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25TH ANNIVERSARY ISSUE

OXOID

Culture

Introduction to 25th anniversary issue

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After 25 years of publication of *Culture*, and as we enter the 21st century, the five contributions to this celebratory issue address some of the key areas and challenges of modern food microbiology.

First, the last two decades have seen the slow but accelerating introduction of some exciting new technologies for food preservation, e.g. high hydrostatic pressure; high voltage electric discharge; high intensity laser and non-coherent pulsed light. Most of these technologies have the advantage that they inactivate micro-organisms rather than inhibit them, which is much to be preferred where pathogens are concerned. The first products to be processed by these new technologies were 'fail-safe' with respect to pathogens, because they had low pH values (e.g. fruit juices and fruit conserves). However, new near-neutral pH products are now being developed and produced commercially and for these processed packs potential pathogen hazards must be taken seriously. A major food poisoning incident related to a new technology could set back development by many years. Margaret Patterson's paper surveys progress in what is currently the most successful of the new 'non-thermal' technologies, high pressure processing, pointing out the substantial food quality advantages for consumers, but also highlighting the sometimes unexpected behaviour of food poisoning micro-organisms subjected to pressure; this exceptional behaviour must be kept in mind as the techniques are more widely exploited.

Second, while food poisoning remains a topic of real public concern it should not be forgotten that there have been some substantial successes, in particular the recent reduction of salmonellosis following the introduction of poultry vaccination programmes. On the other hand, the reasons for some large outbreaks due to other pathogens, e.g. enteropathogenic strains of *Escherichia coli*, remain difficult to explain, and the large numbers of campylobacter infections, though less dangerous, may not be easy to reduce. Tom Humphrey's contribution about the campylobacter problem illustrates why improved control, which is being specially targeted just now, is going to present difficulties. A more complex integrated attack is required if success is to be achieved.

Third, not all food poisoning micro-organisms are of UK origin. Enormous tonnages of foods travel from country to country, and will increase in parallel with the expected increase in globalisation and consequent international trade. Extensive testing of such large volumes of materials is out of the question. Safety can only be maintained by the application of tight controls, backed up by selective microbiological testing in suspect cases and when high risks are indicated. Sandra Westacott surveys the nature and operation of national and international agreements and controls, and sometimes their deficiencies as well, with impressive (and sometimes frightening) examples of potential consequences when things go wrong.

Fourth, it is often said that predictive microbiology, or 'modelling', originated nearly 100 years ago with the development of thermal processing guidelines for food sterilisation, derived from the assumed log-linear destruction of *Clostridium botulinum* spores by heat. Over the last few decades, however, the development of better modelling techniques, particularly for microbial growth rather than death, has been the target of many research groups. After many (sometimes heated!) discussions about preferred mathematical models and modelling techniques, and after some false starts of national and international modelling systems and collaborations, this area has perhaps 'come of age'. Two of the key players in the development and promotion of modern modelling techniques and information bases, Joszef Baranyi and Terry Roberts, survey the up to date situation, illustrating the practical value of current easily available and easy to use models bases.

Fifth, methods for the detection, identification, and enumeration of micro-organisms are going through an interesting phase. It had been suggested that the increased use of methodologies such as HACCP and

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modern risk assessment techniques in recent years would steadily reduce the need for microbiological testing of food products and raw materials. However, increased public awareness and concern about food safety has had the effect of encouraging more attention to microbiological methods and, in particular, the development of improved analytical techniques that are the focus of the contribution of Roy Betts. These include the provision of more discriminatory cultural techniques, easy to store/easy to use kits, immunological and nucleic acid-based rapid methodologies, in a trend that is likely to continue.

Overall, therefore, it seems that rather than being 'old science' about which, one might have guessed 25 years ago, most facts of relevance would by now be well established, food microbiology is vibrant, with many new challenges ahead of it. They include exciting new technologies, with great potential, but also with attendant risks that must be addressed. There are challenges that still have to be overcome in the control of even the most common food poisoning micro-organisms, with new threats, and even with the possibility of new 'exotic pathogens' from 'new exotic foods'. Also, user-friendly advanced models of microbial growth, survival, and inactivation, to facilitate better control of food poisoning and spoilage micro-organisms in foods. New cultural and molecular high tech methodologies for rapid discriminatory microbiological analysis will also play an important role.

Altogether, food microbiology remains an exciting science with a great future.

Grahame W Gould
Guest editor

Under pressure: A novel technology to kill micro-organisms in foods

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Introduction

Consumers in the 21st century demand high quality foods that are free from additives, fresh tasting, microbiologically safe and with an extended shelf-life. One food technology that has the potential to meet these demands is high pressure processing (HPP). HPP, also known as high hydrostatic pressure or ultra-high pressure processing, uses pressures up to 900 MegaPascals: MPa (= 9,000 atmospheres = 135,000 pounds per square inch) to kill many of the micro-organisms found in foods, even at room temperature. These pressures are immense. A mid-range food processing pressure of 600 MPa is equivalent to a stack of 5 family cars bearing down on a postage stamp or 3 elephants on a strawberry! In general, vitamins, flavour and colour molecules are not significantly affected. The conformation of other molecules, such as proteins (including enzymes), may be altered. This can be beneficial or detrimental. For example, enzyme activity may be enhanced, reduced or unaffected depending on the protein structure and the pressure applied.

The idea of using high pressure in food processing is not new. Bert Hite (1899)[1] published the first report of using high pressure as a food preservation method. He reported that milk "kept sweet for longer" after a pressure treatment of ~650 MegaPascals (MPa) for 10 minutes at room temperature. Hite (1914)[2] also reported that while pressure could be used to extend the shelf-life of fruits, it was less successful with vegetables. He concluded that fruits and fruit juices responded well to high pressure because the "yeasts and other organisms having most to do with decomposition are very susceptible to pressure, while other organisms not so susceptible do not long survive the acid media". Vegetables, however, he "abandoned as hopeless" due to the presence of spore-forming bacteria which survived the pressure treatment and could grow in the low acid environment. The problem of pressure resistant spores still remains one of the challenges for the technology today.

The high pressure process

Although the idea of pressure treating foods is over 100 years old, it is only in the last 25 years that it has become a commercial reality. There are considerable engineering problems involved in repeatedly generating and containing the immense pressures in a vessel suitable for food products. However, by the 1980s a range of specialist high-pressure vessels, based on those used routinely in the production of polymers, ceramics and artificial diamonds, became available and reopened the possibility of commercial production of pressure-treated foods.

A typical pressure treatment system consists of a pressure vessel, the pressure transmission fluid (usually water) and one or more pumps to generate the pressure. It is traditionally a batch process and pressure vessels used for commercial food production have capacities of 35L up to 350L.

Food packages are loaded into the vessel, the top is closed and the pressure transmission fluid is pumped into the vessel from the bottom. Once the desired pressure is reached, pumping is stopped, valves are closed and the pressure can be maintained without further need for energy input. The pressure is transmitted rapidly and uniformly throughout the pressure fluid and the food. It is equal from all sides so there is no "squashing" effect and product shape is not significantly affected. The pressure is released after the desired treatment time and the food packages can be unloaded. In the case of liquids, such as fruit juices, the whole vessel can be filled with the juice, which itself becomes the pressure transmission fluid. After treatment, the juice can be transferred to an aseptic filling line, similar to that used for UHT liquids. A series of these vessels can work in sequence, with a vessel filling with juice, a vessel pressurising and another emptying, all operating simultaneously, so the overall system can become semi-continuous.

High-pressure equipment suitable for food use is specialised and the capital equipment cost is relatively high, although running costs are relatively low. Typically a commercial vessel can cost £250,000 to over £1 million, depending on its size. It is likely that these costs would reduce

if the use of the technology grows and more vessels are sold.

Pressure-treated foods in the marketplace

The first pressure-treated foods have been on the market in Japan since the 1980s. These are mostly acidic products such as fruit juices, sauces and jams. The pressure treatment kills many of the spoilage organisms, thus extending shelf-life, whilst retaining the colour, flavour and vitamin content of the fresh food. Not all micro-organisms are killed and the products have to be refrigerated in order to maximise shelf-life to around 30 days. Pressure-treated fruit juices are also available in France and in a limited number of UK supermarkets. Pressure-treated fruit smoothies (fruit and yoghurt drinks) are also available in the UK. Pressure-treated, vacuum-packed sliced ham is available in Spain and the USA. The pressure treatment is used as an extra safety assurance against pathogens and further extends the shelf-life of the ham.

Today the biggest growth area for pressure-treated foods is the USA. A wide range of pressure-treated foods are being developed and a number are already in the marketplace (*Figure 1*.).

The first product, guacamole, has been available since the early 1990s. The fresh taste and colour of the product, compared to frozen or heat-treated alternatives, has been a tremendous success with consumers. The company is now launching a new portfolio of pressure-treated products, including salsas, vegetables and cooked meats, including a complete chicken or beef fajita "meal kit" where all the components are pressure treated except the flour tortillas.

Pressure treatment of oysters is another example of a commercial success (*Figure 2*). The treatment extends the shelf-life of the product and retains the sensory characteristics of the fresh oyster. In addition, pressure kills *Vibrio parahaemolyticus* and *V. vulnificus*, and also loosens the adductor muscle. Loosening of the adductor muscle means the shell will open of its own accord. Therefore, before pressure treatment a heat-shrink plastic band is placed around each oyster to keep the shell closed. When required, the band can be removed and the oyster shell opens easily and the meat is shucked cleanly. This "self-shucking" property is regarded as an even greater commercial advantage than improved microbiological safety and shelf-life. The company producing these pressure-treated oysters has received several national awards for product innovation and new product quality.

Microbiology of pressure-treated foods

Microbial inactivation studies have shown, not unexpectedly, that many factors can influence pressure resistance. A summary of these findings is given below.

Figure 1. Selection of commercially available pressure-treated foods: cooked ham, orange juice; guacamole and oysters. Pictures courtesy of Avure Technologies Inc.

Microbial species

1. Vegetative bacteria

The lethal effect of high pressure on vegetative bacteria is thought to be the result of a number of different processes taking place simultaneously, in particular damage to the cell membrane and inactivation of key enzymes including those involved in DNA replication and transcription.

Vegetative bacteria tend to be most sensitive to pressure when treated in the exponential phase of growth and most resistant in the stationary phase of growth. When bacteria enter the stationary phase they can synthesise new proteins that protect the cells against a variety of adverse conditions such as high temperature, high salt concentrations and oxidative stress. It is not known if these proteins can also protect bacteria against high pressure but this may explain the increase in resistance in the stationary phase.

In general, Gram-positive bacteria, especially cocci such as *Staphylococccus aureus*, tend to be more pressure resistant than Gram-negative rods, such as *Vibrio* spp. (*Table 1*). However, there are exceptions to this general rule. For example, certain strains of *Escherichia coli* O157:H7 are relatively resistant to pressure (*Figure 3*) while other strains are relatively sensitive.

The variation in pressure resistance between different species, and even strains of the same

Figure 2. High pressure vessel used to process oysters. Picture courtesy of Avure Technologies Inc.

species, are important considerations when optimising processing conditions. Generally a 6-log kill of the most resistant vegetative pathogen is required to give the lethality equivalent to heat pasteurisation. With a novel technology, such as pressure, one challenge is to find what the most resistant vegetative pathogen is to ensure that an appropriate kill is achieved.

