

Microbial update

listeria

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In 1926, Murray, Webb and Swarm published a paper in the Journal of Pathological Bacteriology entitled 'A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes*'. Later in the 1930s South African microbiologists isolated a bacterium from the livers of sick gerbils which was named *Liserella hepatolytica*, but it was not until 1940 that these two micro-organisms were recognised as the same, and renamed *Listeria monocytogenes*.

With such innocuous origins starts the history of *Listeria monocytogenes*, but it was to be many years before its importance in foods was recognised.

Infections due to *L. monocytogenes* have been recognised in a wide variety of animals, including cattle, sheep, birds, rodents and fish. Contaminated animal feeds such as silage was a recognised source, but the existence of a human illness was not fully appreciated until the 1980s.

Then in 1981 began a series of food related outbreaks directly linked to *listeria monocytogenes*, involving coleslaw, raw vegetables, milk, and soft cheese. The remarkable thing about these outbreaks was the very high number of deaths, which was as high as 30% of those affected. This began the food industry's battle with *listeria*, an organism that in real terms is not particularly resistant to heat or sanitisers, and in most situations will compete very poorly with other more innocuous micro-organisms.

Put that organism in cold, damp environments, however, and not only will it survive well, but it is able to grow at temperatures much lower than most other bacteria.

What are listeria?

The genus *Listeria* comprises six true species (*L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri* and *L. grayi*). A previous species called *L. murrayi* is now considered to be the same as *L. grayi*. Out of all of these species, only *L. monocytogenes* is considered to be a human pathogen.

Microbiologically, *listeria* are short rod



shaped micro-organisms, they are motile and can grow in the presence or absence of oxygen.

They are, however, 'psychrotrophic' being one of the very few pathogens able to grow at 'good' refrigeration temperatures.

Illness

An infection by *listeria* is called listeriosis, an illness that has many forms. One of the main forms affects mothers and their unborn or newly born children. In such cases, the symptoms suffered by the mother can be mild, with a slight fever and mild gastroenteritis.

For the child, however, the outcome can be very serious and often fatal. In non-pregnancy associated cases, those affected can suffer bacteraemia (viable organisms in the blood) or meningitis (swelling of the membranes surrounding the brain).

In the past, the main concern with listeriosis has centred on its effects on pregnant women and unborn or very young children, and most *listeria* cases involved this group. More recently however, there have been an increasing number of cases in the over 60 year old age group.

This phenomenon has been noted in a number of European countries, and at present there is no understanding behind the reasons for this.

Over the last two years, however, figures from the UK Health Protection Agency have indicated that the numbers of cases of illness

caused by *listeria* in the UK has been slightly decreasing, by 13% in 2010 and a further 7.5% in 2011. It is hoped that this trend continues in years to come.

The annual number of cases of reported listeriosis tends to be low when compared to cases of food poisoning caused by campylobacter, or salmonella.

The main concern with *listeria* is the much higher death rate in those that contract the illness, which as noted previously, can be as high as 30% of those affected.

Growth requirements

Unlike many traditionally recognised food-borne pathogens, *L. monocytogenes* is psychrotrophic and can even grow slowly at 0°C. As a result of this, refrigeration alone cannot be relied upon to prevent growth. Although growth may still occur, lower temperatures will result in slower growth and may interact with other factors to prevent growth. Growth will not occur at temperatures less than -1°C, but the bacterium will survive and is generally considered resistant to freezing and has been isolated from a variety of frozen foods.

The optimum temperature for growth is generally 30-35°C and the maximum temperature for growth is 44°C.

The minimum pH for growth is approximately 4.3 but growth is restricted below pH 5.0 at refrigeration temperatures.

The presence of organic acids will raise the

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minimum pH for growth compared with the inorganic acids used in many laboratory studies.

L. monocytogenes is resistant to salt with all strains capable of growth in 10% salt ($a_w = 0.94$). The minimum a_w for growth is lower when sucrose is used as a humectant (0.92). When present in dry foods (for example, milk powder) the bacterium can survive for many weeks.

The heat resistance of *L. monocytogenes* has been studied in both broth and food systems. It is now generally accepted that High Temperature Short Time (HTST) milk pasteurisation (71.7°C for 15 seconds) will eliminate this bacterium from liquid milk.

With other foods, heating to 70°C for two minutes, or the thermal equivalent, is recommended for the thermal destruction of this bacterium.

Generally, modified atmospheres have little effect on listeria as the organism can grow in the absence of oxygen. If however a very high level of carbon dioxide were used (>60%), then growth can be limited.

The food environment

The interest in and importance of *L. monocytogenes* has prompted numerous studies on its incidence in foods. Listeria originates from the environment and can be com-

monly found in soils and untreated water.

This will of course mean that animals and plants destined for human consumption may well be contaminated with listeria, leaving the food producer to develop ways in which this contamination can be removed, this being particularly important in any food considered 'ready to eat'.

With animal derived foods, normal cooking is enough to destroy the organism, the main control that has to be employed is the correct cooking time and temperature, followed by hygienic methods that prevent recontamination of the cooked material. Some animal derived foods are not cooked, for example cold smoked fish; here good hygienic procedures are the only way forward.

It is well known that listeria can be a problem in some dairy foods. Raw milk can be contaminated with the organism, and there can be a risk that products made from raw milk can contain viable listeria.

