioned above.

9.   WHERE MIGHT A RISK PROFILE BE APPROPRIATE ?(TERMS OF REFERENCE 3)

The food categories identified in chapter 7.5, should be considered for further Risk Profiling, taking account of risk profiles or risk assessments already published (e.g. with regards to eggs, broilers and chickens see FAO/WHO (2002); FSIS (1998a); Ranta and Maijala (2002)) or in progress worldwide. The discussions outlined in this document already constitute elements of a Risk Profile.

Any further risk profiles should consider risks to normal and especially susceptible populations e.g. young, old, pregnant, immunosuppressed, variation in prevalences in different geographical regions, and the consequences of cultural differences in food handling, preparations and consumption.

10. CONCLUSIONS

Terms of Reference 1: Risk associated with particular commodities

In the context of this document, any serotype of the genus *Salmonella* is regarded as capable of causing gastrointestinal illness of varying severity in humans.

- Salmonellae can survive and multiply under certain environmental conditions found in foods and food processing environments. Food formulations and processes applied have a marked influence on the survival of salmonellae during processing.

- Contamination of foods with salmonellae mainly originates from primary production.

- Salmonellae can be introduced along the entire food chain.

- Salmonellae transmission routes vary widely, which makes tracing back and identification of transmission routes for cases of human salmonellosis difficult.

- Taking into account: the prevalences of salmonellae, the reported outbreaks of human salmonellosis, the physiological characteristics of salmonellae in the light of the formulation of the foods and the processes applied, some products represent a higher risk to public health than others.

- Food categories possibly posing a high risk to public health include raw meat and some products intended to be eaten raw, raw or undercooked products of poultry meat, eggs and products containing raw eggs, unpasteurised milk and some products thereof. Sprouted seeds and unpasteurised fruit juices are also of concern.

- It should also be stressed, that in all food chains cross-contamination and poor hygienic practices will represent a risk for human salmonellosis. In particular, if contamination takes place after processing and the conditions thereafter enable survival and/ or growth of salmonellae, the consumer is at risk.

Terms of Reference 2: Appropriateness of setting criteria

- The application of a microbiological criterion does not guarantee the absence of salmonellae in the food. The risk of humans contracting salmonellosis will never be zero.

- Microbiological criteria may either be standards or guidelines. Application of such criteria has been shown to reduce effectively the prevalence of salmonellae in primary production and in other stages of the food chain. Both are useful tools in the appropriate circumstances: where salmonellae – in the light of epidemiological data and in the absence of a process that inactivate salmonellae – may be able to reach the consumer prior to consumption of the food, mandatory measures might be useful. Where there is – in the light of the intended consumption practices – a safe food technology or preparation practice applied before consumption that controls/inactivates salmonellae, guidelines might be useful.

Terms of Reference 3: Risk profile

- A risk profile should be considered for food categories posing a high risk including raw meat and products thereof intended to be eaten raw, raw or undercooked products of poultry meat, eggs and products containing raw eggs, unpasteurised milk and some products thereof, sprouted seeds and unpasteurised fruit juices. The data provided in this document together with risk profiles

already performed can guide the risk management decision regarding the application of guidelines or standards.

11. RECOMMENDATIONS

The decision whether or not a microbiological criterion (standard or guideline) is needed should be taken on the basis of two considerations:

1) if this would indeed protect the consumer, and

2) if it is feasible to comply with the proposed criterion by applying good industrial or agricultural practices.

When establishing microbiological criteria, the issue of sampling strategies and analytical methods need to be considered. The application of criteria does not imply that all lots need to be sampled.

In foods with food processing technologies capable of eliminating salmonella and with control measures applied prior to consumption, the use of guidelines as criteria should be considered, aiming primarily at the reduction of the prevalence in the food. In other foods, where salmonellae might be able to reach the consumer, the use of standards should be considered, at appropriate stages in the food chain. With the failure to meet the microbiological criteria, appropriate actions are required.