2. Bacterial spores

It was clear, even from the earliest studies at the start of the 20th century, that ungerminated bacterial spores can be extremely pressure resistant and may survive pressure treatments of over 1,000 MPa for more than 1 hr at room temperature. However, detailed work by Gould and Sale (1970)[4] showed that relatively low pressures (below 300 MPa) could trigger spore germination. Pressures as low as 50 MPa could make bacillus spores germinate, although there was considerable strain variation in the optimal pressure level needed to initiate the germination process. This led to the suggestion that a two-stage process, sometimes referred to as pressure cycling, could be used to deal with spores. The first "low pressure" step initiates germination. The second step, at a higher pressure kills the germinated spores. The whole process can be repeated a number of times to increase the level of kill. This approach has been investigated by a number of researchers and it has been found that long process times are needed and it can be difficult to achieve extensive inactivation of spores. Thus, the 2-step process may not be a practical solution for commercial sterilisation. The simultaneous application of heat and pressure, however, looks more promising as a method of producing pressure-treated commercially sterile foods. The treatment conditions used are such that the pressure-induced germination mechanism is by-passed. The initial temperature of the food is usually 90-100°C. However, due to adiabatic heating* the temperature in the food may rise by 3-9°C/100 MPa, depending on the food. It is claimed that the simultaneous application of heat

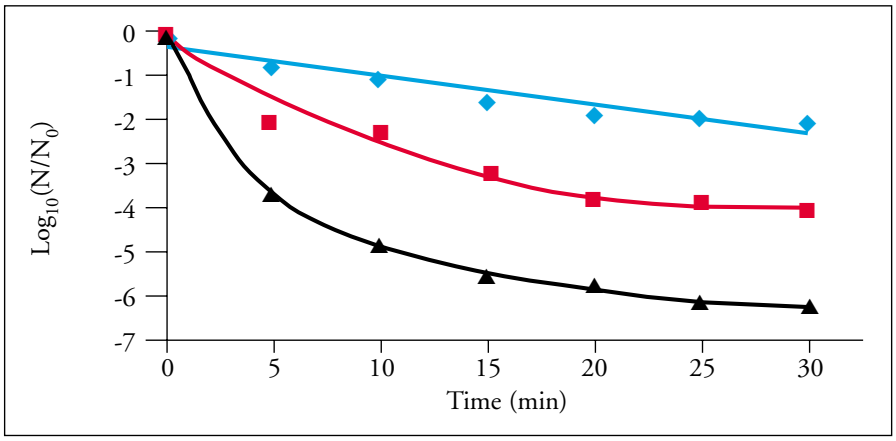

Figure 3. Effect of substrate on the high pressure (700 MPa for 15 min at 20°C) inactivation of *Escherichia coli* O157:H7. N = number of surviving organisms; No = initial number of cells. ◆ UHT milk; ■ Poultry meat; ▲= saline buffer.
This strain is relatively resistant to high pressure. Substrate has a marked effect on the level of inactivation, with greatest survival in milk and least in buffer. The graph also shows the "tailing" effect of survivors in poultry meat and buffer.

Table 1: Sensitivity of micro-organisms to high pressure treatment in various foods.

Micro-organism	Substrate	Treatment conditions	Inactivation (log_{10} units of reduction)
Vegetative bacteria			
Campylobacter jejuni	Poultry meat	300 MPa, 10 min, 25°C	6
Salmonella typhimurium	Pork slurry	300 MPa, 10 min, 25°C	6
Escherichia coli O157:H7	UHT milk	800 MPa, 10 min, 20°C	<2
		700 MPa, 30 min, 20°C	
	Poultry meat		5
Escherichia coli (non-pathogenic	Goat's cheese	400 MPa, 10 min, 25°C	>7
Listeria monocytogenes	UHT milk	340 MPa, 80 min, 23°C	6
Vibrio parahaemolyticus	Canned clam juice	170 MPa, 10 min, 23°C	>5
Spore-forming bacteria			
Bacillus stearothermophilus spores	Not given	800 MPa, 60 min, 60°C	4
		400 MPa, 6 x 5 min, 70°C	4
Clostridium sporogenes spores	Meat broth, pH 7.0	800 MPa, 5 min, 80-90°C	>5
		1500 MPa, 5 min, 20°C	no inactivation
Yeasts and moulds			
Byssochlamys nivea ascospores	Grape juice, aw 0.97	700 MPa, 30 min, 70°C	4
	Bilberry jam, aw 0.84		<1
Saccharomyces cerevisiae	Mandarin juice, pH3.1	100 MPa, 5 min, 47°C	3
Viruses			
HIV-1	Laboratory culture	550 MPa, 10 min, 25°C	Infectivity titre reduced by 4 log units
Poliovirus	Tissue culture medium	450MPa, 5 min, 21°C	No reduction in plaque forming units.
Human rotavirus	Tissue culture medium	300 MPa, 2 min, 25°C	8
Feline calicivirus	Tissue culture medium	275 MPa, 5 min, 21°C	7
Hepatitis A	Tissue culture medium	450 MPa, 5 min, 21°C	7
	Seawater	450 MPa, 5 min, 21°C	2-3

Modified from Patterson (2000)[3]

and pressure is less detrimental to the sensory and nutritional quality of the food compared to conventional retorting. In the last few years a number of patents have been issued for the production of commercially sterile food using high pressure and temperature. Typically pressures of 700 MPa are applied for 15 minutes to products initially heated to 90°C.

3. Viruses

Human Immunodeficiency Viruses (HIV) are reported to be relatively sensitive to pressure with 10^5 or more plaque-forming units (PFU) being destroyed by a treatment of 550 MPa for 10 min at 25°C (*Table 1*). Likewise, Hepatitis A virus, human rotavirus and feline Calicivirus (a surrogate for Norwalk virus) were all relatively pressure sensitive when treated in tissue culture medium. Poliovirus was more resistant and there was no reduction in plaque forming units after a treatment of 450 MPa for 5 min at 21°C.[5] These results would suggest that the pressure treatments necessary to kill vegetative bacterial pathogens would also be sufficient to cause significant inactivation of human virus particles. However, the virus studies were carried out using culture media, rather than a food substrate. More research is needed on the pressure-inactivation of food-borne viruses actually present in foods.

4. Yeasts and moulds

Yeasts are generally not associated with food-borne disease but are important in spoilage, especially in acidic foods. They are relatively sensitive to pressure (*Table 1*) and this is one reason why pressure treatment of fruit products to extend shelf-life is successful.

There is relatively little information on the pressure sensitivity of moulds but it is thought that vegetative forms are relatively sensitive, while ascospores are more resistant. Treatment conditions of 800 MPa at 70°C for 30 min were needed to give a 4-log reduction of *Byssochlamys nivea* ascospores, whereas the vegetative forms only required 300 MPa at 25°C for a few minutes to achieve the same level of inactivation.[6]

Kinetics of microbial inactivation

Increasing the magnitude of the pressure generally increases the lethal effect on micro-organisms. However, sometimes increasing the duration of the treatment does not increase the lethal effect. Thus, killing micro-organisms with high pressure is complex and often does not follow first order kinetics. Plotting the log of surviving numbers against time does not always form a straight-line relationship (*Figure 3*). Often there is an initial linear decrease in numbers followed by a "tail". Studies have shown that when this pressure-resistant "tail" population is isolated, grown and again exposed to pressure, there is no significant difference in pressure resistance between it and the original culture. Such tails are also found with heat processing but this phenomenon seems to be more pronounced with high pressure processing. The tailing effect is not fully understood. It may be due to inherent phenotypic variation in pressure resistance in some cells. Experimental conditions, such as the substrate and growth conditions, may also be a factor.

Substrate

The composition of the foodstuff can significantly affect the response of micro-organisms to pressure and there can be significant differences in the levels of kill achieved with the same organism on different substrates. For example, *E. coli* O157:H7 treated under the same conditions of 700 MPa for 30 min at 20°C resulted in a 6 log reduction in numbers in phosphate-buffered saline, a 4 log reduction in poultry meat and <2 log reduction in UHT milk (*Figure 3*). The reasons for these effects are not clear but it may be that certain food constituents like proteins and carbohydrates can have a protective effect on the bacteria and may even allow damaged cells to recover more readily.

Most micro-organisms are more susceptible to pressure at lower pH values and the survival of pressure-damaged cells is less in acidic environments. This can be of commercial value, such as in the pressure treatment of fruit juices, where in the high acid conditions pathogens such as *E. coli* O157:H7, which may survive the initial pressure treatment will die within a relatively short time during cold storage. Water activity is also important and a value below 0.95 appears to protect micro-organisms from pressure inactivation.

Conclusions and future prospects

Although the concept of using high pressure to kill micro-organisms in foods is not new, it is only in recent years that the technology has been seriously considered as a practical method for preserving foods. There is much ongoing research on the ability of pressure to kill vegetative bacteria but the resistance of bacterial spores still makes the possibility of producing pressure-treated shelf-stable foods a real challenge. Combining pressure with heat is likely to solve this problem. In addition, the fact that high pressure can be used to affect the functional properties of foods is now also being exploited. High-pressure modification of molecules such as food proteins gives the opportunity to produce novel products. For example, pressure can produce the self-shucking oyster, as described above, and this is thought to be more beneficial than improvements in microbiological quality.

The cost of high pressure equipment and the fact that it is essentially a batch process, using relatively small vessels, is currently a deterrent to the technology being more fully exploited on a commercial scale. High pressure, therefore, tends to be considered only when it can give a real advantage over existing technologies, e.g. the superior quality of pressure-treated guacamole. This is likely to be the trend for the foreseeable future.

* Adiabatic heating: The work of compression during HPP treatment will increase the temperature of foods through a process known as adiabatic heating. The extent of the temperature increase varies with the composition of the food but is normally 3- 9°C/100MPa.

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Control of *Campylobacter* spp. in the food chain: a far from simple task

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Introduction

Infection with *Campylobacter* spp., principally *C. jejuni* (*Figure 1.*), is the leading cause of bacterial diarrhoeal disease worldwide and the most common antecedent to the peripheral neuropathies Guillain-Barre Syndrome (GBS) and Miller-Fisher Syndrome (MFS)[1,2]. Campylobacter is a zoonotic pathogen of humans and in England and Wales in 2002 over 56,000 cases were reported. This pathogen accounts for approximately 62% of all cases of bacterial food poisoning and is also responsible for 82% of hospital admissions due to food poisoning in the UK (O'Brien, personal communication). The World Health Organisation estimates that ~1% of the population of the UK will be infected with *Campylobacter* spp. each year[3], although detected outbreaks are relatively uncommon. Symptoms of infection with *C. jejuni* range from mild watery to profuse bloody diarrhoea with mucosal damage and inflammation especially in the ileum and jejunum[4]. The economic burden of campylobacter infection is large. In the United States the annual estimated cost is around US$ 4.3 billion[5]. The average cost of a case of acute infection (excluding long-term sequalae) in England in 1995 was estimated to be £1315[6]. Conservatively, therefore, food-borne campylobacter infection cost the nation over £113 million per annum. There are clear public health and economic benefits to be gained by better controlling these bacteria in the food chain. At a time when some real success has been achieved in reducing salmonella in both chickens and people in the UK, and elsewhere, the control of *Campylobacter* spp. remains difficult. Some reasons for this are discussed below.

Vehicles of infection and survival in the food chain

Most campylobacter infections, although not all, are either food or water borne[7]. In almost all cases the infecting bacterial population will have been exposed to hostile environments, particularly either high and/or low temperatures before being consumed. Identified risk factors in sporadic cases include; chicken[8], barbecued meat[9]; raw or improperly pasteurised milk[10]. It would seem clear that, despite the reported sensitivity of this pathogen to the extra-intestinal environment, infectious potential is not compromised by exposure to potentially lethal high or low temperatures. Despite the importance of *Campylobacter* spp. as human pathogens, little is known about their ability to cope with hostile conditions within the transmission chain from animals to man. In addition, little is known about how these apparently very sensitive bacteria persist in foods or non-food environments and there is similarly scant information about the molecular mechanisms that enable the differential survival of different campylobacter strains under a range of environmental stress conditions, relevant to food production. Much remains to be learned about the interaction of *Campylobacter* spp. with their environment and how this influences pathogenicity.

Work is in progress to unravel the often complex behaviours of campylobacter in the food chain and these studies are beginning to produce information which may explain the success of these bacteria as zoonotic pathogens. Recent work by the Bristol Food Safety Group[11] has demonstrated that a strain of *C. jejuni* did not show a reduction in heat tolerance following exposure to low temperature. This is in marked contrast to the behaviour of *Salmonella* spp. (*Figure 2*). This work has now been repeated with 25 more strains of *C. jejuni*, which all responded in an essentially similar manner (Hughes, personal communication).

