FOOD MICROBIOLOGY

PT.2

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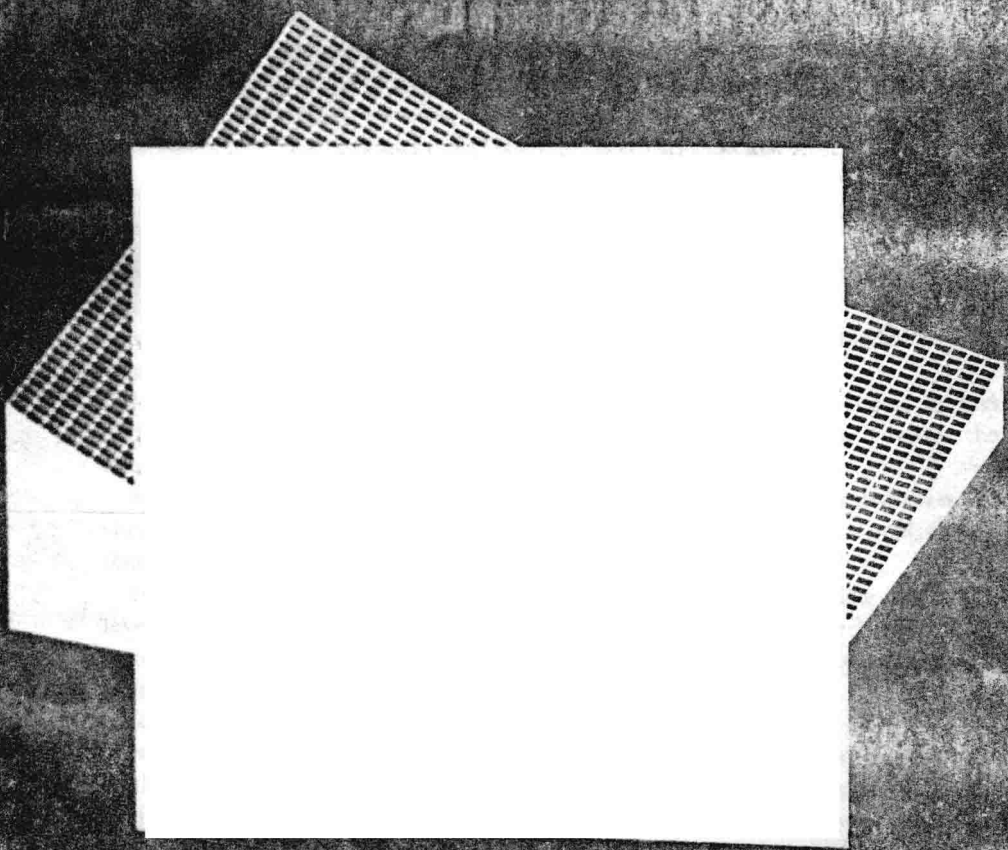

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heat by their intracellular location within milk leucocytes but subsequent studies have failed to demonstrate any significant effect.

Models of the thermal inactivation of *L. monocytogenes* in milk have indicated that conventional HTST pasteurization achieves a reduction of 5.2 log cycles in the number of survivors; an acceptable safety margin assuming low numbers of the organism present on the incoming milk.

Growth of all strains is inhibited at pH values below 5.5 but the minimum growth pH is dependent on both strain and acidulant and has been variously reported as between 5.6 and 4.4. *L. monocytogenes* is also quite salt tolerant being able to grow in 10% sodium chloride and survive for a year in 16% NaCl at pH 6.0.

The organism is ubiquitous in the environment. It has been isolated from fresh and salt water, soil, sewage sludge, decaying vegetation, and silage. Its prolonged survival in the environment has been demonstrated in one study where the level of *L. monocytogenes* in sewage sludge sprayed on to agricultural land remained unchanged for more than 8 weeks. Asymptomatic human and animal carriage is also common with reports of isolation of the organism from the faeces of, among others, cattle, pigs, sheep, chickens, turkeys, ducks, crustaceans, flies and ticks. In a study of faecal carriage in human population groups, it was isolated from 4.8% of healthy slaughterhouse workers, 1.2% of hospitalized adults, 1% of patients with diarrhoea, and 26% of household contacts of listeriosis patients.

7.8.3 Pathogenesis and Clinical Features

Its ubiquity in the environment suggests that human exposure to *L. monocytogenes* must be frequent. Incidence of infection is however low since invasive infection will result only if a susceptible individual is exposed to a sufficiently high dose of a virulent strain.

Incubation periods for the disease have varied from 1 day to as long as 90 days with a typical incubation period of a few weeks; a situation which makes the identification of food vehicles difficult if not often impossible.

Symptoms of the disease, which is most likely to develop in pregnant women, the very young or elderly and the immunocompromised, can vary from a mild, flu-like illness to meningitis and meningoencephalitis.

In pregnant women, it most commonly features as an influenza-like illness with fever, headache and occasional gastrointestinal symptoms, but there may be an associated transplacental foetal infection which can result in abortion, stillbirth, or premature labour.

Listeriosis in the newborn can be an early-onset syndrome, which occurs at birth or shortly afterwards, or a late-onset disease appearing several days to weeks after birth. Early-onset illness results from *in utero* infection, possibly through the aspiration of infected amniotic fluid, and is characterized by pneumonia, septicaemia and widely disseminated granulomas (abscesses). Meningitis is rare.

In the late-onset syndrome, meningitis is more common, 93% (39 of 42) of late-onset cases in Britain between 1967 and 1985 had evidence of infection of the central nervous system. Infection may occur from the mother during passage through the birth canal, but some may also be acquired after delivery. A study in

the UK found a lower mortality rate for late-onset disease (26%) than for early-onset listeriosis (38%).