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13. ANNEXES

13.1. Annex I

Table 3: Reported number of outbreaks of salmonellosis by food type (Table 45-16, D'Aoust, 2000)

Country	Period	Beef	Pork	Poultry	Unspecified meats	Milk & dairy	Eggs & egg products	Vegetables & salad	Fish & shellfish	Bakery	Others	Unknown	Total
Canada	1989	1.0*	3.0	15.0	4.0	3.0	1.0	0.0	1.0	1.0	12.0	10.0	510
England & Wales	1989-1991	2.3	1.7	14.7	10.3	1.3	16.7	0.3	NL	2.3	22.7	58.0	130.3
France	1993	NL	NL	NL	15.0	1.0	136.0	NL	4.0	NL	15.0	29.0	200.0
Germany	1991	NL	NL	6.0	8.0	4.0	28.0	2.0	2.0	NL	12.0	NL	72.0
Italy	1991-1994	NL	NL	4.3	13.8	2.8	182.0	NL	11.5	9.3	13.1	108.0	344.8
Japan	1991	NL	NL	NL	13.0	0.0	7.0	5.0	4.0	NL	51.0	58.0	138.0
Scotland	1989	0.0	1.0	20.0	4.0	0.0	10.0	NL	NL	NL	8.0	111.0	154.0
Spain	1982-1990	NL	NL	0.0	1.0	0.0	10.2	1.2	0.6	1.3	1.4	14.6	30.4
Catalonia	1994	NL	NL	9.0	10.0	11.0	217.0	NL	5.0	24.0	25.0	77.0	378.0
USA	1992	0.0	0.0	0.0	0.0	1.0	5.0	6.0	0.0	3.0	20.0	45.0	80.0

NL = not listed

*= reported annual number, or mean annual number

references- see original article

Table 4: Outbreaks of salmonellosis from cheese made with unpasteurised milk (Part of Table 45-17, p. 1261, D'Aoust, 2000)

Country	Year	Cheese	Cheese-milk processing	Disease agent	Cases	Deaths
Canada	1982	Accawi	Raw	S. Muenster	3	0
Canada	1984	Cheddar	Thermised	S. Typhimurium	2,700	0
Switzerland	1985	Vacherin (soft)	Raw	S. Typhimurium	>40	0
England	1989	Irish (soft)	Raw	S. Dublin	42	0
France	1990	Goat cheese	Raw	S. Paratyphi B	277	0
England	1996	Cheddar	Thermised	S. Gold-coast	84	0

references: see original article

Table 5: Recent outbreaks of salmonellosis from fresh fruits and vegetables (Table 45-18, D'Aoust, 2000)

Food	Country of origin	Country outbreaks	Year	Serotype	Cases	Deaths
Alfalfa sprouts	Australia	Finland and Sweden	1994	S. Bovismorbificans	492	0
	Netherlands	USA and Finland	1995	S. Stanley	242	0
	Netherlands	Canada and USA	1995-96	S. Newport	150	0
	Unknown	Denmark	1995	S. Newport	>150	0
Cantaloupes	Mexico	USA	1989-90	S. Chester	>245	2
	USA	Canada and USA		S. Poona	>400	NS*
Mung bean sprouts	Australia	UK	1988	S. Saint-paul	143	0
Mustard cress	Netherlands	England and Wales	1989	S. Gold-coast	14	0
Orange juice	USA	USA	1995	S. Hartford, S. Gaminara, S. Rubislaw	63	0
Tomatoes	USA	USA	1990	S. Javiana	>174	NS
Watermelon	USA	USA	1991	S. Javiana	39	0

references: see original article

*NS = not specified

Table 6: Major foodborne outbreaks of human salmonellosis (Table 45-19, D'Aoust, 2000)