Under-cooked chicken and improperly pasteurised milk are frequently identified as vehicles for campylobacter infection, which can be difficult to reconcile with the alleged heat sensitivity of these bacteria. It is likely that work to date has under-estimated the heat tolerance of campylobacter because studies have been performed with planktonic cells. Previous work[12] demonstrated that cells of *C. jejuni* survived for much longer at high temperature in chicken muscle slurry than in broth. Similar data are available for salmonella[13]. Case-control studies and outbreak investigations have identified a range of vehicles for campylobacter infection and these include contaminated drinking and recreational water, unpasteurised milk and contact with infected pets, principally dogs. The most important, however, is contaminated chicken meat.

Control in poultry production

Work at Bristol on chicken production hygiene covers the whole food chain and has explored carcass treatments as a means of reducing the campylobacter load. In a series of investigations, naturally contaminated carcasses were removed from the processing line just prior to chilling. They were examined for numbers of *Campylobacter* spp. either without further treatment or after immersion in water at different high temperatures or at 20ºC (*Figure 3*).

The data demonstrate that the hot water treatments were not much more effective in removing *Campylobacter* spp., (even though

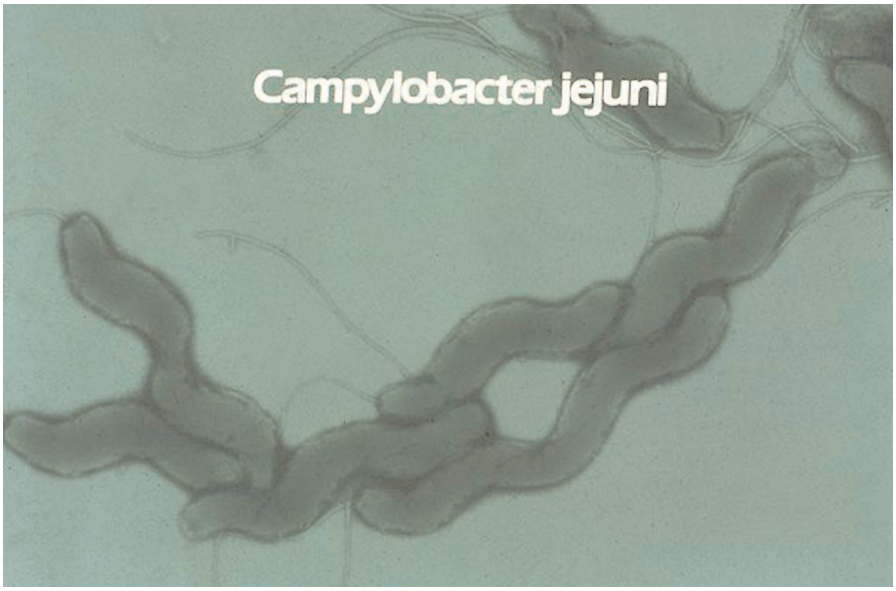

Figure 1. *Campylobacter jejuni*, showing typical 'seagull wings' cell morphology

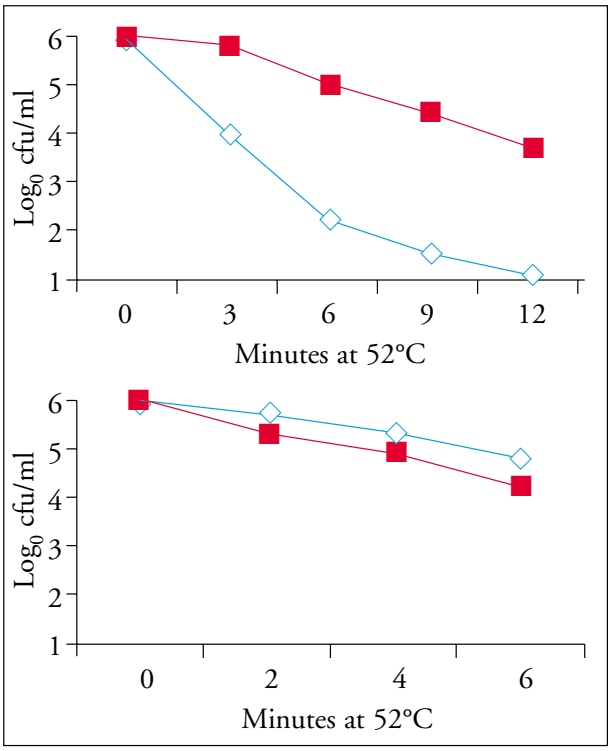

Figure 2. Heat tolerance of *Salmonella* enteritidis (2A) and *Campylobacter jejuni* (2B) either before (closed squares) or after (open diamonds) chilling overnight at 6°C. Cultures were grown for 24 hours at 37°C, prior to chilling

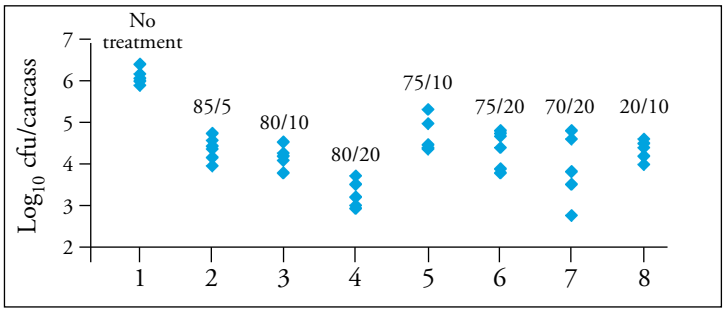

Figure 3. The effects of hot water treatment on the numbers of *Campylobacter* spp. on naturally contaminated chicken carcasses. Each symbol represents the log count of campylobacter cells on an individual carcass and five were tested in each sampling regimen. The first two numbers refer to the water temperature in degrees centigrade and the last one or two numbers indicate the immersion time in seconds.

some markedly affected the integrity of the skin), than simple immersion in cold water. Other studies on the effectiveness of hot water treatments show a similar low reduction in pathogen numbers[14]. Data like these suggest that heat treatment may not be a significant control measure, particularly where chicken carcasses are to be sold whole. Snap freezing of the carcass surface may be more effective, given the known sensitivity of *Campylobacter* spp. to freezing[15]. A variety of chemical treatments have been shown to be effective in reducing the levels of *Campylobacter* spp. on carcasses although European Union legislation limits their use at present. Irradiation is also highly effective in destroying pathogens like *Campylobacter* spp. on chickens but there is currently consumer resistance to the use of this technology. At present there are few, if any, reliable control options available for intervention during poultry processing.

On-farm control of *Campylobacter* spp. in poultry meat production

A more effective means of control is to identify measures which protect chickens on-farm. The UK egg and poultry industries have been very successful over the last few years in reducing salmonella contamination rates in eggs and on poultry carcasses. The introduction of vaccination of commercial egg laying flocks under the Lion Code is thought to be the principal reason for the marked fall, since 1997, in human infections in the UK with *Salmonella* enteritidis. Similarly chicken carcass contamination rates have fallen from c 80% in 1980 to c 5% in 2001[16]. The improvement with poultry meat has been achieved by the removal of breeding flocks infected with *S.* Enteritidis, which stopped vertical transmission, and improvements in on-farm biosecurity and feed hygiene. However, it is proving more difficult to bring about the same improvements with campylobacter in chickens.

The epidemiology of *Campylobacter* spp. in poultry meat production is quite different from that of *Salmonella* spp. With the latter, infection is more common in young birds whereas with campylobacter, flock colonisation is unusual in birds younger than three weeks of age. The reasons for this delay are not yet understood but evidence suggests that it could be due to the presence of maternal antibodies, competitive gut flora or both[17]. It may also be that around the time of infection, 3-4 weeks of age, events occur in the flocks, which predispose the birds to colonisation with *Campylobacter* spp. Around this time birds will experience a change in diet and anti-coccidiostats, which could lead to a shift in commensal flora and receive multiple vaccinations. Any of these might just change host susceptibility sufficiently to allow campylobacter to colonise the intestinal tract. Identification of the key events, which occur around this time could give very useful clues for control.

Biosecurity

There are a number of potential control measures open to the poultry meat industry in the UK to reduce the risks of chickens carrying *Campylobacter* spp., particularly with birds that are housed. Before these are discussed, however, it is important to mention that campylobacter are more ubiquitous in the external environment than salmonella and can be isolated from most domestic and wild animals, which is also in contrast to *Salmonella* spp.

It would also appear that *Campylobacter* spp. colonise chickens rather more easily than *Salmonella* spp. The combination of these factors mean that the margins for error with campylobacter are smaller than they are with salmonella. This is exacerbated by the reported low infectious dose for *C. jejuni* in broiler chickens.

Once present in a broiler flock, *C. jejuni* can spread very rapidly and within four days all the birds can be carrying the bacteria (*Figure 4*). Spread is facilitated by the birds' habit of eating faeces and because passage through the chicken enhances the colonisation potential of *Campylobacter* spp.[18] While it will be possible to modify host susceptibility by better flock management and dietary manipulation (see below) the most important control measure is to prevent ingress into the poultry house. A comparison of data from Norway and Sweden with that from other parts of Europe reveals that the frequency of *Campylobacter* spp. in broiler flocks in those parts of Scandinavia are lower than elsewhere. The principal source of the bacteria in chicken flocks is the environment around the broiler house[19], which will be contaminated with the faeces of wild and domestic animals. In essence, control of campylobacter in housed broiler flocks requires that the environmental load is reduced by good farm hygiene and the ingress into the flock is controlled by good biosecurity. In Norway and Sweden control is achieved by hygiene barriers and dedicated footwear and clothing in each broiler house and a strict limitation on people who can enter the flock. These measures have caused flock colonisation rates to fall to ~10% compared to 50-60% in much of the rest of Europe, including the UK. In theory such intervention should work in the rest of Europe for housed

birds but it is important to bear in mind that the winters in Norway and Sweden can be very harsh, which will markedly reduce environmental load of campylobacter for many months of the year. The industry is also smaller and the birds are generally killed at an earlier age than in the UK, thus reducing the 'window' for campylobacter colonisation.

There is a clear public health need for the incidence of broiler flock colonisation with *Campylobacter* spp. to be reduced and the UK industry is actively involved in research with the scientific community to identify cost-effective control programmes. The Scandinavian biosecurity measures are proving to be reasonably successful on trial sites. For example, a study undertaken by the UK Veterinary Laboratories Agency[20] investigated Scandinavian-type intervention in the UK. Measures tested included boot dipping, changing boots and outer clothing and hand washing. Data from this study show that where personnel strictly followed the biosecurity programme, flocks were three times less likely to be campylobacter-positive. Flock colonisation rates were also halved if boot dips were changed more than once per week. Similar data have been obtained with more recent work (Davies, personal communication). The larger size of UK farms, which increases the chances of biosecurity breakdowns, means that maintenance of biosecurity has to be particularly vigilant and that other interventions may also be needed.

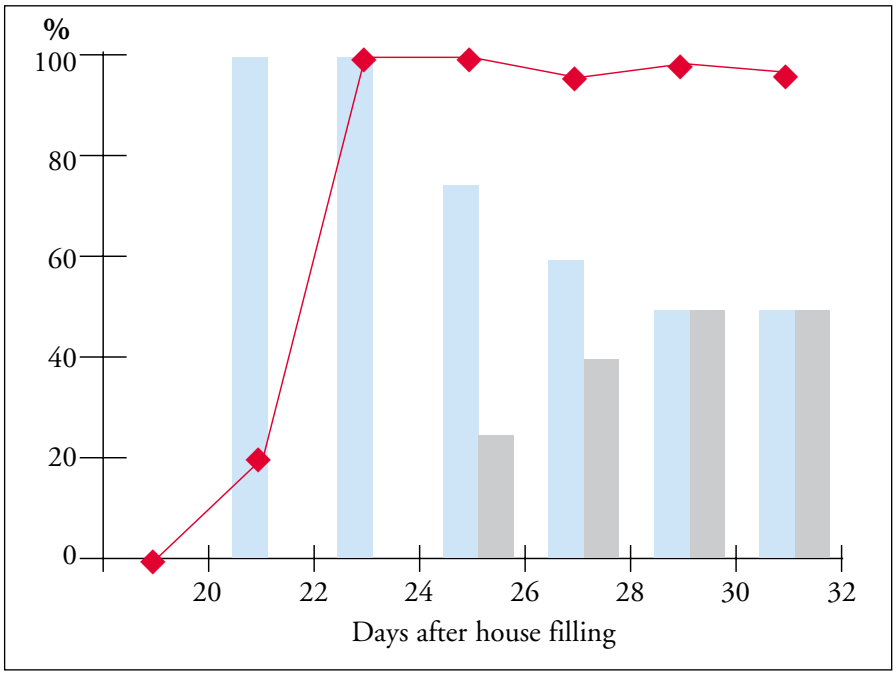

Figure 4. Colonisation of housed broiler flock with *Campylobacter jejuni*. The line shows the % of birds that were campylobacter-positive. *Campylobacter jejuni* was not isolated from birds before 22 days. The histograms show the relative frequency (%) of two PFGE types of *C. jejuni* isolated from the sampled birds.

