MEAT MICROBIOLOGY

1 – Introduction

Meat Microbiology will be an ongoing series of short articles that will keep you informed about microbiological issues associated with meat, be it red meat, white meat or fish.

Each article will focus on a topic designed to refresh the thoughts of those with microbiological knowledge and inform those who do not have a microbiological background.

This first article will set the scene by considering what bacteria are. In essence, bacteria are microscopic animals and in a row of bacteria 1.0cm long there would be some 10,000 or so bacteria.

Bacteria are microscopic and in the early days they were named after their shapes that were seen under a microscope.

Cocci are spherical bacteria. Staphylococcus literally means a 'cluster of cocci' and Streptococcus means a 'row or string of cocci' and this is just what these bacteria look like under the microscope. Bacillus means 'rod' shaped, while vibrios are 'comma' shaped.

To see bacteria more easily under the microscope we stain them and one of the early staining regimens was Gram staining.

Gram staining is named after the scientist who developed this method for staining bacteria.

Using this staining method bacteria which stain purple are Gram positive, while those staining red/pink are Gram negative.

Thus, for example, E. coli and salmonella are Gram negative rods, while clostridia and Bacillus Spp are Gram positive rods.

It is quite good to consider bacteria as microscopic animals as this gives us an insight into their needs. Like other animals, bacteria eat and drink – but for bacteria a feast is the smallest speck of protein/fat containing dirt and a damp patch will provide water for literally thousands of bacteria. Many 'breathe' (require oxygen) and are known as aerobes, whereas others do not and are called anaerobes. Some can survive on reduced oxygen levels and are referred to as microaerophilic.

Some bacteria have small whip-like appendages known as flagella and these can propel the bacteria through water films.

You may never have thought about it but drying is actually an important stage of any washing/cleaning programme – if we leave equipment wet then bacteria that have survived the cleaning process can potentially move on to product contact areas.

Bacteria can 'find shelter' in any small or microscopic crack or hole and this is why stainless steel is preferred in processing plants – it presents a smooth, flat surface with no microscopic hiding places for the bacteria.

The same can be said for ceramic tiles – unfortunately it can not be said for the grouting between them!

Bacteria can grow and multiply very quickly. They do not need to find a mate and multiply by binary division with one becoming 2, and then 4, and then 8 and so on. The generation time (the time between the same stages in successive cycles) can be as low as 10 minutes.

In 10 cycles one bacterium becomes 512, in 20 it becomes 284,288 and in 30 cycles it can be as many as 291 million. By 32 cycles one bacterium can become over a billion bacteria!

1
2
4
8
16
32
64
128
256
512
1,024
2,048
4,096
8,192
16,384
32,768
65,536
131,072
262,144
524,288
1,048,376
2,096,752

Fig. 1. Bacterial multiplica-

In a day there is enough time for 144 ten minute life cycles!

However, bacteria die off but many more replace them resulting in a net massive growth in numbers. Consider a bacterium with a ten generation life – using the figures above when one dies off it would be 'replaced' by 512 leaving a net increase in the population of 511!

Life is not always Utopian for bacteria. These ideal growth rates have some caveats attached to them, for example there must be enough food, water and space.

Also the bacteria must remain separated from their own toxic waste products or they may well poison themselves!

In the next 'Meat Microbiology' we will look at the dynamics of bacterial growth and how it is affected by temperature. ■

MEAT MICROBIOLOGY

2 – Bacterial dynamics

In this MEATMICROBIOLOGY we are going to look at the dynamics of bacterial growth and multiplication from the point of view of the bacterial population on a product rather than the single bacterium.

These dynamics are represented by the graph in Fig. 1. It can be seen that this growth curve has three distinct sections which are known as the lag phase (A), the log phase (B) and the stationary phase (C).

In the lag phase the bacterial population is establishing itself, whereas when we get to the log phase the population is established and growing and multiplying at its fastest.

Eventually, food and space run out and by-products which adversely affect the bacteria accumulate and bacterial growth/mul-tiplication rate dramatically slows down – this is the stationary phase.