Country	Year	Vehicle	Serotype	Cases*	Death
Canada	1984	Cheddar cheese	*S.* Typhimurium PT10	2700	0
USA	1985	Pasteurised milk	*S.* Typhimurium	16284	7
China	1987	Beverage (egg)	*S.* Typhimurium	1113	NS**
Norway and Finland	1987	Chocolate	*S.* Typhimurium	361	0
Japan	1988	Cooked eggs	*Salmonella spp.*	10476	NS
USA	1991	Prosciottino ham	*Salmonella spp.*	>350	NS
USA and Canada	1991	Cantaloupes	*S.* Poona	>400	NS
France	1993	Mayonnaise	*S.* Enteritidis	751	0
Germany	1993	Paprika chips	*S.* Saint-paul, *S. Javiana*, *S. Rubislaw*	1000	0
France	1993	Goat cheese (raw)	*S.* Paratyphi B	273	1
USA	1994	Ice cream	*S.* Enteritidis	740	0
Finland and Sweden	1994	Alfalfa sprouts	*S.* Bovismorbificans	492	0

* = **confirmed cases**

** = **not specified**

references: see original article

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Table 7: Major foodborne outbreaks of human salmonellosis (adapted from Table 45-20, D'Aoust, 2000)

Vehicle	Year	Exporting country	Importing country	Serotype	Cases	Deaths
Chocolate	1973	Canada	USA	S. Eastbourne	122	0
	1982	Italy	UK	S. Napoli	245	0
	1985-86	Belgium	Canada	S. Nima	29	0
	1987	Norway	Finland	S. Typhimurium	12	0
Alfalfa sprouts	1994	Australia	Finland and Sweden	S. Bovismorbificans	492	0
	1995	Netherlands	USA and Finland	S. Stanley	>230	0
	1995-96	Netherlands	Canada and USA	S. Newport	150	0
Black pepper	1982	Brazil	Norway	S. Oranienburg	126	1
Pate	1984	France	UK	S. Gold-coast	506	0
Aspic glaze	1984	UK	International	S. Enteritidis PT4	766	2
Mung bean sprouts	1988	Australia	England	S. Saint-paul	143	0
Mustard cress	1989	Netherlands	England	S. Gold-coast	14	0
Cantaloupe	1989-90	Mexico	USA	S. Chester	>245	2
Infant milk formula	1996	France	UK	S. Anatum	>12	0

References: see original article

Table 8: Recognised international outbreaks of salmonellosis (Source: Enternet)

Organism	Year	Countries involved	Vehicle implicated	Number of cases
S. Stanley	1995	Finland and U.S.A.	Alfalfa sprouts	Over 200
S. Dublin	1995	France and Switzerland	Cheese	Over 30
S. Anatum	1996	Ireland, England, Wales, Scotland and France	Baby milk	19
S. Typhimurium DT204b	2000	England, Wales, Scotland; Germany, Iceland and the Netherlands	Lettuce	392
S. Livingstone	2001	Norway and Sweden	Fish pie	Over 40
S. Typhimurium DT104	2001	Australia, Canada, England, Wales and Scotland	Peanuts	Over 100
S. Oranienburg	2001	Austria, Belgium, Denmark, Finland, Germany, Netherlands and Sweden	Chocolate	Over 500

Table 9 Cases and Sampling Plan Performance Assuming a Standard Deviation of 0.8. Lots Having the Calculated Mean Concentrations or Greater Will Be Rejected with at Least 95% Probability.

Type of hazard	Cases, sampling plans and calculation of their performance		
	Conditions reduce hazard	Conditions cause no change in hazard	Conditions may increase hazard
Indirect	case 4 (3-class, n=5, c=3) e.g. m=1000/g, M=10000/g **Mean conc.=5128/g**	Case 5 (3-class, n=5, c=2) e.g. m=1000/g, M=10000/g **Mean conc.=3311/g**	case 6 (3-class, n=5, c=1) e.g. m=1000/g, M=10000/g **Mean conc.=1819/g**
III. Moderate	case 7 (3-class, n=5, c=2) e.g. m=1000/g, M=10000/g **Mean conc.=3311/g**	Case 8 (3-class, n=5, c=1) e.g. m=1000/g, M=10000/g **Mean conc.=1819/g**	case 9 (3-class, n=10, c=1) e.g. m=1000/g, M=10000/g **Mean conc.=575/g**
II. Serious	case 10 (2-class, n=5, c=0) e.g. m=0/25g **Mean conc.=3.2/100g** **(1 cfu/32g)**	Case 11 (2-class, n=10, c=0) e.g. m=0/25g **Mean conc.=1.2/100g** **(1 cfu/83g)**	case 12 (2-class, n=20, c=0) e.g. m=0/25g **Mean conc.=5.4/1000g** **(1 cfu/186g)**
I. Severe	case 13 (2-class, n=15, c=0) e.g. m=0/25g **Mean conc.=7.4/1000g** **(1 cfu/135g)**	Case 14 (2-class, n=30, c=0) e.g. m=0/25g **Mean conc.=3.6/1000g** **(1 cfu/278g)**	case 15 (2-class, n=60, c=0) e.g. m=0/25g **Mean conc.=1.9/1000g** **(1 cfu/526g)**