Other potential on-farm control measures

The importance of chicken meat as a vehicle for human campylobacter infection has meant that a lot of work has been done on potential control strategies, other than biosecurity. Not all strains of *Campylobacter* spp. present in chickens have been found in human cases and there is thus the possibility that some chicken-associated strains are non-pathogenic for humans. Given that commercial poultry flocks usually contain a dominant campylobacter type, the strains suggested as being 'non-pathogenic' may have a role as agents to exclude those known to cause human illness. Under laboratory conditions birds colonised with one campylobacter isolate were able to resist challenge with another[21]. Such an approach may have potential dangers as the genome of *C. jejuni* contains many hypervariable sequences allowing a high degree of genetic adaptability. Passage through the chicken gut has been shown to increase the infectious potential of *C. jejuni* in chickens[18]. It is vital to establish that the strains used as exclusion agents do not change in the chicken gut to become human pathogens.

Competitive exclusion, which uses mixed bacterial cultures to exclude salmonella from broiler chickens, is a well established technique in the poultry industry[22]. Such an approach has been tried against campylobacter, with mixed results. This may be related to the fact that campylobacter occupy a particular niche in the chickens' lower intestine, namely the mucin layer, which lines much of the intestinal epithelium. This is different from salmonella and suggests that other approaches may be necessary. A potentially more profitable approach may be to exploit the fact that campylobacter-negative chickens have been shown to have a gut flora which is naturally antagonistic to *C. jejuni*[17], due to the production of inhibitory compounds (*Figure 5*). Under laboratory conditions, such bacteria are able to protect against challenge with broth cultures of *C. jejuni*. Work is needed on this approach, particularly on why the antagonistic bacteria disappear at 2-3 weeks of age in most flocks[17] but not all (Bristol Food Safety Group, unpublished data) but it has the advantage of being a 'natural' phenomenon.

Campylobacter spp., like other bacteria, are susceptible to bacteriophages (or phages), which are found naturally in the chicken gut. Recent research (Connerton, personal communication) has shown that the administration of phages to campylobacter-positive chickens dramatically reduces gut levels. If this technique is to be used it would seem best that it is applied as close to slaughter as possible as the host bacterium will re-colonise chickens once phage levels fall. It does, however, offer another potential control measure, although it might lead to an increase in the prevalence of phage-resistant campylobacter strains.

One reason suggested for the delay in the colonisation of broiler chickens with campylobacter is maternal antibodies which protect the chicks during the first few weeks of life. It appears that chickens can mount an antibody response to *Campylobacter* spp. and high antibody levels have been seen in breeders and egg yolks. In broilers, sera from one and seven-day-old chicks contained high antibody levels, which then declined to become undetectable at three-four weeks. This group[23] used artificial challenge to determine whether campylobacter-specific maternal antibody (MAB) protected young chickens. Colonisation with *C. jejuni* was compared in three-day-old broiler chicks which were MAB-positive, and birds at 21 days, which were negative. Colonisation occurred much sooner in the older birds than it did in the younger ones. In additional work, campylobacter-positive and -negative SPF chickens were raised under laboratory conditions, and their progenies with or without campylobacter-specific MAB were challenged orally with *C. jejuni*. Significantly fewer colonised chickens were observed in the MAB + group during the first week post-infection. Despite the above study, data on immune responses by chickens to campylobacter remain equivocal and may require further investigation. Some work has been done to examine whether the administration of antibodies can protect chickens against

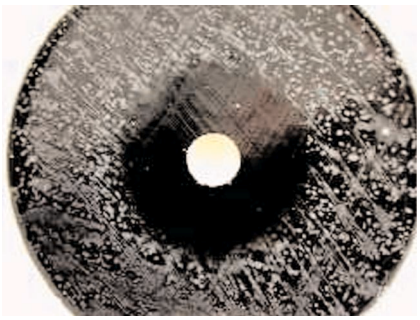

Figure 5. An agar plate showing the lack of growth of *C. jejuni* around a well containing caecal contents from a naturally campylobacter-negative broiler chick.

challenge with campylobacter. Chickens immunised intraperitoneally with killed whole cells of *C. jejuni*, and subsequently challenged with live cells, had only 2% of the levels in caeca found in control birds. Intraperitoneal vaccination with heat-killed cells was found to reduce numbers of *C. jejuni* in the caeca of artificially-infected birds by 2-logs. This is not a practical approach but oral vaccination with formalin-killed cells of *C. jejuni* reduced caecal colonisation in vaccinated birds by between 16-93% compared to controls. Anti-campylobacter antibodies given prior to infection reduced (>99%) caecal campylobacter levels in artificially-infected broilers and the administration of antibodies post-infection also reduced levels in the caecum, although effects were smaller (80-95% reduction).

Broiler flock management and *Campylobacter* spp.

A variety of studies has demonstrated that farmers differ in the frequency with which campylobacter-positive housed broilers are produced. The distribution pattern of the cohorts of farmers suggests that these differences may not be random. In a preliminary study in South West England, where the incidence of campylobacter-positive birds was compared, rather than the incidence of positive flocks, it was possible to assign farmers to different groups with regard to campylobacter status. At the extreme ends of the spectrum of farmers, one produced only 1.4% of campylobacter-positive birds over seven flock cycles whereas another had 97% of birds colonised with *Campylobacter* spp. at slaughter. A comparison of company production data showed that the farmer with the highest incidence of campylobacter had higher levels of hock and pad burn, which are lesions which generally become obvious in the third week of life, than the farmer where almost all the chickens were campylobacter-negative. There were also differences in levels of mortality on-farm and rejection of carcasses at the processing plant. These were rather limited investigations however, and a more detailed study is required to try to determine the relative roles of the different aspects of flock management, which might influence the entry of campylobacter into broiler flocks and/or affect the susceptibility of the birds to colonisation. It may be possible to identify measures or management practices in use on 'low campylobacter' farms, which could be applied on farms where control is more difficult.

Conclusions

Campylobacter spp. remain extremely important human zoonotic pathogens. A dilemma with these bacteria has been to try to reconcile their apparent high sensitivity to extra-intestinal environments with their success as pathogens. It is now becoming clear that campylobacter are rather more robust than previously thought and that they can resist quite high temperatures, for example, when attached to chicken skin. They also do not become sensitised to heat when pre-exposed to low temperature unlike salmonella and *E. coli*. Contaminated chicken meat is a major vehicle of human infection and work in the UK, and elsewhere, is beginning to show that on-farm control is possible when biosecurity is strictly applied.

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Imported foods/imported diseases

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Introduction

Food-borne and infectious diseases amount to an enormous global health problem and do not respect borders. A decade ago, food safety was not the great public concern that it is today. Food, then, was considered to be safe, incidences of chemical or microbiological contamination were localised corresponding to food supply. But in 2002, the World Health Organization, (WHO), observed that food safety was one of the highest priority issues for consumers, producers and governments.[1]

Why has there been such a change in the perception of the risk to our health and well-being from food? In 1985 Bovine Spongiform Encephalopathy (BSE), was discovered in the United Kingdom (UK), and brought with it the link to Variant Creutzfeld-Jacob Disease, creating worldwide concern that food might be a vehicle for the dissemination of such disease-causing agents. Initially confined to the UK, the disease did not generate much interest beyond the country's farmers, veterinarians and epidemiologists. Soon, however, the consequences were felt across Europe and beyond. In 1991, the discharge of ballast water contaminated with *Vibrio cholerae* from a cargo ship was the likely mechanism for the introduction of cholera on the South American continent for the first time in almost a century through the contamination of shellfish in the discharge area.

Through these and other dramatic incidents resulting in debilitating illness and fatalities, an enhanced awareness of the public with food safety is understandable and there is a realisation that global threats from food-borne and infectious diseases are not lessening.

The value of the world trade in food in 2000 was about $558 billion presenting many benefits to consumers through the availability of a wide variety of foods. The global food trade also provides opportunities for food exporting countries (including the UK) for economic development. Significant amounts of food and other goods arrive in the UK daily from all parts of the world. In 2001, 3,167,863 tonnes of food of animal origin and many millions of passengers passed through UK ports and airports.[2] Food and people are distributed over far greater distances than before, creating the conditions necessary for widespread and rapidly occurring outbreaks of food-borne illness. This global movement of food, goods and people provides opportunities for global movement of known and "exotic" infections. Unfortunately, the interests of the consumer are not always the prime consideration of those in business and at the opening of the twenty-first century we must also consider the intentional, malicious introduction of pathogens and contaminants into the food supply.[3]

Paradoxically, in contrast to the heightened awareness of the risks and public preoccupation with food safety, food today is one of the most regulated commodities world wide. Early attempts to regulate food were primarily aimed at preventing and detecting adulteration and fraud, which work continues as many adulterants are frighteningly toxic. Current regulatory measures are focussed on safety, evaluated by a multitude of scientific disciplines including risk analysis, toxicology, and microbiology to attempt to consider credible risks of every step in the chain, from raw material to final consumption.

Border controls

Nations wishing to remain disease free and reduce the increasing burden of food-borne illness, must establish effective strategies for minimising the risks of introduction of disease-causing agents, capable of operating at national and international level. Food safety is an essential public health function in which successful outcomes are more likely if the prosaic tasks are synchronised along the entire food chain. Prevention of food-borne hazards and risks is the most desirable option, in which good co-ordination of the efforts of the food producer, the authorities in the country of origin, quarantine and government authorities in importing countries, and food importers is essential. The mechanism by which this is achieved will include sensible precautions, together with high quality surveillance systems and proportional responses.

Most food safety regulatory systems rely upon evaluating the food against legal definitions of food safety, the hazards presented and/or the likely risks to health from consuming the food. Countries which rely significantly on imported food usually establish quarantine controls to try to prevent contaminated food from entering the importing country. The enormous diversity of food/contaminant combinations, and possible non-compliances make the task of inspecting and testing all consignments neither practical nor sensible. Neither would it be desirable to interrupt trade in safe food without good cause. The priorities and scope of an imported food inspection strategy will also be determined by budget and resource availability.

On entering the European Union (EU) all consignments of animals and animal products must be accompanied by a health certificate that attests to the disease status of the country, and that processing plants comply with legislative requirements. An obligation is placed on Member States to ensure that no consignment from a non-member state is introduced into its territory without having been subjected to the veterinary checks required by import legislation. The checks may include physical assessment comprising of microbiological examination, chemical analysis, and packaging integrity amongst others.

Import controls are intended to be carried out consistently at all points of entry across the Member States. Regulatory systems also exist for food products not of animal origin. These are not yet fully harmonised in the EU but provide a level of control determined by budget and resource availability. Since 1992 a regulatory system has existed in the EU to control the trade in animals and animal products entering from non–Member States. The European Commission is responsible for approving countries and facilities wishing to export to the EU, and this is performed by means of inspections. During the inspections attention is paid to the exporting country's disease surveillance, reporting and outbreak and outbreak control systems.

In 2001, over 3 million tonnes of products of animal origin and more than 19 million tonnes of products of vegetable origin were declared for import into the UK. In all cases provisions allow for destruction, for use other than for human consumption, or for re-dispatch outside of the EU depending on the assessed risk to human or animal health.

