

ILSI Europe
Report Series

MYCOBACTERIUM AVIUM SUBSP. *PARATUBERCULOSIS*
(MAP) AND THE FOOD CHAIN



REPORT

Prepared under the responsibility of the
ILSI Europe Emerging Pathogen Task Force

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AND THE FOOD CHAIN***

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Bernard Mackey, Fergus Shanahan*

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EXECUTIVE SUMMARY

Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of Johne's disease in cattle, sheep and goats, and occurs in some non-ruminants, including primates.

MAP is shed from infected animals, and so may contaminate pasture, water run-off, meat and milk. Most experimental work has been undertaken, and sound data derived, by research institutions and by the milk and dairy industries. However, other sources should not be discounted.

Programmes to improve preventive measures undertaken on the farm, in order to minimise transmission within and between herds and so reduce shedding into the environment, offer the most effective and confident means for better control in the future.

Heat treatment is the most effective measure to reduce MAP during the manufacture of milk and milk products, and of meat and products derived from meat. However, anomalously high heat tolerances of fractions of MAP populations have been reported, though not explained, and low level survival has been demonstrated in some surveys of commercially pasteurised milk. It is uncertain how effective processes, such as milk centrifugation and ultrafiltration, may be in helping to reduce residual contamination levels. MAP is relatively resistant to chlorination and other disinfection methods.

There have been reports of a potential association between MAP and Crohn's disease in humans. At present the complexity of the human disease is such that definitive answers cannot be given, though most recent research does not support a causal link. However, since different researchers have different opinions, the possibility should not be ignored. Issues that remain, therefore, include the possibility of an association of MAP with the disease and, should there prove to be an association, the dose response relationship that would allow a proper risk assessment to be undertaken, targeting all possible sources of MAP.

The report has identified a number of gaps in our understanding, particularly with respect to the heat tolerance of MAP, and to sources of MAP other than dairy, especially water and meat.

INTRODUCTION

M*ycobacterium avium* subsp. *paratuberculosis* (MAP) is a slow-growing, hydrophobic, acid fast, rod-shaped bacterium that is the causative agent of Johne's disease, or paratuberculosis, in ruminants, including cattle, sheep and goats. It also occurs in some non-ruminants - including primates. Johne's disease is a fatal, incurable, chronic inflammatory bowel disorder. It has been reported to contribute to large financial losses in the agriculture industry in some countries, e.g. the USA (see 'Occurrence and spread' below).

There have been reports of an association of MAP with Crohn's disease in humans, although evidence is currently insufficient to confirm or to disprove an aetiological or participatory role.

With regard to the possible exposure of humans to the organism via food, most studies have targeted milk. Consequently there are considerable milk-related data, though this should by no means indicate that milk is the only, or the main source for transmission. MAP may be present in the milk of animals that are infected symptomatically or asymptotically, derived from faecal contamination or directly from the udder.

Many laboratory studies suggest that standard milk pasteurisation times and temperatures of processing, by holder treatment and by high temperature short time (HTST) treatment, should satisfactorily eradicate MAP from milk. Other reports indicate low levels of survival, even after extension of HTST pasteurisation times from 15 to 25 sec at 72°C, and at temperatures as high as 90°C. Furthermore, survival of MAP has been reported in studies employing pilot plant and commercial pasteurisation units, and some surveys of retail pasteurised milk have detected surviving MAP. While such unexpected survival is not fully explained, it may be exacerbated by the strong tendency of the hydrophobic MAP cells to adhere to each other to form large clumps. The purpose of this review is to summarise and document up-to-date information about MAP, and to highlight its significance in the food chain by:

- reviewing the veterinary and human health implications of MAP
- reviewing the ecology of MAP in the food chain
- identifying farm and food safety management options which, in the short and in the longer term, will assist in better controlling MAP.

MAP IN VETERINARY HEALTH

Pathogenesis of Johne's disease

Paratuberculosis, or Johne's disease, is an infectious disease caused by MAP in cattle, goats, sheep, deer and South American camelids. Neonatal and juvenile animals are at highest risk of acquiring infection, through the faecal-oral route. MAP targets the mucosa-associated lymphoid tissues of the gastrointestinal tract. Survival within macrophages is characteristic of the organism. Cytokine production and the initiation of a cellular immune response causes the appearance of intestinal granuloma, and a cellular response is initiated in the nearby lymph nodes in an attempt to clear the infection (Harris & Barletta, 2001). The inflammatory process leads to the clinical manifestations of a corrugated intestinal epithelium and the corresponding characteristic malnutrition syndrome associated with the disease.