Table 8.5 from ICMSF (2002).

Spreadsheet available to download at (www.icmsf/samplingplans.htm),, in which the performance of a particular sampling plan can be determined. For example, if a sampling plan with n=20 samples is used and c= 0 and m=100, then there is a 95% (or higher) probability of rejecting lots if the mean concentration of *L. monocytogenes* in the lot is ≥ 15 cfu/g. It therefore follows that even with 20 samples, the probability of accepting a lot actually containing *L. monocytogenes* increases rapidly if the mean concentration is below 15 cfu/g.

Table 10*: on major outbreaks of salmonellosis from mayonnaise-based salads

Country	Year	Salad type	Produced	Organisms	Source of contamination	Cases	Fatal	References
DE	1974	Potato salad	Industry	*S.* Typhi	Potato and well water; defective chill chain	417	5	Hupper, 1973
NL	1981	Meat/potato	Restaurant	*S.* Indiana	Cross-contaminated salad from contaminated kitchen	600-700	??	Beckers *et al.*, 1985
USA	1987	Tuna macaroni	Hospital	*S.* Enteritidis	Mayonnaise made with raw egg; salad stored at ambient temperature	404	9	Telzak *et al.*, 1990

*: modified from Michels and Koning, (2000). Table 30-3, p. 826.

Table 11. Prevalence of salmonellae in the Pathogen Reduction HACCP programme, USA 1998-200, Code A (random samples)

Product	1998		1999		2000		1998/2000		1998/2000
	N	%	N	%	N	%	N	%	%
Broilers	5,659	10.8	6,768	11.4	10,057	9.1	22,484	10.2	
Market hogs	1,390	5.8	1,923	9.8	5,170	6.2	8,483	7.0	
Cows/bulls	179	1.1	1,521	2.2	1,995	2.2	3,695	2.1	
Steers/heifers	214	0	782	0.3	1,092	0.4	2,088	0.3	
Ground beef	1,296	6.4	16,375	4.3	32,844	3.3	50,515	3.7	
Ground chicken	24	4.2	297	16.2	414	13.8	734	14.4	
Ground turkey	591	36.5	1050	31.6	1,551	25.7	3,192	29.7	

13.2. Annex II test characteristics

If the tests used have a perfect diagnostic sensitivity and specificity (100%) the measured apparent prevalence will equal the true prevalence: that is A+B will equal A+C and (B= C =0%).

The definition of sensitivity and specificity is as follows:

Sensitivity = # test positive (a) / # diseased or contaminated (a+c)

Specificity = # test negative (d) / # healthy or not contaminated (b+d)

	Contaminated	Not contaminated	
Test +	A	B	A+B
Test -	C	D	C+D
	A+C	B+D	N

If as normal the tests are imperfect (sensitivity and specificity < 100%) must be calculated the true prevalence (A+C) the apparent prevalence (A+B) and the diagnostic sensitivity and specificity are known according to the formula:

True prevalence = (Apparent prevalence + specificity – 1)/ (Sensitivity + Specificity – 1)

Important parameters for the interpretation of results are the predictive values for positive (PVPT) and negative test results (PVNT) according the formulae:

PVPT = # contaminated (A) / # test positive (A+B)

Or in terms of prevalence, sensitivity and specificity

PVPT=[prevalence*sensitivity]/[(prevalence*sensitivity)+(1-prevalence)*(1-specificity)]

PVNT = # not contaminated (D) / test negative (C+D)

PVNT = [(1-prevalence)*specificity]/[(1-prevalence)*specificity + prevalence*(1-sensitivity)]

It should be noted that the predictive values vary with the prevalence in particular if the prevalence is low and specificity is less than 100% there will be a large fraction of false positive samples.