Inspection programmes exist to try to establish the safety of imported food consignments, which include point of entry testing. Such inspection and testing relies upon documentary checks of health certification, organoleptic examination, laboratory examination or analysis of the product, and is an attempt to compensate for the lack of knowledge about production and transportation controls, and for the food safety status of the consignment as presented at point of import. Examples of food-borne risks to public health experienced during the inspection programme at Southampton are shown in *Table 1*.

Point of entry testing is an attempt to take into account the lack of knowledge regarding the controls exercised in the country of origin for safe food production or that no deterioration of the product has occurred since it left the exporting country.

Table 2 describes a typical overview of an imported food inspection process in the UK.

At each stage of the process, regulatory decisions have to be made by inspection staff based on current knowledge and legislation that will have relevance for consumers and business. Such knowledge is hardly ever complete and the challenge of evaluating a decision often stretches beyond the ability of individuals within an inspection service. Decisions have to be made without unreasonable delay, and good co-operation with other food safety professionals and legal advisors is an integral part of that process.

This structured approach to imported food inspection complies with internationally determined profiles such as FAO and WHO, yet there are many concerns about the number of infectious diseases entering the EU.

Where might the weaknesses in our systems be?

Frontier checks

In 2002 The Royal Society reported on infectious diseases in livestock following the Foot and Mouth Disease outbreak.[4] In their evidence to the enquiry the Association of Port Health Authorities said that *'it is relatively easy for illicit imports of products of animal origin to evade veterinary checks'*.[5] Whilst the Government has invested in strengthened border controls, people still try to import illegal products in quantity from countries where exotic diseases are endemic as evidenced by the many tonnes of products seized.The volumes are very large but as yet this information is not in the public domain, and what is recorded largely relates to airport seizures. It is likely that unless attitudes toward importing these products change, the threat of disease transmission from illegally imported food will remain.

Certification

Health certification is provided by exporting countries with the intention of providing a certain level of confidence that stated requirements are met and is the foundation of the EU veterinary checks regimen.

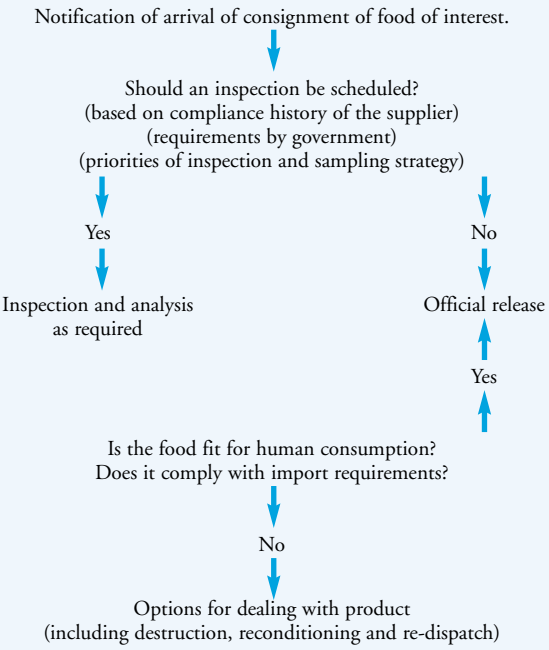
In 2002, a consignment of about 20 tonnes of ready to eat prawns was presented for import at Southampton.[6] The appropriate certification accompanied the consignment declaring that the food had been processed in accordance with all relevant hygiene requirements. Similar consignments from the production establishment had previously been exported to Norway, where, following microbiological examination, *Vibrio cholerae* had been detected. The Southampton consignment was inspected and examined and high levels of aerobic bacteria were detected in samples submitted. In the opinion of the food examiner, the levels indicated an enhanced risk of pathogens being present elsewhere in the consignment. Process control and laboratory examination data were requested in order to determine a course of action for the fate of the consignment. The production data were not produced and a critique of the laboratory operating procedures for export certification revealed defects against UK accredited methods, which were likely to account for the lack of detection of micro-organisms at the processing end point.

In 2003, EU Border Inspection Posts were warned by the European Commission that Escolar,

Table 1: Examples of Potential Food-Borne Risks to Public Health from Imported Foods.

RISK	EXAMPLES
Contamination with pathogenic micro-organism.	Salmonella. Pathogenic *E.coli.* *Listeria monocytogenes.*
Presence of toxins either due to poor handling or naturally occurring toxins.	Staphylococcal enterotoxin. Aflatoxins and other mycotoxins. Marine biotoxins. Histamine in susceptible fish species.
Poor production methods that may allow growth of food poisoning microoganisms or development of toxic substances.	*Clostridium botulinum.*
Environmental contaminants.	Heavy metals such as mercury in fish.
Presence of excessive pesticide or veterinary drug residues.	Organochlorine and organophosphate residues.
Illegal or excessive food levels of preservatives.	Sulphur dioxide implicated in asthma attacks.
Labelling and packaging inadequacies.	Food irradiated but not labelled as such. Packaging incapable of hygienically protecting product. Foods from non-approved establishments.
Contamination with toxic substances.	Mustard oil with erucic acid.

Table 2: Chart of Imported Food Inspection Process



an exotic fish was being exported to the EU, labelled and certified as sea bass. Escolar contain very high levels of oil, mainly wax ester, and if the fish is not cooked in such a way to remove some of the oils, or eaten in large quantities, the highly indigestible wax ester nature of the oils may cause stomach cramps, diarrhoea, headache, nausea and vomiting. Enforcement officers were asked to ensure that there were no cases of mis-describing of the species.

International regulatory levels and sampling methods

Many countries have legal limits for contaminants in food and methods for detecting them. The limits and methods vary widely.

In 2003, three consignments of brazil nuts of about sixty tonnes gross weight were presented for import at Southampton. EU legislation currently requires this product to be subjected to a rigorous sampling and analytical regime. Following analysis of samples submitted, very high levels of Aflatoxin B1 were reported, such that the nuts were considered to be unsafe for human or animal consumption. The importer and exporter both insisted that the consignments had been tested prior to export in the United States of America (USA), and the Federal Inspection Service confirmed this. No aflatoxins had been detected at that testing and export certification was issued. Following protracted discussion between the Port Health Authority and exporting country authorities, it became clear that neither the sampling nor detection methods used for export were comparable with EU import controls and these would have accounted for the variation in detection efficiency. The USA authorities requested that the consignments be allowed to return to USA, since their maximum tolerance levels were higher than that permitted by the EU. A decision to destroy the consignments rather than re-export them was made since there can be no guarantee that following re-exportation such consignments would not be re-imported into another Member State.

Global concensus on the implementation of regulatory standards, sampling methods and analytical techniques is crucial for both the protection of public health and to facilitate trade.

Risk analysis /decision to inspect and if yes, what for?

In 2002,1.3 million twenty-foot equivalent containers entered the United Kingdom through the port of Southampton. By no means were enforcement staff able to or entitled to visit all of these. The task of identifying which container has a pathogen or contaminant in it, and where in the consignment it might be is a real problem. Contaminants and pathogens are enormously diverse in nature, number and type. How can we plan and support the detection and identification of new and old disease causing agents? How do we fund and organise ourselves adequately to offer the level and quality of protection that our communities tell us they want?

Current Government and EU strategies for identifying agreed inspection requirements and assessing risk are largely still limited to animal health controls e.g. Foot and Mouth Disease.

Anti-smuggling activities were transferred to Her Majesty's Customs and Excise (HMCE) in 2002, but that government department focuses its priorities on fiscal controls and the detection of narcotics, pornography, counterfeit and illicit tobacco and alcohol imports. Its enforcement staff have little experience or knowledge in ascertaining food safety issues.Further, successful seizure rates achieved so far relate primarily to those done at airports.The appropriate government departments have yet to determine the prioritisation of resources to target the significant volumes that are bound to be entering through seaports.On numerous occasions, we have been lucky. Goods have been discovered during unloading in warehouses and other dock premises having to all intents and purposes cleared HMCE and border inspection post controls, and been reported to us by responsible warehouse personnel and agents.

One recent event revealed, in a 'cleared' container, quantities of shark fins, in which, under the Convention for Trade in Endangered Species(CITES), it is illegal to trade; abalone from unapproved establishments in China; and legs of pork, also originating in China. Swine Fever and Foot and Mouth Disease are endemic in China, and there have been many occasions when marine biotoxins have been detected in shellfish originating and harvested there. The consignment was intended for a major Chinese Restaurant group for use on England's south coast. The same sort of event, but concerning diverse products such as 'thousand year old eggs', toxic 'Japanese' star anise, mustard oil containing high levels of erucic acid for lamps which, if lighted, would have given off cyanide fumes, occur frequently.

Even if we can determine which consignment to inspect and how much resource we can afford to do the work, where in it to look, and what to look for, there are the inevitable problems of ensuring that there is sufficient quality laboratory ability capacity to perform the examination or analysis adequately, agreement on national and international methodologies to be applied to testing, and the scientific interpretations of results obtained.

Decisions by governments to allow resumption of imports of previously banned products from around the world, such as foodstuffs contaminated with antibiotic residues provoke much discussion and suspicion amongst enforcement officers, particularly when testing of a previously implicated product reveals that the contamination issues remain. Are the assessors of that exporting country's official controls, which previously failed to prevent the abuse or the contamination, so confident that the improved controls will now do the job? Or is there another reason why import controls are so readily lifted? And why do we 'telegraph' to food processors and rogue traders the sort of contaminant we might want our laboratories to search for? Don't policy makers understand that elements of the food industry are endlessly inventive in finding a different food additive, (perhaps more dangerous than the last illegal contaminant detected), which serves their purpose and will likely evade the current published strategy? These importers also show remarkable innovation in developing new routes or masking the route from country to country of origin and manufacturer.

Diverted cargoes and re-exports

A significant minority in the food import community have as much knowledge about the food safety status of their food imports as they do about the furniture, electronic equipment and car tyres that they also import. They appear to show little interest in the final consumer in the sure knowledge that, should they manage to evade official controls, it is unlikely that they will have to face up to the consequences resulting from the consumption of unsafe food. Comments from importers such as, when shown decomposing and faecally contaminated food, " you don't need to worry about it, this is what they are used to" or, "well, it's only for this or that ethnic community", are sadly too common.

When this sort of importer realises that a positive enforcement approach to dealing with the food, i.e. condemnation and destruction, will be followed in a particular port or country, they often seek to divert cargoes to other points of entry where they know that surveillance and control is weak. Some enforcement authorities choose the easy option to re-export contaminated and infected cargoes rather than seek to remove it from the human and /or animal food chain.

In 1995 Southampton Port Health Authority was presented with 72 container loads of twenty tonne gross weight each of canned Russian salmon intended for import. Upon examination gross "crush' damage and classical processing can defects were observed in over 80% of the consignment. Microbiological examination of the can contents detected the presence of clostridia. The consignment was rejected and destroyed and

later found to have been examined by United States of America import inspection staff and rejected. The cans then found their way to Southampton, where if import had been successful, they would have been packed in Christmas gift food hampers. Five months were spent by the Port Health Authority in securing proper control of the consignment, for which no charges for the work carried out by enforcement staff were recoverable. Often, rejected cargoes are dumped on poor countries with far fewer resources than the UK has to organise import controls. Even in UK territories such as the Falkland Islands, this Authority has been told that there are no import controls for food. Controls there are currently in position for exports only.

EU enlargement

In May 2004 ten new member states will accede to the EU. Food safety issues are spread over two areas of the accession negotiations so far:

'Free movement of trade' including general rules for hygiene and control, genetically modified foods and food labelling, and 'Agriculture', covering veterinary and phytosanitary issues, and animal nutrition.

From the outset, the EU has stressed that enlargement must not lead to lower food safety standards or to any risks for consumers. One of the main issues on food safety was the capacity of the new Member States to implement EU-compliant controls for trade inside the EU and for imports from third countries. There remain serious concerns as the EU realises its plans to enlarge further towards areas where many exotic animal diseases are endemic. These and other issues regarding biosecurity and the relationship between imported food products and human disease are described in the Royal Society's report 'Infectious diseases in livestock' July 2002.

Emerging agents of disease

So much for the problems and risks, which are so readily identifiable.