Occurrence and spread of Johne's disease

Johne's disease is common in dairy herds worldwide, where it causes significant losses through decreased milk production, animal deaths, low weight at slaughter and replacement costs. The disease has been spreading through domestic livestock for many years (Kennedy *et al.*, 2001). For example, it is estimated that about 22% of dairy and 8% of beef herds in the USA are infected, and 10% and 3% respectively in Belgium (Boelaert *et al.*, 2000). As a consequence, financial losses to the agriculture industry are substantial, for instance they are estimated at about \$1.5 billion annually in the USA (Stabel, 1998). The primary means of introduction of infection into a herd is through the acquisition of infected cattle. These may test negative at the time of purchase, but later shed the organism and spread the disease. Successful control of Johne's disease represents a particular challenge for a number of reasons. For example: infection may occur in the calf at an early age, but recognisable clinical disease occurs usually about two years later; MAP is able to survive well in the environment; the disease has a long incubation period; diagnostic tests have poor sensitivity during that period.

MAP IN HUMAN HEALTH

Pathogenesis of Crohn's disease

The pathogenesis of Crohn's disease is complex and does not appear to involve a simple cause and effect relationship. There are three major interacting elements: genetic susceptibility factors; immune-mediated tissue injury; and environmental modifiers, such as the enteric microbial flora. Intestinal inflammation seems to be due to unrestrained immune response to components of the intestinal bacterial flora in individuals who are genetically susceptible. The role of genetic factors is well established and the first susceptibility gene (CARD15, NOD2) has been identified. Mutations of CARD are associated with defective innate immune responses to bacterial components such as peptidoglycans. The contribution of intestinal bacteria is supported by several clinical observations in humans and by studies in genetically defined animal models (Shanahan, 2002). Genetic defects either at the level of mucosal barrier function or at the level of immunoregulation have been associated with diseases like Crohn's in animals, but colonisation with bacteria is necessary for disease development irrespective of the underlying genetic defect. Commensal microorganisms such as *Bacteroides* spp. and other Gram-negative organisms, including those which are unculturable at present, that are part of the normal intestinal flora, rather than pathogenic infectious agents drive the inflammatory disease in these animal models (Shanahan, 2002). Hugot *et al.* (2003) have suggested that predominant use of refrigeration may have contributed by encouraging increased exposure to components of psychrotrophic bacteria that normally do not provoke the immune response.

The role of non-genetic (environmental) factors in Crohn's disease is shown by the incomplete concordance rate in monozygotic (identical) twins (<50%). In addition, an environmental influence is likely to account for the changing epidemiology of Crohn's disease. Increases in incidence and prevalence of Crohn's disease as countries become socio-economically developed have occurred over too short an interval to be explained by changes in population susceptibility genes (Ekbohm, 2003). However, the changing epidemiology of Crohn's disease within developed countries has been accompanied by increases in other inflammatory disorders that involve a disturbance in immunoregulation. These include asthma, insulin-dependent diabetes and multiple sclerosis (Bach, 2002). It is unlikely that each of these conditions is due to separate infectious agents targeting different end-organs. A more likely explanation is that environmental conditioning occurs at the level of immunoregulation. Changes in modern lifestyle and reduced microbial exposure have influenced mucosal immune development and may be a risk factor for inflammatory bowel disease. In addition, genes that once conferred resistance to infectious diseases in an unsanitary environment might become risk factors for excessive immunoinflammatory responses in the setting of a modern lifestyle and sanitised environment.