Once bacterial numbers reach a certain level (N_x in Fig. 1) we can detect the signs of the product going off, including an undesirable odour.

Obviously we want a level of bacteria less than this level (N_{UB} in Fig. 1). This wants to be the maximum level of bacteria present by the Use By Date (T_{UB}).

We can measure this in shelf-determination tests and we can then say, 'providing this product has the level of bacteria at the outset that was present on the test product at the outset and providing it is subjected through its life to the same temperature profile then T_{UB} is a reasonable Use By Date to allocate to that product'.

Now let us look at this bacterial growth curve to substantiate what has just been said. In Fig. 2 we have a product with a higher initial bacterial load and its bacterial growth curve (II).

We can see that this bacterial curve bisects the N_{UB} line sooner and so TII_{UB} occurs sooner than TI_{UB}. This product (II) has a shorter shelf-life than the product depicted in graph I.

Conversely, a product with a lower bacterial load (III) has a longer shelf-life ($TIII_{UB}$). So, shelf-life is inversely proportional to the initial bacterial load.

In other words, the higher the initial bacterial load the shorter the product's shelf-life or the lower the initial bacterial load the longer the shelf-life.

Now let us turn to Fig. 3, which shows the effect of temperature on bacterial dynamics. In this graph we have the original graph (I) and the effects of higher (III) and lower (IV) temperatures.

When temperature is raised we move the graph to the left (A) because bacteria are growing and multiplying faster.

When temperature is lowered we move the graph to the right (B) because bacteria are growing and multiplying more slowly.

As a consequence we can see that storing meat at a higher temperature reduces shelf-life/Use By Date (TIV_{UB}), whereas storing meat at lower temperatures increases it (TV_{UB}).

The two scenarios we have discussed (initial load and temperature) are, for all practical purposes, additive.

This being the case, the worst scenario for a product is to have an elevated initial bacterial load and to be stored at high temperatures, whereas the converse – a low initial bacterial load plus storage at low temperatures – is the best scenario (see Fig. 4).

This is all summed up in the phrase 'get it clean, keep it clean, get it cool, keep it cool' which epitomises good handling of meats and meat products. ■

Fig. 1. The basic bacterial growth curve.

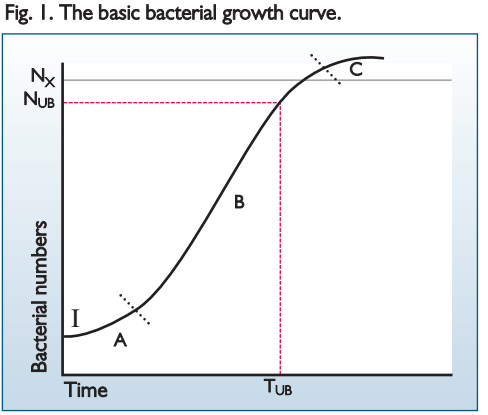


Fig. 2. Initial bacterial load effects.

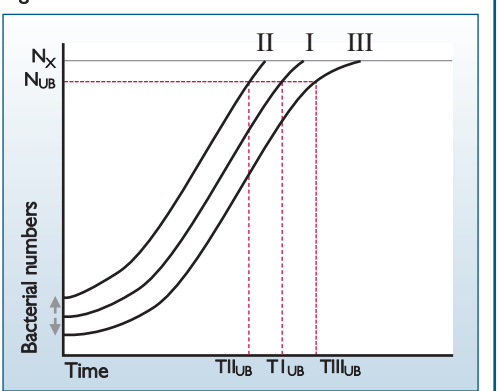


Fig. 3. Temperature effects.