What about the ones we haven't identified? The House of Lords Select Committee on Science and Technology report "Fighting Infection" had already detected signs of weakness in our defences, as have many other international organisations concerned with world health.[7] Professor Stephen Palmer's work has shown infectious agents emergent in human disease since 1970.[8,9] The abbreviated table below is from his work and published in Communicable Disease and Public Health, September 2003.

The publication reported that recent decades have shown an unprecedented rate of emergence of new zoonoses. The obvious point is that not only do infectious diseases persist and return, but that new ones emerge. Year on year in Southampton we observe more frequent large outbreaks of norovirus amongst cruise line passengers and crew. There is no indication that this is likely to decrease, current control strategies being limited to containment whilst the outbreak runs its course through susceptible populations. And there is every reason to anticipate a great increase in the volume of people passing through our ports as the cruise industry expands. To my knowledge the UK does not gather data relating to these outbreaks. The Port Health Authority does, and knows the distress it causes to passengers. The UK media gave mass coverage to the problem during the summer of 2003.The amount of resource having to be expended by the cruise industry and our organisation in trying to prevent and bring outbreaks under control has never been quantified.

Conclusions

Foot and Mouth Disease appears no longer to be present in the UK, the world appeared by September 2003 to be in the recovery phase from SARS, but as the winter of 2003/2004 progresses we observe new cases of SARS occurring. The malicious contamination of food for terrorist purposes is a real and current threat for importing nations and their trading partners. There are lessons to be learnt from these and all the repetitions of mass infections in history

As I come to the end of this report, the news has just broken that another outbreak of 'avian flu' has been reported in the far east. Our staff are busily ensuring that all consignments of poultry and other poultry products identified by Government instructions are secured. Fortunately such consignments are few in number as far as we know, and they will be properly controlled and rejected in Southampton. The WHO has stated that 'the unprecedented spread of avian influenza requires broad collaboration'.

Outbreaks of both unintentional and deliberate food-borne disease can be managed by the same mechanisms. Sensible precautions, coupled with strong surveillance and response capacity, have always and continue to be the most efficient and effective way of countering all such emergencies.The well established tasks of harmonised epidemiological surveillance and laboratory networking, effective early warning and response systems, high quality scientific opinions, adequate international technical assistance, collaborative preparedness against health emergencies etc really should not need to be repeated. I would however add a few personal wishes:

- Recognition that the opinions of all stakeholders and not just the established experts are significant.
- That the WTO, WHO, the World Bank and all other trade groups recognise that a fulsome approach to their collective responsibilities to human and animal health is badly needed. There is, after all, less profit to be made from sick animals and from diseased or dead humans.
- That anyone who is occupied in the great industry of public safety offers as much of themselves, their knowledge and expertise as possible in preventing unsafe food being made available for use.

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Table 3. Some infectious agents emergent in humans since 1970[8]

1970s	1980s	1990s	2000s
Rotavirus	*Helicobacter pylori*	Sin nombre virus	Human Meta-pneumovirus
Parvovirus B19	*Borrelia burgdorferi*	Bat Lyssa viruses	SARS coronavirus
Legionella pneumophila	Hepatitis C virus	Equine morbillivirus	
Campylobacter jejuni	Hepatitis E virus	Nipah/Hendra viruses	
Cryptosporidium parvum	Human Herpes virus 6	Human Herpes virus B	
Noroviruses		Avian Influenza virus	
Clostridium difficile			
Ebola virus			

Predictive microbiology – quantitative microbial ecology

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Introduction

An area of food microbiology has come to be known as "predictive microbiology" in the last two decades. Checking the search programme of the Web of Science (http://wok.mimas.ac.uk/), hundreds of papers were published with the keyword "predictive microbiology", in the last 10 years and the number is steadily increasing.

So what science does "predictive microbiology" cover exactly? In the first book on the subject, published just over 10 years ago, McMeekin *et al.* (1993)[1] defined it as a quantitative science that enables users to evaluate objectively the effect of processing, distribution and storage operations on the microbiological safety and quality of foods. Later, the same authors used the expression "Quantitative Microbial Ecology of Food", which is a somewhat more generic description. The most recent book on the field[2] puts more emphasis on the need to describe the microbial responses to the food environments by mathematical models. The evolution of predictive microbiology into a more and more exact science is well illustrated by this shift of emphasis in its definition.

Food microbiology has adopted modern methods and novel concepts with some reluctance. Many food microbiologists follow the "old fashioned" approach of enumerating microbes at different stages of food storage, identifying the major fractions of the microflora by their phenotypic characters, and gradually building up an understanding of the shelf-life and safety of foods. However fascinating this is to the dedicated food microbiologist, it is slow and expensive, and has not led to a cumulative, structured database of information that can be interrogated quickly.

Study of the effects on microbial growth of single controlling factors such as temperature, pH or water activity, resulted in acceptance that particular microbes of concern would not grow below certain temperatures, or below a certain pH value or water activity. Some scientists recognised that other factors were important e.g. the composition of the atmosphere above the food, preservatives, food structure, but the experiments needed to cover the effects of all those factors appeared enormous and beyond the scope of individual food microbiologists.

Not until the problem was viewed from another perspective was progress made. All foods contain water, have a pH value and a temperature of storage. If the growth response determined by those "controlling factors" could be measured, then modelled, the result would indicate how much growth could be attributed to those three factors. If the differences between the calculated and observed responses were significant, other factors would have to be taken into account.

Comparisons of growth rates published in the scientific literature with "predictions" from such relatively simple models for the same conditions of pH, temperature and water activity were often surprisingly close and encouraged further efforts.

Gradually, use of models that had been validated by comparing outputs with independent data became recognised as just as reliable as accumulating results from the scientific literature or spending weeks generating more microbiological data. Occasionally it is important to have an "accurate" estimate of the growth/survival, but more often it is sufficient to have a "reasonable" estimate, but quickly. It is necessary to obtain quick and "good enough" estimations of the shelf-life of foods, in which pathogenic bacteria might grow, in new product development and in risk assessment.

History

Predictive microbiology started as a purely empirical (though quantitative) science. Its earliest appearance is probably Esty and Meyer (1922)[3], who described the thermal death of *Clostridium botulinum* type A spores by a log-linear model, which is still used to estimate the necessary heat processing of low-acid canned foods. This model simply says that, at a given temperature, the *relative* (or: *specific*) death rate of the bacteria is constant with time. In other words, the percentage of the cell population inactivated in a unit time is constant. This is a simple, logical and understandable model, similar to those commonly used in physical and chemical sciences for processes such as dissipation, diffusion, etc, when the force that causes the decrease of a certain quantity is constant with time.

A step forward was taken by Scott (1936)[4], who investigated how the specific death rate depended on the available water, quantified today by the so-called water activity, a dimensionless number between 0 (dry) and 1 (wet). He subsequently studied the effect of the temperature on the specific microbial death rate. Today the most frequently assumed relation in thermal inactivation theory is that the logarithm of the specific death rate decreases linearly as the temperature increases (this is equivalent to the so-called constant z-value theory).

"Classical" predictive microbiology

The above two-step approach to develop predictive models is still in use, and not only for death but also for growth curves. Commonly, the first step in the developmental procedure is to establish the growth/death model in constant environment (primary model); the next step is to determine how the parameters of the primary model are affected by altered environmental factors (secondary model – see *Figure 1*).

While it is accepted that in "smooth" cases, the bacterial population should die/grow at a constant specific rate, several complicating factors arise, even in a constant environment. In both situations, the prior history of the cells affects the transition period, during which bacteria arrive at the exponential phase. In the case of death curves, this is frequently referred to a "shoulder"; with growth curves it is called the "lag" (see *Figure 2a*). In most circumstances limited information is available about the pre-inoculation period, with no satisfactory solution to modelling these transition periods.

Another problem is the post-exponential phase, which is the stationary phase for growth curves (the bacterial population reaches the maximum carrying capacity of the environment) and the so-called "tailing-off" or "tails" with death curves. For growth curves, the problem is not significant from a practical point of view, since the food is inedible by the time the microbial load reaches the maximum population level. However, researchers still do not agree whether "tailing-off" phenomena sometimes observed in thermal inactivation is real, or just experimental artefacts. The problem is that the tails occur at low cell concentrations, where the measurements are unreliable and inaccurate – often around the detection level threshold.

The transitional phases were first described by commonly used sigmoid functions, (see

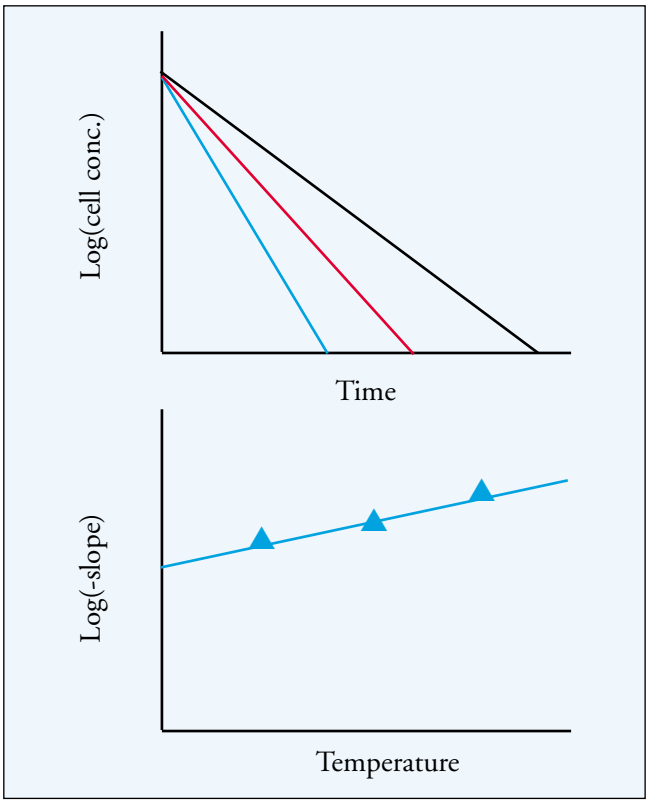

Figure 1. Primary and secondary thermal inactivation models.
Figure 1a. Constant specific death rate: the logarithm of the population size linearly decreases with time; the slope is a function of the temperature.
Figure 1b. Constant z-value: the logarithm of the specific rate changes linearly with temperature. The slope of this linear function is characteristic of the micro-organism.

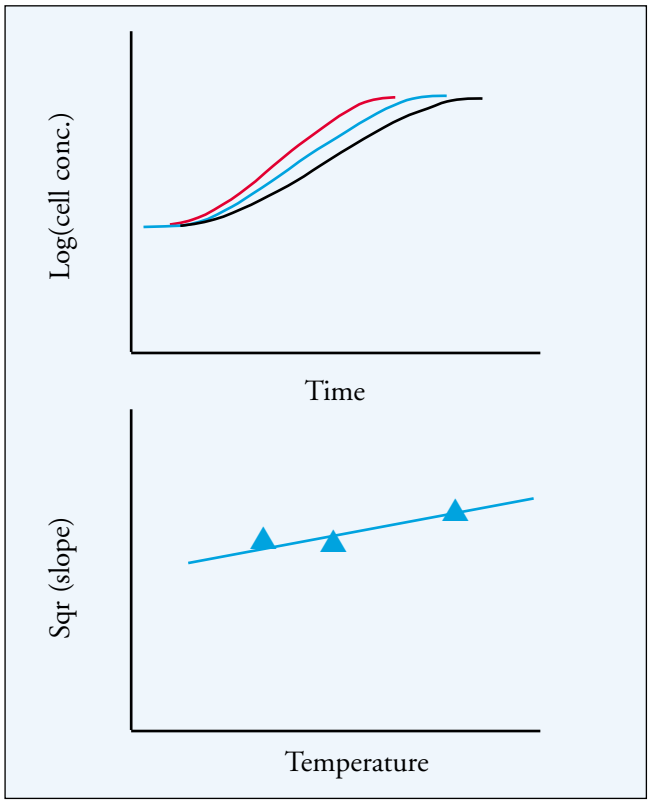

Figure 2. Primary and secondary growth models.
Figure 2a. Constant maximum specific growth rate: After the lag, in the exponential phase, the logarithm of the population size increases linearly with time, until the stationary phase.
Figure 2b. Square-root model: the Square root (spec.rate) increases linearly with temperature. Notice the analogy with **Figure 1.**

Figure 2a), as an empirical approach to primary modelling. The effects of the environment on the parameters of the primary models were then described by secondary models, usually simple, empirical, multivariate polynomials. The most frequently quoted paper in this respect is that of Gibson *et al.* (1988)[5], which has since been cited more than 200 times, according to the Web of Science (WoS). That paper used the sigmoid function of Gompertz for the primary model and a quadratic polynomial for the secondary model. The fitting performance of the Gompertz function was also reported to be the best in another frequently cited paper (Zwietering *et al.* 1990; at January, 2004, its WoS citation index number was 360)[6]. This contributed to the fact that, until the mid-90s, the Gompertz function was the most popular to fit sigmoid bacterial growth curves.