Possibility of MAP involvement

The discovery of a link between *Helicobacter pylori* and peptic ulcer disease helped to heighten awareness of the possibility that infectious agents might be the cause of other complex disorders like Crohn's disease. While various infectious agents have been proposed as possible causes of Crohn's disease, MAP is the most enduring candidate among the pathogens. In particular, studies of the occurrence of MAP in mucosal biopsy specimens from individuals with and without Crohn's disease have been claimed to be especially significant. For example, Bull *et al.* (2003) used culture methods and PCR techniques for MAP specific IS900 insertion sequences to detect MAP in 34 of 37 (92%) of such samples from patients with Crohn's disease, and in 9 of 34 (26%) of samples from control patients without Crohn's disease, but with non-inflammatory bowel disease. Sechi *et al.* (2004) found no MAP microscopically, but detected IS900 DNA in 69% of wax-embedded intestinal tissue samples from Crohn's disease patients. On the other hand, other studies have failed to find any correlation. For example, Bernstein *et al.* (2003) performed

colonoscopy and biopsy sampling of patients with Crohn's disease (n=24), patients with ulcerative colitis (n=28), unaffected siblings (n=9), and controls without inflammatory bowel disease (n=28). PCR testing for MAP in mucosal samples was positive for one patient with ulcerative colitis, but for no patients with Crohn's disease, nor for any of the siblings, whereas 6 of 19 healthy controls were MAP-positive. Baksh *et al.* (2004) found no IS900 DNA in granulomas from paraffin-embedded resection specimens of 18 patients with well-established Crohn's disease. Bernstein *et al.* (2004) found no differences in MAP seropositivity rate among Crohn's disease patients, ulcerative colitis patients, healthy controls, and unaffected siblings, in a Manitoba study. The data seemed to refute an association of MAP with Crohn's disease, but the high seroprevalence in Manitobans raised the possibility that the high rates of Crohn's disease in Manitoba could somehow be related to high exposure rates to MAP. While the occasional presence of MAP may be compatible with a participatory role, it may also be secondary in that its presence, and that of other bacteria within the mucosa, simply reflects opportunistic association, encouraged by defective immunity.

Representative arguments and counter-arguments (facts that have been presented against a relationship of MAP with Crohn's disease) for involvement of MAP, and explanatory comments, are summarised in Table 1.

Facts that have been proposed as counter-arguments include the following. Firstly, there is paucity of evidence for the horizontal or vertical transmission that one would expect from an infectious agent. Secondly, Crohn's disease is less common in rural areas and is not an occupational hazard of farming, where maximal exposure to MAP would be expected (Ekbom, 2003). Thirdly, environmental conditions, such as poor sanitation, endemic parasitism and overcrowding, which should favour infectious transmission, actually appear to protect against Crohn's disease. Fourthly, there is no evidence for MAP in animal models of Crohn's disease. Fifthly, the most compelling clinical argument against persistent MAP or other infections as a cause of Crohn's disease is the clinical experience with anti-TNF- α (infliximab) therapy. Tumour necrosis factor (TNF) is a pivotal mediator of the inflammatory process in Crohn's disease. It is also required for activation of macrophages in defence against intracellular infections such as mycobacterial infections. Intravenous administration of the monoclonal antibody, infliximab, has been shown in well-conducted clinical trials to be therapeutically effective in Crohn's disease and has been approved for treatment of that condition in Europe and the United States. This anti-TNF therapy is not only effective in healing intestinal lesions but also has been shown to be effective in maintaining remission when repeated infusions are given at two-monthly intervals for up to a year. Therapeutic blockade of TNF- α creates sufficient immunosuppression to be a risk for disseminated tuberculosis caused by the closely-related *Mycobacterium tuberculosis* (Keane *et al.* 2001), but has not been associated with disseminated MAP in patients with Crohn's disease. It is difficult to understand why an infection putatively stimulating the intense inflammatory reaction characteristic of Crohn's disease should respond to long-term suppression of immune defences. While none of these arguments is necessarily convincing alone, together they cast considerable doubt on a causal role. In support of the counter-arguments for a role for MAP in Crohn's disease outlined in Table 1, there are specific clinical and epidemiological features of the disorder that are at odds with a putative transmissible agent as a direct cause.

It is noteworthy that only two case reports claiming chronic MAP infection in humans have been described (Hermon-Taylor *et al.*, 1998; Greenstein, 2003). The significance of the finding and relation to Crohn's disease in one case has been questioned (McDonald, 2001), and the other case in an immunodeficient individual highlights the extreme rarity of MAP, even in subjects with defective immune defences (McDonald, 2001). Van Kruiningen (1999) reported that MAP isolated from Crohn's patients were unable to infect ruminants. De Hertog and Geboes (2004) recently reviewed the complexity of the numerous proposed links between Crohn's disease and common gastrointestinal pathogens, some of which can cause infections that even mimic the disease.