For example if the prevalence is 10% and the sensitivity and specificity is 90% the predictive values are

PVPT = (0.1*0.9)/[(0.1*0.9)+(1-0.1)(1-0.9)]=0.09/0.18 = 50% and

PVNT = (1-0.1)*0.9/[1-0.1]*0.9 + 0.1*(1-0.9) = 0.81/0.82 = 99%.

If the prevalence is only 1% the predictive values will be:

PVPT = (0.01*0.9)/[(0.01*0.9)+(1-0.01)(1-0.9)]=0.009/0.108= 8% and

PVNT = (1-0.01)*0.9/[1-0.01)*0.9 + 0.01*(1-0.9) = 0.89/0.892 = 99.9%.

Thus, if the prevalence of a pathogen is very low, and the specificity cannot be assumed to be equal to 100% there is a tangible risk of false positive results when applying the tests. Thus, a positive test results would in this case not mean the presence of a pathogen and there is a need for confirmatory tests before the presence of a pathogen is concluded. Another approach would be the three class sampling plans that are applied for dealing with the similar problem of false positives in the context of microbiological criteria.

A particular effect of a sensitivity that is less than 100% is that the efficient sample diminishes. For example if taking 60 samples from a food lot and one positive sample rejects the lot, the probability of at least one sample testing positive for prevalence of 5% is:

$1-(1-\text{prevalence})^{\text{\#samples}} = 1-(1-0.05)^{60} = 0.954$ or 95%

if however, the sensitivity is 50% this probability will decrease:

$1-(1-\text{prevalence}*\text{ sensitivity})^{\text{\#samples}} = 1-(1-0.05*0.5)^{60} = 0.781$ or 78%

this might be compensated by increasing the sample size for example to 120:

$1-(1-\text{prevalence}*\text{ sensitivity})^{\text{\#samples}} = 1-(1-0.05*0.5)^{150} = 0.952$ or 95%.

> If the sensitivity is less than 100% it is possible to compensate for this by increasing the number of samples taken, whereas problems regarding specificity should be addressed by using confirmatory tests or e.g., three class sampling plans.

13.3. Annex III: Correlation of risk reduction linked to microbiological criteria with prevalence of contaminated foodstuffs

Based on the assumption of perfect tests i.e., diagnostic sensitivity=specificity=100% and the binomial formulae $1-(1-\text{prevalence defectives})^{\text{sample size}}$, (Vose, 1996) one can estimate the risk reduction afforded by the microbiological criteria as illustrated in Tables 6-8. This risk reduction is the fraction of food lots that will be rejected given a prevalence of defectives, number of samples taken and the number of positive samples accepted.

Table 12 The effect of prevalence contaminated food items and sample size on the risk reduction (%) for different 2-class sampling plans [a].

Sampling plan	n=1 c=0	n=5 c=0	n=10 c=0
Prevalence of defective items	Risk reduction on fraction of rejected food lots in %		
0.1%	0.1	0.5	0.9
1%	1	4.9	9.6
5%	5	23	40
10%	10	41	65
20%	20	68	89

[a] The risk reduction is described by the formula $1-(1-\text{prevalence defectives})^{\wedge \text{sample size.}}$ Here n denotes sample size and c the number of sampled food items that can be defective and the whole lot still accepted. The number of food items in the lot is assumed to be large (>5000) thus the binomial distribution can be applied. Note that these numbers presume random sampling from the whole food lot.