It is generally agreed that the most important environmental factor determining growth is temperature, followed by pH and water activity; followed by preservatives, antimicrobials and the composition of the atmosphere. However, while the temperature is controllable during the storage of the food, the other environmental conditions are not. Furthermore, they can be changed by the growing bacteria, and they can affect each other (interactions). This increased the need for dynamic models, when the constant environment would just be a special case (zero-variation) of the general scenario, in which the environment can change with time. Another aim was to use more mechanistic models, i.e. to describe the mechanism behind the observed process by models based on laws of fundamental sciences, as opposed to the empirical models driven primarily by data-fitting. The drives for dynamic and mechanistic features in fact strengthened each other, since most mathematical models of various physical, chemical and biological kinetic systems are dynamic (differential) equations: giving the *direction* or the *rate* of the system as a function of the *state* of the system.

To be fair, purely mechanistic models are very rare in practical applications. Models in daily use are, in fact, between the two, using mechanistic elements when possible and completing them with empirical approaches when only observations are available.

A quest for mechanistic foundations

Baranyi and Roberts published three papers[7-9] that gave a good mathematical basis for mechanistic modelling of the lag phase. According to its WoS citation index, the Baranyi-model has subsequently been cited in more than 300 papers, and has become the most widely used primary growth model. Though it is useful to fit various (semi-) sigmoid curves (linear phase preceded and/or followed by stationary phases) the main step in the model was its dynamic origin. Namely it describes the transition phases, for either the growth or death situation[10], in a way that can be also used for a fluctuating environment.

By the 1990s, the square-root (secondary) model of Ratkowsky (1983)[11] had become the most popular to describe the effect of temperature on the specific growth rate (*Figure 2b*). Others included the Arrhenius model and its variations, and the Cardinal Temperature model of Rosso (1995)[12]. The problem with this approach was that there was no straightforward extension from temperature alone as a single controlling factor to multivariate situations that would retain the good qualitative properties and performance of the original "growth rate *vs.* temperature" models. Consequently many authors stayed with the simple multivariate polynomials, even when the question was the combined effect of several factors on growth. In fact, all the secondary models were dominantly empirical.

Another direction for the mechanistic basis was the wish to relate the kinetics of the whole population to the physiology and kinetics of

individual cells. Baranyi and Pin (2001)[13] gave a mathematical theory how to connect stochastic process models for individual bacteria at the single cell level and a deterministic model for the population level; i.e. how to conclude the behaviour of the population as a whole from observing many individual cells. That theory was recently validated experimentally by Elfwing *et al.* (2004)[14] using a flow chamber and an automated image analyser to enable observation of divisions of thousands of single cells, and to derive statistical distributions for them. The significance of this technique is that not only kinetic parameters could be characterised by a secondary model, but also their variability, which is vital for quantitative microbial risk assessment.

Unifying efforts for a single database of microbial responses to food environment

Predictive microbiology received a big impetus when the UK Ministry of Agriculture Fisheries and Food initiated, in 1988, a coordinated programme on growth and death of bacterial pathogens, collecting and computerising data in a standardised way. Those collected data served as the base on which the first validated, commercialised programme package, Food MicroModel was built. The task of supporting these developments was taken over, when established, by the UK Food Standards Agency (FSA). The FSA, in 2003, released all the data behind the Food MicroModel and funded the development of a program called Growth Predictor, by the Institute of Food Research. The program is freely available today at (www.ifr.ac.uk/Safety/GrowthPredictor). It is the result of a re-modelling effort on all the available growth data (mainly on bacterial pathogens), utilising the scientific developments of the 1990s.

Parallel to these events in the UK, the US counterpart of Food MicroModel, called PMP (Pathogen Modelling Programme: www.arserrc.gov/mfs/pathogen.htm) was developed at the Eastern Regional Research Center of the USDA Agricultural Research Service. Soon, the co-ordinators of these biggest predictive microbiology research centres and funding agencies on the two sides of the Atlantic recognised that a common, joint, database and unified models would be beneficial for everybody. This is how ComBase, the Combined Database of Microbial Responses to Food Environments (see www.combase.cc) started its life. It is now an internet-based, publicly and freely available database, for research and training/education purposes, for food microbiologists, manufacturers, risk assessors and legislative officers. The original Food MicroModel and PMP datasets have been supplemented with additional data submitted by supporting institutes, universities and companies, as well as by data compiled from the scientific literature. Under the funding of the European Union, many EU institutions are also adding their data to ComBase. As written by McMeekin (2003)[15], *'Properly supported, ComBase will be a watershed in the evolution of predictive modelling and its widespread applications'*. *Figure 3* shows a query and output screen of the stand-alone version of the ComBase-browser program.

Although collaboration began as an academic exercise, having a single database of information and joint models offers huge benefits to assuring the safety of foods in international trade.

Future

In the state-of-the art summary of our current predictive microbiology knowledge[2], readers can find a comprehensive picture of the direction in which the subject is expected to continue and what is likely to change. The classical primary-secondary model approach will almost certainly be restricted to "smooth" cases, when the microbial population is more or less homogeneous, the population density is high enough to use deterministic models and there are no significant interactions between the environmental factors. Progress is expected in the area of

- *dynamic modelling:* interaction between bacteria and environmental factors;
- *lag modelling:* by means of quantifying and modelling the effect of history via the actual physiological state of the bacteria;
- *growth/no growth boundaries for bacteria and environment, probability of growth:* for answering the question "what is the probability that the microbial load is over a specified value, at a specified time ?" (for Quantitative Microbial Risk Assessment purposes);
- *more advanced quantification of the structure of the food environment;*
- *modelling individual cell kinetics by stochastic birth/death processes:* Connecting deterministic modelling at population level to statistical assessment and variability characterisation at single cell level;
- *relating predictive microbiology and molecular microbiology:* using data on how genes are switched on as function of the (dynamically changing) environment; characterisation of variability and stress-tolerance;
- *computational microbiology and bioinformatics development:* data storage and retrieval in a more advanced way.

These tasks require the interdisciplinary collaboration of food microbiologists and mathematicians; food technologists and computing scientists; molecular microbiologists and statisticians.

Just 20 years ago very few food microbiologists believed that models of microbial growth and death would ever be sufficiently reliable to be used in the food

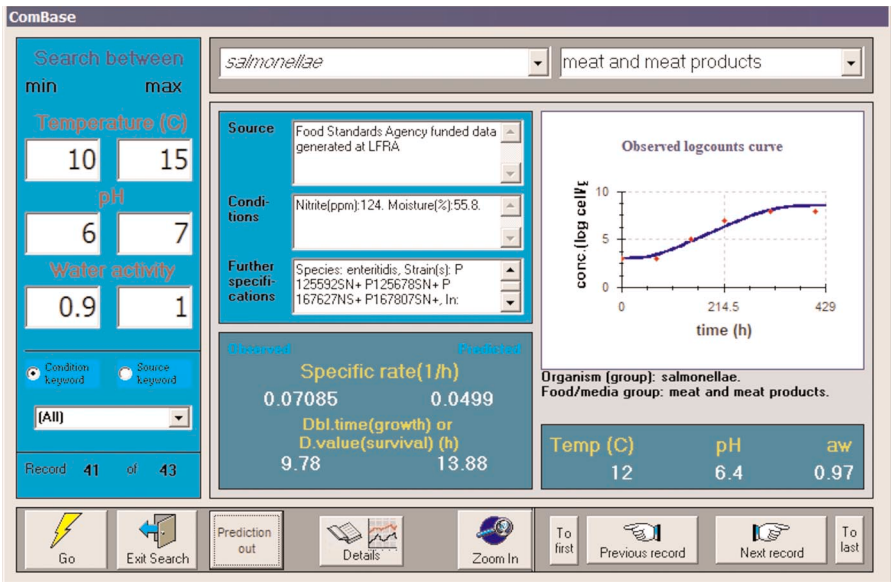

Figure 3. Query and answer screen produced by the stand-alone (not internet-based) version of the ComBase browser. It shows that, at storage temperatures between 10 and 15°C, altogether 43 records were found on the microbial responses of salmonellae in meat, with pH between 6 and 7, and water activity between 0.9 and 1. This particular "record 41" shows a growth curve measured at 12°C, pH 6.4, and aw 0.97. The raw data (red dots) can be compared with prediction (blue curve) generated by Growth Predictor.

industry, or by food regulators. From the early empirical models, a new generation of modelling approaches, together with international collaboration, have opened the door to the possibility of predicting growth and death properties for the key micro-organisms in food.

Acknowledgement

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Rapid test techniques for microbiological safety in the food industry: current status and the future

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Not so many years ago, some authors considered that the widespread use of quality assurance systems like Hazard Analysis and Critical Control Point (HACCP) in the food industry would eliminate the need for the widespread use of quality control procedures such as routine microbiological testing. History has proved that this premise was incorrect, and there is now significantly more microbiological testing ongoing in the food industry than ever before. This does not mean that HACCP is not used, or that is has not added to the overall safety of food products. Indeed, the use of the HACCP approach to risk management and quality assurance has undoubtedly increased the safety of all food products; however, food producers still see the importance of backing up standard HACCP systems with scientifically-founded routine testing. This provides verification that the HACCP system is working on a day-to-day basis and gives the food producer important back up data, showing due diligence in the way they produce food.

Microbiological testing

The routine conventional methods for testing foods for micro-organisms have remained basically unchanged for over a century. The ability of the microbiologist to count or detect extremely low levels of micro-organisms is based upon their high replication rate. Such rapid growth allows a single cell to become a colony of 10^{12} organisms after 24-48 hours incubation on agar media. It also allows detection of individual pathogenic cells, present in tens of grams of food, in a few days.

Although these growth based counting and detection systems are exceptionally good, from the point of view of the current food producer, they are slow. Modern food production methods mean that factories can produce tens of thousands of individual food packs per day. To the producer, the necessity of having to wait a number of days to get a microbiological result is a problem, as the actual microbiological status of that food remains unknown over that time. With the increase in marketing of shorter shelf life products, the microbiological result may not be known until the shelf life of the food has passed.

This problem with the analysis time of conventional microbiological methods has, over the past decades, resulted in a large amount of research into 'Rapid Methods'.

Rapid microbiological methods

A rapid method can be defined as any method or system which reduces the time taken to get a microbiological test result. As such there are many different types of method which can be considered to be 'rapid methods' and have a use within the food industry. In this paper, the major different types of method will be briefly described, and some of their positive and negative points defined.

Detection of metabolism

This title covers a wide range of test types, whose common method of operation is to detect some output from a micro-organism, which is formed during growth. Detection of metabolism in this way can be used to establish the presence or number of micro-organisms in a sample.

The key thing about these methods is that growth is required to enable the organism to metabolise and therefore be detected. Growth will always take time, and therefore these techniques will never give an instantaneous result. There are, however, always positive sides to any situation, and the requirement for growth to occur does mean that the growth media used can be 'engineered' to be specific to particular types or groups of micro-organisms. So these systems can often be used to detect a wide range of different microbial types and they can detect very low numbers, as even a single cell will grow to a detectable level over time. An 'instant' result will, however, never be obtained; the time taken to get results will vary with the exact system used. An impedance instrument or automated colorimetry may detect 10^4/g Total Viable Count or Coliforms in food, in under 12h, whilst a food containing tens per gram would take 24h to 30h. When using impedance or colorimetry, the number of micro-organisms initially in the food is related to the time taken to grow sufficiently for metabolism to be detected. To do this a calibration curve for every type of food to be tested has to be constructed.