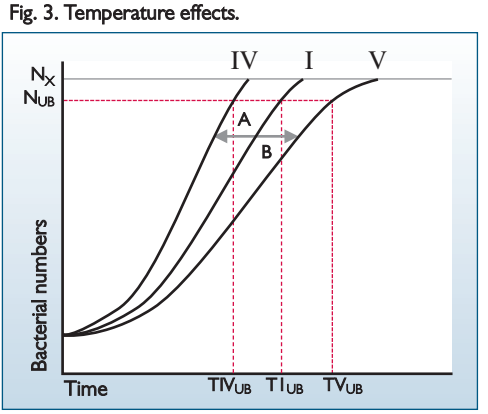
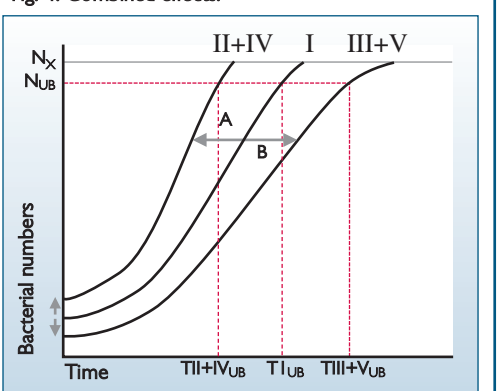


Fig. 4. Combined effects.



3 – E. coli – introduction

Escherichia coli was discovered just over 100 years ago and was named after its discoverer, Dr Theodor Escherich.

In the early 1900s it was appreciated that the presence of E. coli in foods or meats was indicative of faecal contamination and, as such, an indication of a product's hygienic status.

The genus Escherichia was defined in 1964 as Gram negative, non-sporing rods that were often motile with peritrichate flagellae that were easily cultured on laboratory media. They were aerobes that fermented glucose with the formation of acid or acid and gas. They are oxidase negative, catalase positive.

In the mid 1980s Escherichia was positioned in the family Enterobacteriaceae.

In addition to E. coli there are four other species – E. blattae (originally isolated from cockroaches), E vulneris, E. hermannii and E. fergusonii.

It is only in recent years that we have given greater importance to E. coli when certain strains, such as E. coli O157:H7, were associated with serious food poisoning outbreaks that frequently included deaths.

Most strains of E. coli are not pathogenic and the lower gut of mammals, including man, carries large numbers of E. coli.

E. coli infections are transmitted via three routes: directly from animals, person to person spread and from contaminated foods.

The first of these three routes is one of the reasons why in some countries there is concern about children visiting 'town farms'.

All animals used for meat production carry E. coli in their guts and meat can become contaminated by E. coli from faecally contaminated feathers or hides and poor quality evisceration that results in spillage of digestive tract contents. For these reasons increasing importance is being placed on delivering clean animals to the abattoir and maintaining high evisceration standards.

E. coli is commonly found in environments that could have been contaminated by animal or human faeces, such as the soil, surface waters such as rivers and lakes and even subterranean water supplies that have become contaminated by seepage.

Today we test foods for meat and meat products for E. coli for two reasons – firstly, as an indicator of faecal contamination (typically there are far more E. coli per gram of faecal material than there are salmonella) and, secondly, in order to confirm the absence of particularly nasty strains of E. coli such as E. coli O157:H7.

Interestingly, the EU now uses another faecal indicator (enterobacter) as a required test for animal feeds, as above a certain level the probability of salmonella also being present is greatly increased.

Some examples of outbreaks of food poisoning due to E. coli are detailed in the table below.

When it comes to meat products there are very simple interventions that will control E. coli, such as cooking.

However, it should be remembered that for steaks contamination is usually on the outside and so easily controlled by cooking.

Burgers, by their very nature, have bacteria throughout the whole of their substance and so burgers with a red centre (rare or medium rare) present a real food poisoning risk if they are contaminated with E. coli O157:H7 or, for that matter, salmonella as the cooking process will not have effectively got rid of the food poisoning bacteria. ■

Table 1. Some E. coli related food poisoning outbreaks.

Year	Country	Food type
1947	UK	Canned salmon
1982	USA	Hamburger patties
1983	UK	Curried turkey mayonnaise
1985	Canada	Undercooked beef patties
1993	USA	Hamburgers
1995	Australia	Undercooked Mettwurst
1996	Scotland	Meat products
2007	USA	Steaks
2010	USA	Bison meat

4 – Diseases caused by E. coli

E coli is important in meat products for two reasons. Firstly, it is an indicator of faecal contamination and, secondly, certain strains of E. coli cause food poisoning.