It appears that the risk reduction is somewhat limited when using a few samples such as 5 or 10 without accepting the finding of defective food items and the prevalence is low. For example by taking 5 samples and assuming a 5% the prevalence the risk reduction is around 23%, that is 23% of the contaminated food lots will be rejected (Table 12).

Table 13: The effect of prevalence contaminated food items and sample size on the risk reduction (%) for different 2-class sampling plans using a higher number of samples [a].

Sampling plan	n=15 c=0	n=30 c=0	n=60 c=0	n=100 c=0
Prevalence of defective items	Risk reduction or fraction of rejected food lots in %			
0.1%	1.5	3	5.8	9.5
0.5%	7.2	14	26	29
1%	14	26	45	63
2%	26	45	70	87
5%	54	79	95	99

[a] The probability of acceptance is described by the formula $1-(1-\text{prevalence defectives})^{\wedge \text{sample size.}}$ Here n denotes sample size and c the number of sampled food items that can be defective and the whole lot still accepted. The number of food items in the lot is assumed to be large (>5000) thus the binomial distribution can be applied. Note that these numbers presume random sampling from the whole food lot.

The risk reduction produced by the microbiological criteria is limited if the prevalence of contaminated food items is low. However, this can be compensated for some extent by increasing the number of samples to be taken (Tables 12 and 13).

For example, if a food lot of 10000 items has 1% prevalence of contaminated items where 10 samples analysed and no positive samples found (n=10, c=0), the risk reduction is 9.6%, consequently 90% of the food lots will be accepted (Table 12). If the number of samples is higher (n=100, c=0) the risk reduction will be 63%, consequently 27% of the lots will be accepted (Table 13).

However, whether the risk reduction is meaningful can be questioned if the prevalence approaches 0.1% (Table 13). For prevalences between 0.5% and 20% the question whether the risk reduction is meaningful or not, ought to be answered on a case-by-case basis. Cost-effectiveness and comparison with other risk management options available would be important parameters to consider when addressing the question of meaningful risk reduction.

One complication is that Tables 12 and 13 assume perfect tests with diagnostic sensitivity and specificity equal to 100%. However, the effect of lesser sensitivity will be a diminished the risk reduction afforded by microbiological criteria. Table 14 gives an example of the effect of criteria of the sensitivity is 50%, that is one will detect 50% of the truly contaminated food items; while the specificity is 100%, that is all truly negative food items will test negative. In this case the formulae will be $1-(1-\text{prevalence defectives} * \text{sensitivity})^{\wedge(\text{sample size})}$ (Gardener and Greiner, 1999). In this case the risk reduction will be reduced. For example for food lots with 1% prevalence and applying a microbiological criteria of 60 samples, without accepting defectives will give a risk reduction of 45% and 26%, for diagnostic sensitivities of 100% and 50%, respectively.

Table 14: The effect of prevalence contaminated food items and sample size and diagnostic sensitivity here assumed to be 50% and specificity is 100% on the risk reduction (%) for different 2-class sampling plans [a].

Sampling plan	n=5 c=0	n=15 c=0	n=30 c=0	n=60 c=0	n=100 c=0
Prevalence of defective items	Risk reduction or fraction of rejected food lots in %				
0.1%	0.2	0.7	1.5	3.0	4.9
0.5%	1.2	3.7	7.2	14	22
1%	2.5	7.2	14	26	39
2%	4.9	14	26	45	63
5%	1.2	32	53	78	92

[a] The risk reduction is described by the formula $1-(1-\text{prevalence defectives}*\text{sensitivity})^{\wedge\text{sample size}}$. Here n denotes sample size and c the number of sampled food items that can be defective and the whole lot still accepted, here c=0. The number of food items in the lot is assumed to be large (>5000) thus the binomial distribution can be applied. Note that these numbers presume random sampling from the whole food lot.

It can be concluded that the risk reduction afforded by microbiological criteria is reduced if the diagnostic sensitivity is less than 100%. Moreover, the risk reduction afforded by microbiological criteria is correlated with the prevalence of contaminated food items, the number of samples and the diagnostic sensitivity of the testing procedure.

14. ACKNOWLEDGEMENTS