Listeriosis in non-pregnant adults is usually characterized by septicaemia, meningitis and meningoencephalitis, but can also include endocarditis. It is particularly associated with those with an underlying condition which leads to suppression of their T cell mediated immunity, so that malignancies or immunosuppression (after renal transplantation, for example) are often predisposing factors. Although not a common infection in AIDS patients, its incidence is around 300 times that in the general population. Other conditions such as alcoholism, diabetes and cirrhosis can act as predisposing factors, but illness does often occur in otherwise healthy individuals who only account for about 18% of adult cases in England and Wales.

Adult listeriosis has a high mortality rate, figures calculated using data for 1989 gave values around the world of between 13 and 34%. Early treatment with antibiotics, normally ampicillin, with or without an aminoglycoside, or chloramphenicol, is essential but in the most severe forms, the prognosis remains poor.

The pathogenesis of listeriosis is not well understood. Much of the information we have comes from studies in mice and it is not yet clear how readily this can be extrapolated to human cases. *L. monocytogenes* is a facultative intracellular pathogen which like *Mycobacterium*, *Brucella*, and others can survive and multiply in cells of the monocyte–macrophage system. The organism attaches to intestinal cells and induces its endocytosis; processes promoted by the virulence factor p60, a 60 kDa protein. Once inside the phagocytic vacuole some protection from toxic superoxide anion is given by production of high levels of the enzymes superoxide dismutase and catalase, but in order to multiply intracellularly the organism must escape from the vacuole into the more conducive environment of the cytoplasm. Production of the haemolysin, listeriolysin O, is essential to this process. Recovery follows inactivation of the listeria by macrophages activated by listeria-sensitized T cells.

7.8.4 Isolation and Identification

Low-temperature enrichment at 4 °C is the traditional technique for isolating *L. monocytogenes* from environmental samples, but the increased interest in routine isolation of the organism from foods has led to its replacement by more rapid, selective enrichment procedures based on antibiotic cocktails as selective agents and incubation at near-optimal growth temperatures.

Selective agars have likewise relied on a combination of selective agents such as lithium chloride, phenylethanol and glycine anhydride and antibiotics. Identification of presumptive *Listeria* colonies was based on microscopic examination of plates illuminated from below at an incident angle of 45° (Henry illumination), when they appear blue–grey to blue–green. Some media avoid the use of this technique by incorporating aesculin and ferric ammonium citrate so that *Listeria* colonies appear dark brown or black as a result of their ability to hydrolyse aesculin.

Confirmation of *L. monocytogenes* requires further biochemical testing including sugar-fermentation tests to distinguish it from other *Listeria* species and, in particular, the CAMP test to differentiate *L. monocytogenes* from *L. innocua*. Specific

miniaturized test kits have been produced to simplify this procedure including one which replaces the CAMP test, which is not always easy for the inexperienced to interpret, with one for acrylamidase activity (*L. monocytogenes*, negative; *L. innocua*, positive). Enzyme-linked immunosorbent assay (ELISA) and gene probe kits are also available.

L. monocytogenes may be serotyped according to a scheme based on somatic and flagellar antigens. This is of limited epidemiological value since the majority of human cases of listeriosis are caused by just three of the thirteen serotypes identified (1/2a, 1/2b, and 4b).

7.8.5 Association with Foods

Its widespread distribution in the environment and its ability to grow on most non-acid foods offer *L. monocytogenes* plenty of opportunity to enter the food chain and multiply.

The transmission of listeriosis by food was first convincingly demonstrated in an outbreak that occurred in the Maritime Provinces of Canada in 1981. The outbreak involved 41 cases in all. Of the 34 perinatal cases, there were 9 stillbirths, 23 neonatal cases with a mortality rate of 27%, and 2 live births of healthy infants. The mortality rate in adult cases was 28.6%. Coleslaw was implicated as the result of a case control study and *L. monocytogenes* serotype 4b (the outbreak strain) was isolated from a sample of coleslaw in a patient's refrigerator. It was not possible to isolate the organism at the manufacturer's plant but it transpired that a farmer who supplied cabbages to the manufacturer also kept sheep, two of whom had died of listeriosis. The cabbage had been grown in fields fertilized by fresh and composted manure from the sheep and the harvested cabbages had been stored in a large shed through the winter – factors thought to account for the introduction of the organism and its multiplication to dangerous levels.

Raw vegetables, in the form of a garnish containing celery, tomatoes and lettuce, were also implicated on epidemiological grounds in an outbreak that occurred in eight Boston hospitals in 1979.

Surveys in the UK, the United States, Australia and elsewhere have reported a high frequency of isolation of *L. moncytogenes* from meats and meat products, where serotype 1 generally predominates. A number of sporadic cases of listeriosis have been associated with products such as pork sausage, turkey frankfurters, cook-chill chicken, and chicken nuggets.

L. monocytogenes is relatively resistant to curing ingredients and has been found in a range of delicatessen meats such as salami, ham, corned beef, brawn, and paté. In an Australian survey 13.2% of samples were found to be positive, largely as a result of cross-contamination in the shop. In Britain in 1989/90, high levels on vacuum-packed ham and on paté, from which serotype 4b was isolated, prompted the recall of both products from the market.