This can take considerable time and effort; however, for many users this input is worthwhile, as results can be obtained more quickly than from conventional methods, and the high degree of automation offered by impedance and colorimetry can offer laboratories an excellent way of testing large numbers of samples quickly and cost effectively.

Chromogenic and fluorogenic media also fall into this group of methods. Over the past decade there has been a large expansion in the range of commercially available media of this type. Fluorogenic media containing Methylumbelliferyl glucuronide (MUG) were the first of the type, developed for the analysis of *Escherichia coli*. Since then a variety of other media for a range of micro-organisms have been produced and are commercially available. Whilst these media do not necessarily speed up the test, they can provide a result which is easy for technical staff to read, and perhaps give advantages over conventional media in circumstances where samples contain a high level of contamination which could interfere with colony counts on conventional media. Whilst this type of media can be excellent, users are advised to check how specific the chromogenic/fluorogenic response actually is. It will often be found that, whilst a majority of the target group of micro-organisms will cause the correct medium response to occur, in some cases a minority of the target group will not give the correct response, whilst some non-target organisms will give a response. A good example is media for *E. coli* containing either MUG or BCIG, where it is well documented that approximately 95% of all *E. coli* contain the enzyme β-glucuronidase which is responsible for giving this response.

Microscopy

Perhaps one of the first 'rapid methods' ever used in microbiology laboratories was based around the use of the microscope. This piece of equipment is usually present in all microbiology laboratories and is very underused in most cases. The microscope can provide users with a large amount of information on product microbiology or microbial identity very quickly. This 'speed' of response was recognised by microbiologists a long time ago, as techniques such as the 'Breed Smear' were developed (1914) to assess the microbiological quality of raw milk samples. One of the next major innovations was the development of the Direct Epifluorescent Filter Technique (DEFT), again for milk analysis. DEFT was the first method which attempted to do two key things during sample preparation: firstly to treat the food in order to reduce the effect of food debris on the microscopic test, by enzyme/ surfactant pre-treatment and coarse pre-filtration of a food homogenate. Secondly, to concentrate the micro-organisms to be analysed by fine membrane filtration, thus increasing the sensitivity of the test.

DEFT has been applied successfully to a wide range of food types, both liquid and solid. Often very good result correlations with plate counts have been obtained; however, two major problems exist. The first is that the commonly used staining method, based on the dye acridine orange, is not a true viability indicator. This gives a concern that DEFT counts would overestimate true viable counts due to the enumeration of dead cells. Secondly the method is highly labour intensive; a single trained operator may be able to set up and read 30 samples/day. Thus although the DEFT is a rapid method giving a count result in perhaps 30 minutes, its sample throughput is low, making it difficult to use in laboratories testing large numbers of samples per day.

Some automated methods based on microscopy have been developed to overcome the low sample throughput of DEFT. Perhaps the most successful has been flow cytometry. This method relies on the pre-staining of micro-organisms with a fluorescent dye. The stained organisms are then caused to flow through a very narrow channel underneath an epi-fluorescent microscope, connected to a light detection system. As each stained cell passes under the microscope and the dye is illuminated, a 'pulse' of light is emitted which passes up the microscope and is registered as a count by the counting system.

The method relies on a good viability stain and separation of micro-organisms from sample debris, which if present could block the narrow channel. The technique has been used to give rapid counts of bacteria and yeasts in a range of foods and, via the use of specific fluorescent antibodies, can be used to detect the presence of specific pathogenic or spoilage micro-organisms.

Luminescence

The quantification of adenosine triphosphate (ATP) using the enzyme luciferase has been used widely in the food industry. Currently many companies use ATP based systems to test the hygienic status of the food processing environment very rapidly. Such procedures detect any contaminating ATP, i.e. that contained in foods, and that from a microbial source. ATP bioluminescence can be used to detect and enumerate micro-organisms. To achieve this, some form of separation of microbial ATP from food derived ATP must be achieved. This separation is possible and a number of commercial ATP systems which allow microbial enumeration within foods are available. One of the major advantages of ATP bioluminescence is its speed. As a hygiene test, a result can be obtained within a minute, providing excellent way of assuring that correct cleaning of equipment has been done before production starts. As a microbial test, ATP bioluminescence has found a more limited use, perhaps because of its limited sensitivity to microbial numbers, requiring approximately 10^4 bacteria/ml to be present before detection occurs.

ATP bioluminescence has always been considered as a non-specific test, able to give total counts only. However, there have been attempts to make specific tests based around ATP detection systems. One such approach under investigation by Alaska Food Diagnostics involves an ATP amplification system and confers specificity by the use of specific, lytic bacteriophage, able to release cell constituents only from those cells that the phage can infect. Using this system, the specific detection of very low levels of *E. coli* O157 in food samples has been achieved within 8 hours.

Immunological methods

When considering procedures for the detection of specific micro-organisms, the use of immunological methods has proved very reliable. Immunological methods are based on a specific antibody or antibodies which will bind only to the target micro-organism. The whole success and reliability of an immunological method is absolutely dependent on the quality of the antibody used. A poor antibody will result in a poor method, perhaps giving levels of false positive or false negative results.

The first immunological detection methods for specific micro-organisms were based on the

Table 1. Examples of methods which detect microbial metabolism

Method	Example of Brand Name	Method of Operation
Impedance/Conductance Methods	Bactometer, Rabit, Malthus, Bactrac	Detects changes in the electrical properties in a growth medium as micro-organisms grow
Colorimetry	MicroFoss	Detects a change in colour or optical density, caused as microbial metabolism changes the colour of an indicator dye
Chromogenic/ Fluorogenic Media	Various media manufacturers produce such media	Growth of micro-organism causes a specific substrate to be metabolised into a coloured or fluorescent product

use of microtitre plate-based enzyme linked immunosorbant assays (ELISA), aimed at food pathogens such as *Salmonella* spp. and *Listeria* spp. There are many companies that now produce such systems for a whole range of food pathogens and toxins: e.g. *Salmonella* spp., *Listeria* spp., *L. monocytogenes, E. coli* O157, *Campylobacter* spp., staphylococcal enterotoxins, *Bacillus cereus* diarrhoeal toxin. Generally these ELISAs have been well proven, are used quite widely and give results faster than corresponding conventional methods. As an example, a conventional salmonella test will give a presumptive result in approximately three to four days, whilst an ELISA would give a result in about 50 hours. One of the problems faced by the ELISA is its limit of detection. A majority of ELISA test kits presently on the market require approximately 10^5 to 10^6 target micro-organisms to be present before a detection occurs. As specifications for foods usually require testing for the presence or absence of particular pathogens in 25g of a food sample, all ELISA tests require an enrichment to be done before the ELISA test is completed. The enrichment is designed to amplify very low levels of target cells, up to a level where they can be detected with the ELISA kit. It is the enrichment stage that makes up most of the analysis time in the ELISA test.

Various other types of immunological method have been developed, perhaps the most widely used being the lateral flow device. These systems are based on a flow of an enriched food sample along a filter strip sealed inside a plastic holder. The filter strip contains small coloured latex particles coated with specific antibodies to the organism under test. Further along the strip there is a line of immobilised antibodies; these capture the target organism, which is itself labelled with the coloured latex, resulting in a visible coloured line on the strip. Lateral flow devices tend to be easier to use than ELISAs, requiring less reagent transfer and therefore hands-on time. Their limit of detection is, however, the same as an ELISA and an enrichment is still required in order to detect very low levels of pathogens in foods.

Nucleic acid based tests

The detection of specific micro-organisms by analysis of their nucleic acid has been used for many years. It has, however, taken some time for these techniques to be put into a format that can be easily used in food laboratories. Early kits were based on the use of nucleic acid hybridisation probes. These are short sections of DNA, that are specific to sequences of nucleic acid in target micro-organisms. The target nucleic acid can be either chromosomal DNA or ribosomal RNA, and detection is usually based on colorimetric or chemi-luminescent methods. The nucleic acid based procedures are available for a wide range of food-borne pathogens such as *Salmonella* spp., *Listeria* spp., *L. monocytogenes*, *Campylobacter* spp., *Staphylococcus aureus* etc. These methods suffer from the same limit of detection issues as immunological tests, requiring 10^5 to 10^6 target organisms to be present before detection occurs. Therefore enrichment is always required, and this enrichment makes up the majority of the test time required. The advent of the polymerase chain reaction (PCR) technique allowed the development of very fast pathogen detection systems. PCR involves the biochemical amplification of specific areas of a target cell's nucleic acid. This amplification, which involves chemical reactions and not cell growth, is very fast, with a 10^9-fold increase in target being possible in around three hours. This possibility of very fast PCR-based pathogen tests was limited by one major problem with this approach. Processed foods could well contain pathogens that had been killed by the food process and these organisms would not be detected by immunological tests, as the minimum level of detection (10^5–10^6 cells/ml) requires growth during enrichment; as dead cells do not grow, they are not detected. The non-growth based amplification offered by PCR caused some concern amongst food microbiologists, in that dead target cells would be detected. In fact, commercially available methods based on the PCR method all have limits of detection of approximately 10^3 to 10^4 target cells/ml, thus all require an enrichment phase to detect low pathogen levels. As growth is required, and it would not be expected to find levels of 10^3 to 10^4 of dead pathogens in foods, the issue of dead cell detection is not of practical significance; however, the need for enrichment does increase the length of the test. Commercial PCR-based kits for the detection of pathogens have been available for a number of years. Originally they were fairly complex systems requiring gel electrophoresis to be used. Newer systems are not gel based and detection is colorimetric or fluorescent. The systems have been fairly widely used and give results equivalent to conventional methods, but with considerably shorter test times, detection of *Salmonella* spp. for example can be achieved within 30h.

Sampling techniques

In covering microbiological methods, it would be wrong not to consider food sampling methods. When trying to detect very low levels of pathogens, the question must be asked, 'how can we assure that the organism is in the sample we test'? Various methods have been considered which could concentrate cells from food samples, thus increasing the chance of their detection. Immunomagnetic separation (IMS) systems are useful in concentrating target cells from enrichment systems, and have become part of reference methods for the detection of *E. coli* O157. Various other commercial systems have employed IMS procedures to separate and concentrate food pathogens. The Pathatrix method uses a novel circulating system to maximise contact between potential pathogens in a sample, and the IMS particles. This allows a rapid detection of low levels of pathogens such as *Salmonella* spp. and *E. coli* O157 in one day. Other method manufacturers such as Alaska Food Diagnostics use IMS as a part of their concentration procedure, before the detection system is employed, thus both speeding up the method and improving its selectivity. The IGEN method on the other hand uses IMS as part of its detection system, bringing the target cells and attached chemiluminescent labels to the detection electrode by magnetic attraction.

The future

There are a wide range of novel and rapid methods available to microbiologists in the food industry. We now have methods available that can detect single individual target cells applied to the detection system. However, the challenge we now face is, how do we get the single cell in a 25g food sample (the usually specified level) into a detection system which will often only hold a few tens of microlitres, and even if we can do this how do we ensure we only detect viable and not dead cells. These are major challenges to the method developer and will take some time to solve. Whilst looking for solutions we must remember that food industry laboratories are often small, do not contain complex equipment, and have considerable financial constraints. Methods must be made easy to use, as automated as possible, and be cost effective to the user. In recent surveys of the industry, one of the biggest issues to hamper the implementation of new methods was noted to be their cost. Method manufacturers must constantly keep this is mind, methods believed to be expensive will not be used routinely; it is however very common for potential users not to fully analyse the cost benefit of new methods. Highly automated methods increase sample throughput and mean a laboratory can handle many more samples. A more rapid result can greatly speed up product release, potentially decreasing product storage requirements and possibly increasing shelf life. All of these can arise from the use of rapid methods and should be considered as benefits when considering the cost of the method itself.

DEDICATED TO MICROBIOLOGY

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