This MeatMicrobiology will focus on the latter.

The importance of E. coli as a cause of human food poisoning has come to the fore in recent years as cases of food poisoning caused by E. coli O157:H7 have increased.

These were typified by the food poisoning outbreak in western Scotland in which cross contamination of cooked products occurred in a butcher's shop and these products then infected several hundred people and deaths ensued.

E. coli associated with disease in man can be classified according to the problem they cause:

Enteropathogenic E. coli (EPEC): These are responsible for many cases of diarrhoea, especially in infants, and are typified by watery stools which may contain mucus but not blood.

Symptoms appear within 12-36 hours of infection and can last for some time. Certain strains of EPEC may result in a persistent life threatening diarrhoea.

Enterotoxigenic E. coli (ETEC): The symptoms of diseases caused by ETECs reflect the toxin(s) produced. ETEC infections are characterised by a sudden explosive onset and a diarrhoea without mucus or blood. Abdominal pain and vomiting may also be seen.

Enteroinvasive E. coli (EICE): The symptoms in this instance are those of bloody, mucoid stools and tenesmus (rectal straining), fever and colitis are usually present.

Enterohaemorrhagic E. coli (EHEC): This group contains the notorious E. coli O157:H7.

EHECs produce two types of illness – haemorrhagic colitis and haemolytic uraemic syndrome.

- Incubation time for haemorrhagic colitis is typically three to four days but can be longer. The onset of symptoms is characterised by a sudden severe pain followed by watery diarrhoea. Vomiting and abdominal distension are seen. After the onset of the disease, which can be quite painful, bloody diarrhoea then appears a day or two later. Deaths occur and in some outbreaks this has been as high as a third of cases.
- Haemolytic uraemic syndrome (HUS) starts with diarrhoea and then progresses into a syndrome characterised by haemolytic anaemia, thrombocytopenia and acute renal failure. Needless to say, the latter of these three is serious and results in death.

Sometimes thrombocytopenic purpura occurs as an extension to HUS and its signs include fever and nervous signs.

Strains that produce HUS typically produce Shiga-like toxin II. Bloody stools and a high body temperature can virtually be considered predictive of the development of HUS.

- Other rarer and less serious complications of EHEC infections have been encountered and these include haemorrhagic cystitis and balanitis, convulsions, sepsis due to a concurrent infection and anaemia.

Persons at particular risk from E. coli infections include the young, the old, the immunosuppressed and those with concurrent infections.

It would appear that for EHEC strains those at the extreme ages (<5 years old and geriatrics) are the most susceptible to infection. HUS is commonest in <16 year olds and the elderly and rare in patients aged 17 to 61 years of age.

With the exception of EHEC strains, the prevalence of pathogenic E. coli is greater in countries with poor hygiene standards. Cattle are an important reservoir of EHECs and products such as burgers, in which the EHEC can be mixed into the middle of the product, present a particular risk, especially if eaten rare or medium rare – with raw centres. A general association exists between EHEC infections and improper cooking and handling of foods.

However, it should be noted that not all EHEC infections in man are associated with the consumption of meat. Cases have also been seen in staff harvesting potatoes, children visiting city farms, where animals are petted, and in bathers who were swimming downstream from a herd of cows that had stood in the river on hot days!

5 – E. coli in Hamburg

In the last issue of International Meat Topics we highlighted the different diseases caused in man by food poisoning E. coli. Since then one of the worst outbreaks of food poisoning in man caused by E. coli has occurred in Hamburg, Germany.

Fortunately, it would appear that meat is not involved and to date both Spanish cucumbers and German grown bean sprouts have had the finger pointed at them.

In the case of the former the Germans are now saying that Spanish cucumbers were not involved and needless to say this has caused some heated political arguments with the Spanish wanting compensation for the unwarranted damage to their salad/ vegetable industry.

Let's look at the German situation and see what lessons can be learned from it.