Dairy products such as raw and pasteurized milk and soft cheeses have been associated with a number of major outbreaks of listeriosis. The overall incidence of *L. monocytogenes* in raw milk derived from surveys in Australasia, Europe and the United States averages at around 2.2%, although one Spanish study reported an

incidence in excess of 45%. Pasteurized milk was responsible for an outbreak in Massachusetts in 1983 involving 42 adult and 7 perinatal cases with an overall mortality rate of 29%. The milk had come from farms where bovine listeriosis is known to have occurred at the time of the outbreak. It was the absence of evidence of improper pasteurization at the dairy that gave rise to the concern that *L. monocytogenes* might display marked heat resistance in some instances (see Section 7.8.2 above).

Soft cheeses are also frequently contaminated with *L. monocytogenes*. In 1985 there was an outbreak in California in which a Mexican-style soft cheese which had been contaminated with raw milk was the vehicle. One hundred and forty-two cases were recorded comprising 93 perinatal and 49 adult cases with an overall mortality rate of 34%. This outbreak served to focus attention on soft cheeses and there have since been other incidents identified in which they have been implicated, including a major outbreak covering the period 1983–87 with 122 cases and 31 deaths associated with the Swiss cheese Vacherin Mont d'Or.

This association with soft cheeses appears to be due to the cheese ripening process. *L. monocytogenes* survives poorly in unripened soft cheeses such as cottage cheese but well in products such a Camembert and Brie. During the ripening process, microbial utilization of lactate and release of amines increase the surface pH allowing *Listeria* to multiply to dangerous levels.

7.9 *PLESIOMONAS SHIGELLOIDES*

7.9.1 Introduction

Plesiomonas shigelloides is the only species of the genus whose name is derived from the Greek word for neighbour; an allusion to its similarity to *Aeromonas*. Its position as a causative agent of foodborne illness also bears some similarity to *Aeromonas*. It is not normally recoverd from human faeces, except in Thailand where a carriage rate of 5.5% has been reported. The association with diarrhoea is largely based on its isolation from patients suffering from diarrhoea in the absence of any other known pathogens and the strongest of this evidence has come with isolation from several patients in the same outbreak. However volunteer feeding trials have failed to demonstrate a causal link.

7.9.2 The Organism and its Characteristics

A member of the family Vibrionaceae, *P. shigelloides* is a short, catalase-positive, oxidase-positive, Gram-negative rod. It is motile by polar, generally lophotrichous flagella in contrast to *Aeromonas* and *Vibrio* which are monotrichous. It grows over a temperature range from 8–10 °C to 40–45 °C with an optimum at around 37 °C. It is not markedly heat resistant and is readily eliminated by pasteurization treatments. Growth is possible down to pH 4.5 and the maximum salt concentration it will tolerate is between 3 and 5% depending on other conditions.

The organism is ubiquitous in surface waters and soil, more commonly in samples from warmer climates. Carriage in cold-blooded animals such as frogs, snakes,

turtles, and fish is common and it has been isolated from cattle, sheep, pigs, poultry, cats and dogs. It is not normally part of the human gut flora.

7.9.3 Pathogenesis and Clinical Features

Cases of *P. shigelloides* infection are more common in warmer climates and in travellers returning from warmer climates. The usual symptoms are a mild watery diarrhoea free from blood or mucus. Symptoms appear within 48 h and persist for several days. More severe colitis or a cholera-like syndrome have been noted with individuals who are immunosuppressed or have gastrointestinal tumours.

Little is known of the pathogenesis of *P. shigelloides* infections. Motility appears to be an important factor and evidence has been presented for an enterotoxin causing fluid secretion in rabbits' ligated ileal loops.

7.9.4 Isolation and Identification

The relatively recent growth of interest in *P. shigelloides* is reflected in the use of 'second-hand' media in its isolation. Alkaline peptone water and tetrathionate broth have both been used for enrichment culture of *P. shigelloides* at 35–40 °C and salmonella–shigella and MacConkey agars have been used as selective plating media. Selective plating media have been developed such as inositol/brilliant green/bile salts, *Plesiomonas* agar. Isolates can be readily confirmed on the basis of biochemical tests.

7.9.5 Association with Foods

Fish and shellfish are a natural reservoir of the organism and, with the exception of one incident where chicken was implicated, they are the foods invariably associated with *Plesiomonas* infections. Examples have included crab, shrimp, cuttle fish and oysters.

7.10 *SALMONELLA*

7.10.1 Introduction

Most salmonellas are regarded as human pathogens, though they differ in the characteristics and the severity of the illness they cause. Typhoid fever is the most severe and consequently was the earliest salmonella infection to be reliably described. This is credited to Bretonneau, the French physician who is also regarded as the founder of the doctrine of the aetiological specificity of disease. During his life, he published only one paper on typhoid, or 'dothinenterie' as he called it, in 1829, and his treatise on the subject was only published in 1922 by one of his descendants.

In 1856, the English physician William Budd concluded that each case of typhoid is epidemiologically linked to an earlier case and that a specific toxin is disseminated with the patients faeces. To support his proposition he demonstrated that treating the excreta of victims with chlorinated lime (bleaching powder) reduced the incidence of typhoid. The typhoid bacillus was first observed by the German

bacteriologists Eberth and Koch in 1880 and four years later Gaffky succeeded in its cultivation. The paratyphoid bacilli, responsible for the clinically similar condition, paratyphoid fever, were first isolated by Achard and Bensaud (1896) and by Gwyn (1898), and confirmed as culturally and serologically distinct from the typhoid bacillus by Schottmüller in 1901. Other salmonellas were isolated during the same period; Salmon and Smith (1885) isolated *Bacillus cholerae-suis* from pigs with hog cholera, a disease now known to be viral in origin, and similar bacteria were isolated from cases of foodborne infection and animal disease. The genus *Salmonella* was finally created in 1900 by Lignières and named in honour of D.E. Salmon, the American veterinary pathologist who first described *Salmonella cholerae-suis*.