Table 1. Potential role of MAP in Crohn's disease

Argument	Counter-argument	Comments
Crohn's resembles Johne's disease in animals, which is caused by MAP.	The similarity is superficial, with several points of dissimilarity.	Crohn's is spontaneously relapsing and remitting, whereas Johne's is progressive. Unlike Crohn's, Johne's is not associated with extraintestinal associated diseases. Crohn's responds to immunosuppressants and steroids, in contrast to Johne's.
Crohn's shares features with intestinal tuberculosis.	Unlike Crohn's disease, intestinal tuberculosis does not respond to immunosuppressants or steroids.	Tissues have limited response to insult and some similarity across different diseases is expected.
MAP infection in humans has been reported suggesting that it may be a zoonotic organism.	Only one or two reports implicate a linkage with Crohn's disease, but do not prove a cause-and-effect relationship.	The extreme paucity of reports suggests that this is a rarity, even in immunosuppressed patients.
MAP has been reported to be detectable by molecular methods in tissues from Crohn's disease.	Presence of MAP and other bacterial DNA within the mucosa probably reflects either defective innate immunity to enteric bacteria or may be secondary to disease-induced defects in barrier function and does not represent a specific infection.	Reports have been inconsistent or conflicting, but this might reflect variations in technique. Reports of MAP in controls and in ulcerative colitis cast doubt on the specificity and pathogenic significance.
MAP has been cultured from Crohn's disease tissue.	MAP is also isolated from healthy subjects and not from all patients with Crohn's disease.	Long-term culture is required, creating the risk of contamination artefacts. There is also a risk of contamination from faecal contents.
Seroreactivity to MAP in Crohn's disease has been reported by some investigators.	Seroreactivity is weak, inconsistent, and may be due to cross reactivity.	Cellular reactivity to MAP would be more relevant, but is weak or non-detectable in Crohn's disease. This is at variance with an infection putatively stimulating such an intense inflammation.
Responsiveness of Crohn's disease to anti-mycobacterial therapy.	Good responses are evident primarily with broad spectrum macrolides, suggesting non-specific antibiotic effect.	Some antibiotics that have been used have immunomodulatory properties which might influence disease activity without implying microbial involvement (e.g. metronidazole).

A recent report (Naser *et al.*, 2004) describes the detection of MAP DNA in peripheral blood of a subset of patients with Crohn's disease (40-50%). However, the finding lacks disease specificity because a similar proportion of patients with ulcerative colitis were also found with the same result. Indeed, the authors of the report also found MAP DNA in 20% of subjects without inflammatory bowel disease. This lack of disease specificity calls for caution in the interpretation of the tests results.

In conclusion, evidence for infection with MAP in humans is sparse. The case for MAP involvement in Crohn's disease is unproven. Most assessments suggest that it is unlikely to be a causative factor. Furthermore, the pathogenesis of Crohn's disease in humans can be explained without invoking an infectious agent, and animal models indicate that a Crohn's-like disease can occur without involving a pathogenic infection. Although the possibility that MAP might account for a subset of Crohn's disease or might have a modifying effect on established disease cannot be definitively excluded, MAP infection is unlikely to be a causative factor for the majority of cases of Crohn's disease.

ECOLOGY OF MAP

Animals

A major source of MAP in the environment is the excretion of large numbers of organisms in the faeces of infected animals. While domestic ruminants, especially cattle but also goat and sheep are important sources, MAP also contaminates a number of wild animals, including deer, rabbits, foxes, stoats, badgers and wood mice, and birds such as jackdaws and rooks (Beard *et al.*, 2001; Stehman *et al.*, 1996). A question remains as to whether there is transmission between these species, because there is no evidence of active disease in wild non-ruminants.

An important aspect of the ecology of MAP is that there will most likely be no further multiplication of the microorganisms after they have left the host, especially since specific growth factor requirements are unlikely to be satisfied in the wider environment. Consequently, along the food chain, a progressive dilution will occur. Excreted organisms may contaminate pastures, water run-off, meat and milk, or milk may be contaminated directly via the mammary gland (Sweeney *et al.*, 1992). Numbers in milk ranged from 2 to 8 cfu per 50ml in culture-positive samples. Positive samples ranged from 3 to 19% in light to heavy shedder cows (Sweeney *et al.*, 1992).