The cause of the food poisoning outbreak is E. coli O104 and the first person hospitalised in association with infection by this organism was on 13th May 2011, which was a few days after the Hamburg Harbour Festival which was attended by 1.5 million people.

By the 18th of May the first person was suffering from HUS (haemolytic uraemic syndrome – See Meat Microbiology No.4 in our last issue of International Meat Topics) and a day later eight had been hospitalised with HUS. By the next day – 20th May – the number of HUS cases had risen to 276!

On 21st May the first death occurred. By 7th June 2,270 people had been infected, there had been 660 HUS cases and 22 people had died.

By this time cases were occurring in many countries but could be linked back to Germany. These were Germans who had recently come to those countries or citizens of the country who had recently returned from Germany. At least one death, in Sweden, had occurred outside Germany.

As we go to press the cause of the problem remains a bit of a mystery because although epidemiologically the finger pointed quite strongly at bean shoots microbiological tests have failed to isolate E. coli O104 from samples taken.

Was this because the problem had already passed through the bean operation and sampling was too late, or was it a case of not enough or the wrong samples being taken?

The other dilemma faced by the authorities, which has been highlighted by this episode, is what to say publically and when!

If you wait until everything associated with an outbreak is fully proven, then people could well become infected, and even die. These may be people who would not have suffered such a fate had information been made public earlier.

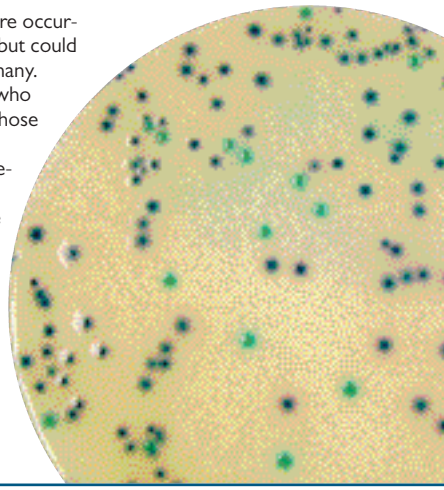
Conversely, if the authorities go public too early they can get it wrong, as unfortunately appears to be the case in this instant, when the blame was placed on Spanish cucumbers.

There is also the impact of public pronouncements on food industries and the public purse to consider. The Spanish claim that many of their farmers are now facing hard times, which some of them will not come through.

At the level of the public purse the EU has already promised significant funds to help adversely affected farmers and processors.

It would not be a surprise if eventually this becomes a figure in excess of €500 million!

It is time to reflect and consider what we need to do so that, should this situation ever hit the meat sector, we will be better equipped to handle it. ■



MEAT MICROBIOLOGY

6 – Sources of bacteria

If we know where bacteria come from we can do something to stop them entering our facility!

The first route into the abattoir is with the live animal. Faecal material contains large numbers of bacteria and these can include bacteria that cause food poisoning such as salmonella, campylobacter and E. coli O157:H7. Secondly, the animal's coat or feathers can carry a significant burden which will obviously be significantly elevated if it is faecally soiled.

Removing the feed from animals some time before they start their journey to the abattoir reduces the desire to defecate.

In addition, in some parts of the world there is a requirement to send clean animals to the abattoir. This can necessitate washing animals before shipping them to the abattoir. In this context, it is important to only bring healthy animals to the abattoir as animals that are scouring or have diarrhoea represent a real microbiological risk.

The importance of clean, well ventilated and well drained lairages can not be overemphasised.

The intestinal contents, which ultimately become the faeces, have a heavy bacterial burden so one of the key goals during evisceration must be to remove these intact and without seepages. In other words, the intestines can be used to provide a 'bag' in which to isolate and contain the intestinal contents and their bacterial load.

In poultry this means removing the digestive tract without damaging it. In larger four legged animals sometimes the digestive tract is tied off or a rectal bung is used. Other parts of the body, such as the upper respiratory tract can harbour undesirable bacteria and the sooner these are separated from the remainder of the carcase the better.

Diseased animals often carry elevated bacterial loads. For this reason it is important to have a competent ante mortem inspection system so that sick and infected animals do not get into the abattoir.