Salmonellas are now established as one of the most important causes of foodborne illness worldwide. In Europe in 1989 the annual incidence of salmonellosis was around 50 per 100 000 inhabitants in most countries, though actual figures varied from below 10 in the case of Luxembourg to more than 120 in Hungary and Finland. In the United States in 1989 the incidence was 19 per 100 000.

On the basis of DNA/DNA hybridization, the genus *Salmonella* is now recognized to contain a single species, *S. enterica* (formerly known as *S. cholerae-suis*), which comprises seven subspecies.

The Kauffman–White serotyping scheme has proved the most useful technique for differentiating within the genus. This describes organisms on the basis of their somatic (O) and flagellar (H) antigens, and by capsullar (Vi) antigens (possessed by *S. typhi*, *S. dublin* and occasional strains of *S. paratyphi* C). In 1941 the scheme contained 100 serotypes and the number has since risen to the current level of around 2200.

The taxonomic nomenclature of the genus is rather different from that of other genera. Many of the different serovars are named as if they were distinct species. The earliest to be described were given species epithets derived from the disease they caused, either in humans (*S. typhi*, *S. paratyphi* A and B), or in animals (*S. typhimurium*, *S. cholerae-suis*, or *S. abortusovis*). Limitations in this approach led to the use of serovar names based on the geographical location of the first isolation, for example *S. dublin*, *S. montevideo*, *S. minneapolis*, and even *S. guildford*. This has some advantage over the use of long serological formulae but since 1966 has only been applied to serovars of subspecies I (*S. enterica* subsp. *enterica*) which accounts for 59.5% of the 2200 serovars known and the vast majority (>99%) of human isolates.

The most recent proposal to introduce some taxonomic rectitude is to use the non-italicized serovar name after the species name so that *S. typhimurium* becomes *S. enterica* subsp. *enterica* ser. Typhimurium or, more concisely, *Salmonella* ser. Typhimurium. By retaining the old serovar name much of the potential for confusion inherent in other schemes is reduced. In the case of other subspecies which comprise mainly isolates from the environment and cold-blooded animals, the serovar formula is used after the name of the subspecies, *e.g. Salmonella fremantle* would be *S. enterica* subsp. *salamae* ser. 42;g,t:-.

Though the traditional system is taxonomically incorrect, description of an isolated serovar as *S. enteritidis* is an economical and efficient way of conveying important clinical and epidemiological information. For this reason it remains in

widespread use and is the system we will adopt here. However, one final word of caution: please remember that in this section use of an italicized binomial *does not* imply species status.

7.10.2 The Organism and its Characteristics

Salmonellas are members of the Enterobacteriaceae. They are Gram-negative, non-sporeforming rods (typically 0.5 μm by 1–3 μm) which are facultatively anaerobic, catalase-positive, oxidase-negative, and are generally motile with peritrichous flagella.

Growth has been recorded from temperatures just above 5 °C up to 47 °C with an optimum at 37 °C. Salmonellas are heat sensitive and are readily destroyed by pasteurization temperatures. *S. senftenberg* 775W is the most heat resistant serotype at high a_w and has a D_{72} in milk of 0.09 min (*S. typhimurium* D_{72} = 0.003 min). Heat resistance has been shown to be enhanced by sub-lethal heat shocking at 48 °C for 30 min and can also be markedly increased in low a_w media, for example *S. typhimurium* has a D_{70} of 11.3–17.5 h in chocolate sauce. In frozen foods, numbers of viable salmonella decline slowly, the rate decreasing as the storage temperature decreases.

The minimum a_w for growth is around 0.93 but cells survive well in dried foods, the survival rate increasing as the a_w is reduced. The minimum pH for growth varies with the acidulant from 5.4 with acetic acid to 4.05 with hydrochloric and citric acids. Optimal growth occurs around pH 7.

It was noted in Section 7.10.1 above that the most important technique for sub-dividing the genus is the serotyping scheme of Kauffman and White. This does not provide a complete account of the antigenic structure of each salmonella, but does provide a workable scheme using antigens of diagnostic value. In the case of the more common serotypes such as *S. typhimurium* and *S. enteritidis* a more discriminating scheme of classification is required for epidemiological purposes and this is provided by phage typing.

This was first applied to *S. typhi* where most strains could be classified into one of 11 phage types using a set of phages that acted only on bacteria possessing the Vi antigen. A high degree of correlation has been observed between phage type and epidemic source. Similar successful phage typing-schemes have been developed for, among others, *S. typhimurium*, which employs 36 phages to distinguish the 232 definitive types currently recognized, *S.enteritidis* and *S. virchow*.

Biotyping according to biochemical characteristics has sometimes proved useful in epidemiological investigations where it can supplement phage typing or subdivide a large group of otherwise untypable strains. This has proved most useful for *S. typhimurium* where Duguid's scheme based on 15 biochemical tests has identified 184 full biotypes.

Plasmid profiling based on the isolation and separation of plasmids by electrophoresis on agarose gels has also met with some success as an epidemiological tool. One notable example of its use was in the early 1980s when it was used to identify a strain of *S. muenchen* responsible for an outbreak in the United States where the food vehicle was marijuana. The plasmid profile was sufficiently

distinctive and stable to allow the outbreak strain to be distinguished from strains of other serotypes and non-outbreak strains of *S. muenchen.*

Salmonellas are primarily inhabitants of the gastrointestinal tract. They are carried by a wide range of food animals, wild animals, rodents, pets, birds, reptiles, and insects, usually without the display of any apparent illness. They can be disseminated via faeces to soil, water, foods and feeds and thence to other animals (including humans).