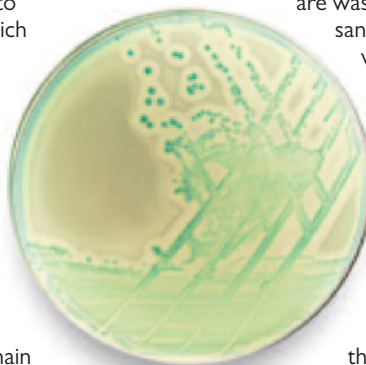
Plant based material such as vegetables, can be contaminated in the field. In vegetables items that are going to be cooked

before eating, this is not an issue; however salad vegetables are eaten raw and could be a source of a problem.

Commercial ready to eat salad products are washed before packing, often using a sanitiser in the washing water. This will remove soils and can kill contaminating micro-organisms and may be a control for low levels of contaminating listeria.

A serious listeria outbreak that occurred in the USA in 2011 was traced to contaminated cantaloupe melons. This outbreak affected around 146 people and resulted in 30 deaths.

It was traced to poor practices in the site preparing and cutting the melons, allowing listeria to proliferate and cross contaminate incoming fruits.



Wide variety of products

A wide variety of products have been implicated in outbreaks of listeriosis including milk, soft cheeses, butter, cooked ready to eat meats, cold smoked fish, pate, raw vegetables and prepared sandwiches. In 2012 in the USA over one million hard boiled eggs were recalled due to positive listeria test results indicating the risks can be found in almost all chilled food products.

Much of the concern with listeria in food

production comes from its ability to inhabit and perhaps even 'colonise' the food production environment itself. If we remember that the natural niche for listeria is the 'outside environment' of soils and waters, then the environmental conditions of cold and moisture found in many chilled food production factories are very similar.

These are the conditions that favour listeria over many other organisms. It is able to grow at low temperature, it favours damp and wet conditions, and it can be extremely hard to eradicate from such areas. It is not particularly heat resistant, nor is it resistant to commonly used cleaning chemicals, so it is controllable.

However, it must always be remembered that unlike many enteric bacteria that are unable to grow in the cold environment of the chilled food production site, listeria can.

So, even if only very low numbers are left behind after cleaning, these will slowly increase, and unless cleaning is constant and frequent, they will reach levels that put a product in danger of contamination.

The main controls within a chilled food production environment are well designed hygiene measures, including constant, frequent cleaning, combined with the use where possible of a correct cooking process. But constant care is required. It is known that listeria can develop and survive in wall/ ceiling insulation, so any engineering/building work must be very carefully monitored and controlled, equipment that is not hygienically designed and built may include 'nooks and crannies' that harbour contamination and may be difficult to clean.

Condensation build up on drip trays and overheads can be a reservoir of contamination that can drip onto unprotected product below. Tools used by cleaners or engineers can become contaminated, and then cross contaminate other areas if they are used throughout a production site.

Well targeted cleaning and disinfection will remove listeria from production areas, but this organism has a tendency to be able to survive in difficult to clean 'nooks and crannies' making eradication very difficult.

Instances of widespread listeria contamination, have been seen on many occasions following building and renovation work within production areas.



Any building/renovation work within a chilled high care area should be considered a potential listeria risk, as the organism can be easily dislodged from insulating materials and cladding creating a widespread contamination within a site.

As well as using standard disinfectants, there has been much consideration of the use of specific anti-listeria bacteriophage to control the organism either on foods or in the food production environment.

Bacteriophage (also known as phage) are viruses that attack and kill bacteria, and they are very specific to their host cell (they will only attach to and kill the host).

There are now commercially available anti-listeria phage preparations that are designed to be used to specifically kill listeria in food production areas. Indeed, earlier in 2012 the European Food Safety Authority published a favourable opinion on one particular type of anti-listeria phage, but we are still awaiting a decision from the EU on the legal position of using phage.

Other countries such as the USA, Australia and New Zealand are looking quite favourably on phage use, but potential users should consult the legal positions in their own country before considering using these agents.

Microbiological standards

All over the world, food safety bodies have developed guidance and standards for listeria in foods. These will usually apply to foods considered 'ready to eat' (RTE) and take into account levels of listeria considered to be a risk to consumers.

Universally, standards and guidelines developed by regulatory bodies will consider only *L. monocytogenes*. In the United States, the detection of *L. monocytogenes* in an RTE product (usually presence in not less than 25g) will require a product to

either not be placed on the market or be recalled.

In Europe, Commission Regulation 2073 has a specific section on *L. monocytogenes* and for certain types of food (for example that destined for infants or for special medical purposes) requires an absence in 25g.

For other foods, a limit of less than 100 *L. monocytogenes*/g at the end of shelf life is considered acceptable, whilst foods in which *L. monocytogenes* can grow should have no detectable *L. monocytogenes* at the point of production.

This latter approach is also the one being considered by Codex at present. In spite of these acceptable levels, most producers of RTE food products will work to an internal specification of absence of *L. monocytogenes* within their product.

Additionally, the Regulation requires food producers to monitor the production environment for listeria presence, and currently (2012) guidance is being formulated within the EU to give advice on how best to do environmental testing for listeria.

Conclusions

Listeria are an interesting group of organisms. They have emerged as a problem in foods relatively recently and our understanding of them has increased considerably, but we are still lacking so much information.

Why, for example, is there a current increasing trend in listeriosis in the over 60 year olds? Why has the previous increasing trend in numbers of cases started to decline in the last two years?

They are an unusual organism amongst the many that food microbiologists study in that they grow at chill temperatures and appear to be able to successfully colonise food production environments. However, they are also not particularly resistant to heat or sanitisers, so can be controlled with good hygiene measures within a production area.

Most chilled food producers now do extensive environmental testing for listeria, backed up with a full programme of end product testing.

This will allow a fast detection of factory contamination that can be tackled by well targeted hygiene measures. ■

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