Where inspection occurs on line, for example with poultry, it is important that diseased carcases are rejected and taken off the line before evisceration. Where diseased parts, for example legs and wings, are removed it is important that healthy tissue proximal to the lesion is cut through and that contamination does not spread from a cut abscess or septic joint.

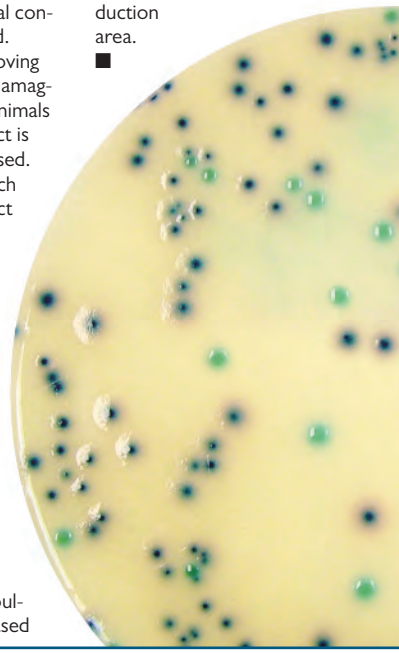
Other sources include airborne contamination. In this context it is important in hot dry weather to have air cooling units and not to rely on draughts through open doors or windows.

Water can bring bacteria into the plant and, as this is used to wash carcases and rinse down product contact surfaces, its bacterial status is critical. We need to know its standards are satisfactory from routine testing but we also need to treat the water with a process like chlorination.

People are another source of bacteria. Personal hygiene standards are important, as is mandatory hand washing after visiting the toilet.

Staff with diarrhoea should not be allowed in production areas and if we are talking high care areas such as those post-cooking it is prudent to screen such people to ensure they are, at least, salmonella free.

In essence you should consider everything entering your facility and satisfy yourself that it is not a vehicle (fomite) for bringing bacteria into your production area. ■



7 – Salmonella: an introduction

Salmonella are Gram negative rods belonging to the Enterobacteriaceae family and they are rod shaped, oxidase negative and catalase positive bacteria that metabolise carbohydrates. They can cause disease in man and animals.

They are named after Dr Salmon who was one of the early scientists associated with this particular bacterium.

Classifying salmonella

One simple way to classify salmonella is as follows:

- Those which only cause specific diseases in man such as Salmonella typhi, S. paratyphi and S. sendai.
- Those which cause disease only in animals such as S. choleraesuis, S. pullorum, S. gallinarum and S. abortovi.
- Those which cause disease in both such as S. typhimurium and S. enteritidis.
- Those which cause food poisoning in man or are apathogenic.

This last category contains the vast majority of salmonellas.

Each individual salmonella is known as a serotype and there are over 2,000 serotypes with, probably, no more than 100 associated with human food poisoning.

Then some serotypes are further divided into phage types or definitive types and good examples of these are S. enteritidis PT4 and S. typhimurium DT104.

For a salmonella to be particularly associated with food poisoning certain things have to occur:

- It needs to be present in a food type(s) with some degree of regularity.
- It needs to avoid salmonella lethal process(es) during the production of that human foodstuff.
- It needs to be pathogenic to man and have some degree of tissue invasiveness.

This is very aptly demonstrated with S. enteritidis that became widespread in table egg laying flocks.

Eggs are eaten semi-raw as 'soft boiled eggs' or are used in products which are not cooked, such as mayonnaise, and S. enteritidis is pathogenic in man.

Likewise, S. typhimurium is found in cattle and pigs; it survives in burgers which are not cooked properly and can be pathogenic in man. The likelihood of infection and pathogenicity in man is influenced by various host factors.

For example, the very young and very old are more prone to infection, as are the immunosuppressed (often as a consequence of anti-cancer therapies) or those with concomitant diseases or infections.

In food poisoning cases in man the source invariably ultimately traces back to animal faeces.

Good examples are poor evisceration of poultry carcases, soiled eggs and vegetables/salad crops from fields which have received slurry applications.