Most salmonellas infect a range of animal species but some serotypes are host adapted such as *S. enteritidis* PT4, *S. pullorum* and *S. gallinarum* in poultry and *S. cholerae-suis* in pigs. In these cases direct animal-to-animal transmission can be more important and vertical transmission may occur – parents infecting offspring. For example, *S. enteritidis* PT4 can pass from breeding flocks to newly hatched broiler and egg-laying chicks via transovarian infection of the egg or its shell.

7.10.3 Pathogenesis and Clinical Features

Salmonellas are responsible for a number of different clinical syndromes, grouped here as enteritis and systemic disease.

7.10.3.1 Enteritis. Gastrointestinal infections are predominantly associated with those serotypes which occur widely in animals and humans. They can range in severity from asymptomatic carriage to severe diarrhoea and are the most common type of salmonellosis.

At any one time human illness is usually associated with a limited number of serotypes; in the UK only about 200 serotypes may be reported in any one year. Currently *S. enteritidis*, *S. typhimurium*, and *S. virchow* are the most common, the first two accounting for about three-quarters of laboratory reports.

The incubation period for salmonella enteritis is typically between 6 and 48 h. The principal symptoms of mild fever, nausea and vomiting, abdominal pain and diarrhoea last for a few days but, in some cases, can persist for a week or more. The illness is usually self-limiting but can be more severe in particularly susceptible groups such as the very young, the very old and those already ill. One example of this is the outbreak which occurred in the Stanley Royd Hospital in the UK in 1984 where about 350 patients and 50 staff were affected and 19 of the patients died.

Ingested organisms, which survive passage through the stomach acid, adhere to the epithelial cells of the ileum via mannose-resistant fimbriae. They are then engulfed by the cells in a process known as receptor mediated endocytosis. The detailed mechanism of this process remains to be clarified, but recent studies have indicated that a key event may be phosphorylation of tyrosine residues in a variety of proteins, following stimulation of the epidermal growth-factor receptor. Endocytosed salmonellas pass through the epithelial cells within a membrane-bound vacuole, where they multiply and are then released into the lamina propria via the basal cell membrane. This prompts an influx of inflammatory cells leading to the release of prostaglandins which activate adenylate cyclase producing fluid secretion into the intestinal lumen. It is unlikely that this is the whole picture since an inflammatory response does not always stimulate fluid secretion.

More probably the cause of salmonella diarrhoea is multifactorial. Evidence has

been obtained that diarrhoeagenic salmonellas also produce enterotoxins which stimulate fluid secretion in assays such as the ligated ileal loop test. In particular, a heat-labile toxin has been purified from *S. typhimurium* which bears some structural and serological similarity to *E. coli* LT and cholera toxin. However, some of the evidence in this area is contradictory and resolution of the problem will probably require optimization of the conditions for toxin production and more sophisticated tests for enterotoxin activity.

As a general rule, the infectious dose of salmonella is high, of the order of 10^6 cells, but this will vary with a number of factors such as the virulence of the serotype, the susceptibility of the individual and the food vehicle involved. A number of outbreaks have occurred where epidemiological evidence points to an infective dose as low as 10–100 cells. This appears to be particularly associated with more susceptible individuals such as children and the elderly, and with fatty foods such as cheese, salami and chocolate. In an outbreak in Canada where the vehicle was cheddar cheese it was found to contain 1.5–9.1 cells per 100 g. It seems likely that the high fat content in some foods affords the bacteria some protection from stomach acidity. A low infective dose (<200) was also indicated in a waterborne outbreak in the early 1970s. In this case fat was clearly not a factor, but the more rapid transit of water through the stomach may have served a similar purpose.

After symptoms have subsided, carriage of the organism and its passage in high numbers in the stools may occur for a few weeks, or occasionally months.

7.10.3.2 Systemic Disease. Host-adapted serotypes are more invasive and tend to cause systemic disease in their hosts; a feature which is linked to their resistance to phagocytic killing. In humans, this applies to the typhoid and paratyphoid bacilli, *S. typhi*, and *S. paratyphi* A, B, and C, which cause the septicaemic diseases, enteric fever.

Typhoid fever has an incubation period of anything from 3 to 56 days, though it is usually between 10 and 20 days. Invasive salmonellas penetrate the intestinal epithelium and are then carried by the lymphatics to the mesenteric lymph nodes. After multiplication in the macrophages, they are released to drain into the blood stream and are then disseminated around the body. They are removed from the blood by macrophages but continue to multiply within them. This eventually kills the macrophages which then release large numbers of bacteria into the blood stream causing a septicaemia. In this, the first phase of the illness, the organism may be cultured from the blood. There is a slow onset of symptoms including fever, headache, abdominal tenderness and constipation and the appearance on the body of rose red spots which fade on pressure.

During the second stage of the illness, the organism reaches the gall bladder where it multiplies in the bile. The flow of infected bile reinfects the small intestine causing inflammation and ulceration. The fever persists but with the onset of a diarrhoea in which large numbers of the bacteria are excreted with the characteristic 'pea soup' stools and, to a lesser extent, with the urine. In more serious cases, haemorrhage of the ulcers may occur and perforation of the intestine leading to peritonitis. In milder cases, the ulcers heal and fever falls with recovery after 4–5 weeks.