Consequently, meat often, and sometimes unfairly, comes under the spotlight in the investigation of outbreaks of human food poisoning caused by salmonella.

Salmonella present the laboratory with an interesting challenge as they are invariably present in material to be tested in very low numbers but that material contains many more bacteria of other types. Salmonella is the microbiologist's 'needle in a haystack'.

This is overcome by using a three stage isolation technique.

In the first stage (enrichment) the sample is incubated in an enrichment broth.

This basically strengthens the salmonella bacteria so they are better able to withstand the second stage which is selective enrichment. In this the material is incubated in a selective enrichment broth which favours salmonella growth but impedes the growth of other bacteria or actually kills them off.

When we get to the third stage (selective culturing) the proportion of salmonella in the sample is greatly increased and can be 100%.

Samples of the incubated enrichment broth are then streaked on to a selective agar.

This also favours salmonella growth at the expense of the growth of other bacteria. Selective enrichment agars are also designed so that colonies appear as a distinctive colour (for example, black on XLD Agar).

Colonies of suspect salmonella are then removed from this selective agar and subjected to various tests to confirm that they really are salmonella. ■

8 – Salmonella: food poisoning

When disease occurs in man as a consequence of consuming a food contaminated with a food poisoning micro-organism, such as salmonella, it is known as food poisoning.

For food poisoning to occur the food poisoning micro-organism has to get into man in adequate numbers so that it can overcome man's natural defences and cause the food poisoning.

Various factors related to the infecting micro-organism and the victim dictate whether or not the infection is likely to succeed.

There are several salmonella related factors, such as the serotype involved, its pathogenicity towards man, the dose of infection (number of salmonella organisms invading the victim) and whether or not the salmonella strain involved has antibiotic resistance.

Host (victim) factors include whether the immune system is compromised (HIV infection or treatment with immunosuppressive anti-cancer drugs) or failing (old age), whether defence mechanisms are established (there is no stomach acid for the first few days of life) and whether any concurrent illness is present.

Salmonella in fat globules

An interesting situation was seen some years ago with Salmonella napoli in Italian soft chocolate. When the number of S. napoli organisms per gram of chocolate was calculated they were so low that people would have succumbed to chocolate poisoning before they got salmonella food poisoning! However, cases of salmonella poisoning were still being encountered.

What was happening was that the S. napoli was being encapsulated in micro-globules of fat in the chocolate and this was protecting them from stomach acid!

Normally the stomach acid wipes out a large proportion of the salmonella but this was not occurring in this instance.

In this case all the salmonella were reaching the small intestines where the action of lipases and emulsification by bile salts on the fat globules liberated the S. napoli which was then able to cause the food poisoning.

Another interesting salmonella outbreak occurred in businessmen in America – they were overdosing themselves with antacids which did what they were meant to do, but in excess!

The outcome was neutralisation of gastric acid and an unhindered passage of salmonella through the stomach.

Safe food preparation

Preparation of food can be critical and of greatest danger is undercooking, for example, rare or medium-rare burgers!

Another factor is cross contamination, for example in the household refrigerator. A good example of this is juices from a salmonella contaminated chicken (that has been placed on the top shelf) dripping on to salad or cheesecake (neither of which will be cooked prior to being eaten) on the shelf below.

Much could be done to improve the situation if we could better educate the general public on how to manage food in their homes.

Factors such as prolonged storage of contaminated foods, which can result in a population of salmonella equivalent to or exceeding the infective dose developing, are important.

This can occur more quickly if the storage temperature of the refrigerator is too high. Surveys over the years have shown an alarmingly high proportion of domestic refrigerators to be running at too high a temperature!

However, salmonella infection need not come from food. One American study looked at salmonella infection in very young children and found a very poor correlation between salmonella serotypes from the children and those isolated from foods in the families' refrigerators.

A much better correlation occurred between isolates from the children and isolates from hoover dust bags or from the soil in the backyard.

This puzzled scientists but when you think of what young (crawling) children do and what goes in their mouths, the results are not that surprising! ■