Unlike the more localized enteric infections, typhoid is usefully treated with antibiotics such a chloramphenicol, ampicillin and amoxycillin. After remission of symptoms, a carrier state can persist for several months and occasionally years as parts of the gall bladder are colonized and bacteria are discharged intermittently with the bile into faeces. This occurs more commonly in women and the elderly and there have been a number of typhoid carriers who have achieved some notoriety as a result of their condition and its consequences. These include the 'Strasbourg Master Baker's Wife', the 'Folkestone Milker', and, probably best known of all, 'Typhoid Mary'. Mary Mellon worked as a cook in a number of households and institutions in the New York area at the beginning of the century. She first attracted the attention of the authorities when she disappeared after an outbreak of typhoid fever in a family for whom she had been working. When she was eventually tracked down by following a trail of outbreaks in places she worked, she was forcibly detained in the New York City Hospital for three years. Despite an undertaking not to work as a cook or handle food on her release, she disappeared again, assumed a false name, and started work as a cook. In 1915 she was working at another New York hospital when a typhoid outbreak occurred in which eight people died. She failed to return from leave, but was later found and held in the City Hospital until her death, from a stroke, in 1938, aged 70. It has been estimated that during her career Mary Mellon was responsible for more than 1300 cases of typhoid.

Nowadays chronic carriers can be treated with antibiotics, but in particularly recalcitrant cases cholecystectomy (surgical removal of the gall bladder) is necessary.

A number of non-human adapted serotypes such as *S. blegdam*, *S. bredeny*, *S. cholerae-suis*, *S. dublin*, *S. enteritidis*, *S. panama*, *S. typhimurium*, and *S. virchow* can also be invasive in susceptible individuals. They can cause less severe forms of enteric fever and septicaemia, and focal infections at a wide variety of sites around the body such as the heart, appendix, gall bladder, peritoneum, lungs, urinary tract, brain, meninges and spleen. Localization is more likely to occur at sites where there is pre-existing disease or damage and some sites of infection are associated with particular population groups such as meningitis in infants, pneumonia in the elderly, and osteomyelitis in patients with sickle-cell anaemia.

7.10.4 Isolation and Identification

Methods for the isolation and identification of salmonellas in foods have arguably received more attention than those for any other foodborne pathogen. Using traditional cultural techniques, a five-stage procedure has emerged as the widely accepted norm. This is outlined in Figure 7.4.

Pre-enrichment in a non-selective medium increases the recovery rate of salmonellas by allowing the repair of cells which have been sub-lethally damaged. Such damage can result from any exposure to adverse conditions that might occur during food processing, such as chilling, freezing, or drying, and increases the cell's sensitivity to selective agents used in media in subsequent stages of the isolation procedure. Failure to include a resuscitation step could therefore result in the non-detection of cells that might recover and cause infection if the food is mishandled.

The selective enrichment stage is intended to increase the proportion of

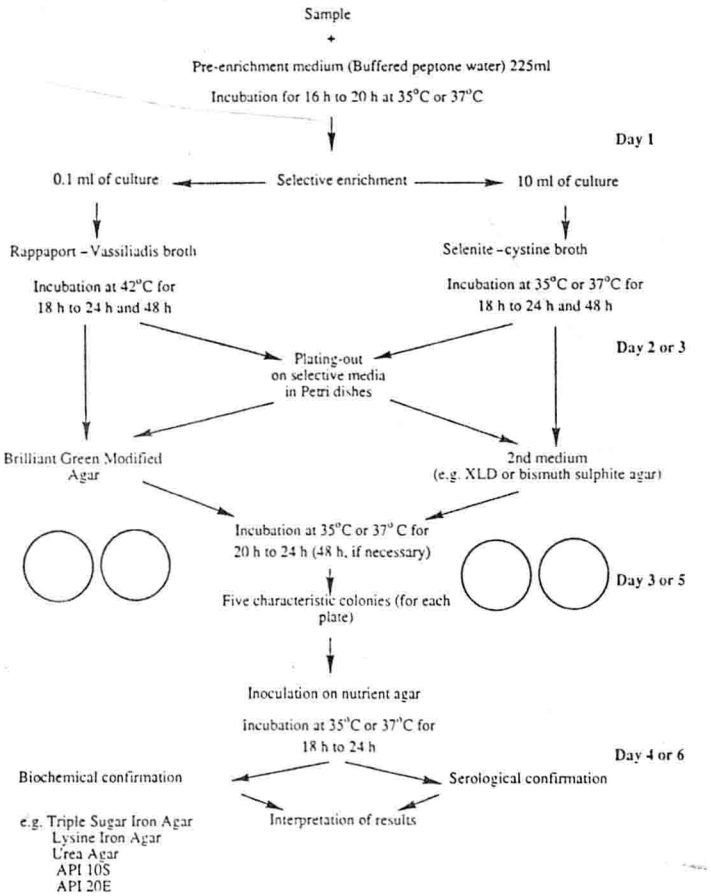

Figure 7.4 *Traditional cultural protocol for isolation of* Salmonella *from food*

salmonella cells in the total microflora by allowing them to proliferate while restricting growth of other micro-organisms present. To this end a number of different media have been proposed employing selective agents such as bile, brilliant green, malachite green, tetrathionate and selenite. The most widely used are selenite–cystine broth, which contains cystine to stimulate growth of salmonellas; Muller–Kauffman tetrathionate broth, containing tetrathionate, brilliant green, and bile; and Rappaport–Vassiliadis (RV) broth, which contains malachite green, magnesium chloride and a slightly reduced pH as selective factors. Since they differ in their selectivity, two broths are usually used in parallel; commonly a combination of the less selective selenite–cystine broth and one of the others.

From the selective enrichment broths, cultures are streaked on to selective and differential solid media. Once again it is usual to use two different media in parallel. The selective agents used are bile salts or deoxycholate and/or brilliant green and the diagnostic reaction is usually provided by the inability of most salmonellas to

ferment lactose and/or the production of hydrogen sulfide. In choosing the media to use, it is advisable to select two based on different diagnostic reactions to ensure that atypical strains, for instance lactose-positive ones, will not be missed.

Presumptive salmonellas from selective plating media must be confirmed by biochemical testing and serologically by agglutination with polyvalent O antisera.

The whole protocol is rather complex and lengthy, requiring at least four days for a negative result. In view of this, a number of procedures have been described which attempt to simplify the procedure and reduce the elapsed time involved. Two of these employ the motility of salmonellas which means that they would fail to detect non-motile salmonellas (incidence <0.1%).

In one, a conventional pre-enrichment culture is inoculated into an elective medium, salmonellas swim into a compartment containing a selective medium and from there into one containing a diagnostic medium. A diagnostic medium giving the appropriate colour change is then tested for ability to agglutinate latex particles coated with salmonella antibodies. A positive result indicates a presumptive salmonella, which must then be confirmed by conventional serological and biochemical testing using a sub-culture from the diagnostic medium. With this technique, presumptive identification of a salmonella is obtained within 42 h compared with 3–4 days by the traditional cultural method.

In another system, salmonella detection is by formation of an immunoprecipitate as *Salmonella* antibodies diffusing down through a medium meet salmonellas swimming up from a chamber containing a selective medium.

Impedance–conductance techniques (see Chapter 10) have been successfully applied to the detection of salmonellas. The original medium of Easter and Gibson comprises a modified selenite–cystine broth containing dulcitol and trimethylamine oxide (TMAO). Salmonellas are able to ferment dulcitol and reduce TMAO to the base trimethylamine. This increases the conductivity of the medium and provides the basis for detection. The detection time is reduced if the samples are pre-enriched in a medium containing dulcitol and TMAO to induce the relevant enzymes. In a comparison using 2586 samples of milk powder, this method was found to be as effective as a traditional cultural method but with considerable savings of time and labour. With a 24 h' pre-enrichment step, *Salmonella*-negative samples can be detected within 48 h.

A number of modifications to the original medium and protocol have been described. These include the incorporation of a *Salmonella*-specific bacteriophage in a parallel sample to demonstrate that observed changes in electrical properties are in response to salmonella; the replacement of dulcitol with mannitol or deoxyribose in order to detect dulcitol-negative salmonellas; and the use of detection media based on lysine decarboxylase activity.

ELISA and gene probe kits for the detection of salmonellas are also available, but like all the techniques described, they require a certain threshold concentration of salmonellas. One approach to avoid or curtail the enrichment steps that this usually entails is immunomagnetic separation. *Salmonella* antibodies are attached to magnetic particles which are added to a liquid culture containing salmonellas which are then captured by the antibodies. The beads with adhering *Salmonella* cells can then be readily separated from the culture with a magnet, achieving a substantial

enrichment in min. Their presence can then be confirmed using conventional media or one of the more rapid techniques.

7.10.5 Association with Foods

Salmonellosis is described as a zoonotic infection since the major source of human illness is infected animals. Transmission is by the faecal–oral route whereby intestinal contents from an infected animal are ingested with food or water. A period of temperature abuse which allows the salmonellae to grow in the food and an inadequate or absent final heat treatment are common factors contributing to outbreaks.

Meat, milk, poultry, and eggs are primary vehicles; they may be undercooked, allowing the salmonellas to survive, or they may cross-contaminate other foods that are consumed without further cooking. Cross-contamination can occur through direct contact or indirectly via contaminated kitchen equipment and utensils.

Human carriers are generally less important than animals in the transmission of salmonellosis. Human transmission can occur if the faecally contaminated hands of an infected food handler touch a food which is then consumed without adequate cooking, often after an intervening period in which microbial growth occurs. This was the cause of a major outbreak affecting an international airline in 1984. The outbreak involved 631 passengers and 135 crew and was due to contamination of an aspic glaze by a member of catering staff who returned to work after illness but was still excreting *Salmonella enteritidis* PT4.

Direct person-to-person spread by the faecal–oral route is also possible but is usually restricted to institutional outbreaks such as occur in hospitals, old people's homes, and nurseries.

Food animals may acquire salmonella infection on the farm from wild birds and rodents, but the principal sources are other animals, which may be symptomless excreters, and contaminated feeding stuffs (Figure 7.5). Measures that can be taken to minimize transmission between animals on the farm include good animal husbandry, protection of feeds and water from contamination, hygienic disposal of wastes, and maintenance of a generally clean environment. Transfer of *Salmonella* between animals is particularly associated with situations where animals may be stressed and crowded such as during transport, at markets, and when in lairage at the slaughterhouse. It is best minimized by avoidance of overcrowded conditions, ensuring a clean environment, and otherwise limiting the stress to animals on such occasions.

An important factor in maintaining the cycle of *Salmonella* infection in food animals is the practice of using animal by-products as animals feeds such as meat and bone meal. The heat process that these materials undergo in their conversion to feeds should destroy any salmonellas present. Nevertheless they are subject to post-process contamination either in the plant or on the farm by contact with unprocessed material or with bird and rodent faeces. Recognition of this problem led to the introduction in the UK of the Processed Animal Protein Order (1989) requiring the testing of animal proteins intended for use in feeds for the presence of *Salmonella*.

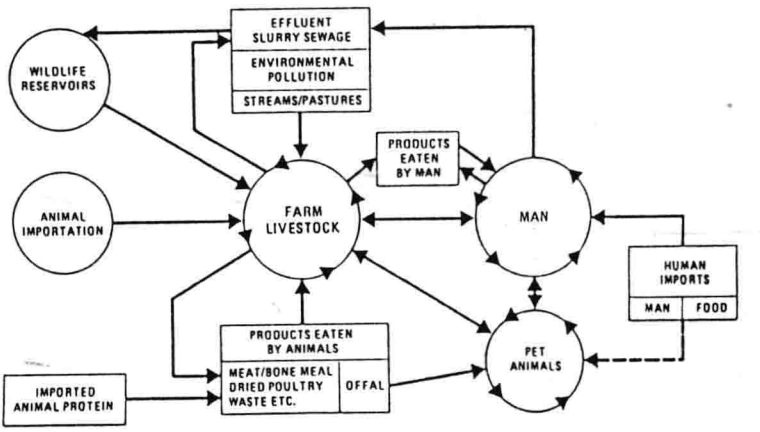

Figure 7.5 *The* Salmonella *cycle of infection (Reproduced with permission from WHO, 1983)*

In the UK, the major source of *Salmonella* infection is poultry and poultry products. Here the problem includes vertical transmission of host-adapted serotypes from the breeding flocks to their progeny. Particularly noteworthy in this respect is *S. enteritidis* PT4 which has been responsible for the rise in salmonellosis since 1985. Isolations of *S. enteritidis* increased 14-fold between 1981 and 1988, while those of *S. typhimurium* less than doubled, and *S. enteritidis* is now the commonest serotype recorded. Poultry was the food most commonly implicated in outbreaks of salmonellosis in 1986 and 1987 but in 1988 and 1989, eggs were the most frequent vehicle. Most outbreaks were associated with raw eggs in products such as home-made mayonnaise and ice cream or, in one instance, a 'body-building' drink.

Contamination of eggs with salmonellas is a long-recognized problem but in most cases this was due to contamination of the eggshell exterior with faecal material in the hen's cloaca or after laying in the nest or battery. The shell could then contaminate the contents when the egg was broken. This is a particular problem when breaking large quantities of eggs where it is difficult to avoid some contamination with shell fragments. During the Second World War, there were a number of outbreaks attributed to dried whole egg powder and a survey conducted in 1961/2 found 16% of frozen whole egg samples to contain salmonellas. This led to the introduction in 1963 of regulations requiring that liquid whole egg be pasteurized at 64.4 °C for 2.5 min. The prescribed heat treatment also inactivates the yolk enzyme α-amylase and provides the basis of a simple test to ensure that the regulations have been complied with.

In the more recent cases however, contamination of the yolk of intact hen's eggs has also been indicated. In the UK and in other European countries, particularly Spain, this problem has been associated with *S.enteritidis* PT4 but other phage types (PT8 and PT13a) have been reported to cause similar problems in the United States. It is thought that these organisms infect the bird's ovaries and oviduct and thereby contaminate the egg contents. The temperatures reached in the yolk during

mild cooking procedures such as 'soft boiling' or light frying are probably insufficient to kill the organism and the fat content of the yolk may protect the organism from gastric acidity.

The precise extent of this problem is difficult to determine, but one survey found *Salmonella* in the contents of one in a thousand eggs from flocks associated with human illness. When one considers that 30 million eggs are eaten daily in the UK, then eggs are clearly an important source of human infection. In an attempt to control salmonella in poultry in Great Britain, compulsory bacteriological monitoring of all commercial egg-laying and breeding flocks and hatcheries was introduced. If *S. enteritidis* was isolated from an egg-laying or breeder flock, the birds were required to be slaughtered. This practice has since been stopped although layer breeder flocks infected with either *S. enteritidis* or *S. typhimurium* are still subject to compulsory slaughter. An additional approach that is being explored extensively in Europe is known as the competitive exclusion. It is well known that the mature intestinal microflora has a protective effect against colonizing *Salmonella*. However in modern mass-production systems where newly hatched birds are not reared with adults, the development of a mature gut flora is slower. To overcome this, it is possible to supply young chicks with the necessary cultures artificially in their feed or water. Commercial preparations of live cultures prepared from the caecal bacteria of the normal chicken are available for this purpose.

Raw milk will inevitably contain *Salmonella* and any slight nutritional advantage it may have over pasteurized milk is far outweighed by the very real risk of salmonellosis (and campylobacteriosis). Outbreaks in a number of countries have been associated with pasteurized milk that has been inadequately processed or subject to post-process contamination. *Salmonella* is unable to grow in dried milk but is able to survive and resume growth when the milk is reconstituted. *S. ealing* was responsible for an outbreak in the UK in 1985 where the vehicle was a dried baby-milk. The organism had contaminated the insulation surrounding a spray drier and penetrated the drying chamber itself through a small defect in the chamber wall. Rigorous cleaning and disinfection were unable to eliminate the contamination from the spray drier which was eventually decommissioned.

Fish and fish products are only occasionally associated with salmonellosis, although fish meal for animal feed often contains *Salmonella* as a result of contamination from rodents and birds. Filter-feeding shellfish harvested from polluted waters and frozen precooked prawns have been identified as higher risk products.

Since birds, rodents, insects, infected food handlers or infected foods can all contaminate foods directly or indirectly, potential food vehicles for salmonella are numerous. Contaminated cocoa beans which had been processed into chocolate were responsible for outbreaks of *S. eastbourne* in the United States and Canada, of *S. napoli* in England, and *S. typhimurium* in Scandinavia. Although the production of chocolate involves a heating stage, this was insufficient to kill all the salmonellas present, possibly as a result of a protective effect from the cocoa butter.

Desiccated coconut is used in a range of confectionary products and was identified as a hazard following cases of typhoid and salmonellosis in Australia. In 1959/60, a survey of desiccated coconut imports into the UK from Sri Lanka