MAP may become widely distributed within the tissues of infected animals, so that its occasional presence in meat and meat products is likely. For example, examination of thin cows after slaughter in the USA yielded positive results for MAP in 11% of dairy cows and 0.7% of beef cows. Sampling sites were gut, liver and meat-associated lymph nodes (Rossiter & Henning, 2001). Ingestion is therefore possible, at least in raw or undercooked meat. It should also be considered that cow's meat in particular is often used to manufacture meat products such as sausages and ground or minced meats, which could lead to the contamination of larger lots of respective products. Furthermore, it could be important that dry-cured and fermented meat products are normally manufactured without heat treatment and then eaten without cooking, so that no MAP-lethal step occurs prior to ingestion, but there are no data on the incidence or the resistance of MAP in such foods.

Water and the environment

Since cattle with severe disease may shed more than 10^{12} organisms onto pastures daily (Chiodini *et al.*, 1984), there is the possibility of contamination of water supplies from run-off (Grant, 1997). MAP has been shown capable of remaining viable for at least 163 days in river water, at least 270 days in pond water, and at least 330 days in bovine faeces and soil (Chiodini *et al.*, 1984). Ward and Perez (2004) reported that MAP survival was enhanced in loamy soils with high contents of sand or silt. The possibility of MAP entering domestic water supplies has been suggested by studies in the USA demonstrating survival of other mycobacteria through municipal water treatment plants (Mishina *et al.*, 1996), and by the observation that MAP is relatively resistant to chlorination (Whan *et al.*, 2001).

A recent study from the Czech Republic showed that MAP may be present in the stems, leaves and fruits of tomatoes, radish and lettuce when grown on soil contaminated by the use of manure (Pavlik *et al.*, 2002). MAP survived in the soil, at $< 6^{\circ}\text{C}$, for at least 113 days, and contaminated the plants within four weeks of planting (Pavlik *et al.*, 2002).

INTERVENTION MEASURES

Farm

Control of Johne's disease within and between herds is considered to be feasible with existing technologies, but with considerable difficulties (Kennedy, 2001; Kennedy *et al.*, 2001), and with greater difficulty for large rather than for small sized herds (Groenendaal *et al.*, 2002). Progress is likely to be slow, and depends on strong commitment of and incentives for farmers. Key elements of control being pursued include:

- Improved calf hygiene to prevent infection (prevention of exposure to manure of adults)
- Identification and removal of infected animals
- Inspection and identification and removal of suspected animals
- Introduction of animals only from herds thought to be free of infection
- Protection of herds thought to be free of infection and regions by:
 - Maintenance of biosecurity and best hygiene practices
 - Regular monitoring of infection status
 - Assistance with control if infection/disease is detected

Farm hygiene

Since Johne's disease is very common within animal populations of domestic ruminants, management practices are considered to be the most important tools for controlling paratuberculosis in domestic livestock herds. For control and eradication programmes to be effective therefore, it is generally accepted that extensive husbandry measures should be undertaken along with intensive diagnostic testing. Husbandry measures have two main objectives: firstly to prevent the spread of a possible infection within a herd, and secondly to prevent introduction of infection into a disease-free herd. The most important management practices that have been identified are overall cleanliness of the farm, careful manure handling, hygienic calving procedures, newborn calf care, and restriction of contact between calves and mature animals (Goodger *et al.*, 1996). To prevent spread of the disease, prevention of infection of young, newborn animals is especially critical. It is important to ensure clean calving pens, separate housing of young and older animals and feeding of colostrum and milk only from non-infected mothers. Fischer *et al.* (2004) detected MAP in blowflies that had fed on infected cattle or waste, and suggested they should be targeted during herd sanitation procedures and in slaughterhouses.

Primary sourcing

To prevent the introduction of infection, it is important to maintain a closed herd. This includes not purchasing animals from farms with an unknown history of paratuberculosis, but also not spreading as fertiliser manure from other farms. Banning positive herds from trading will prevent spread while activation of test-and-cull programmes helps to eradicate the disease, with replacement stock then being purchased from certified free herds.

Transfer of animals of unknown disease status between herds is a major impediment to control, so that action to prevent acceptance of infected animals into herds, which depends on effective diagnosis of diseased animals, is fundamental. A critical management tool is therefore herd testing. However, diagnosis presents a major challenge, firstly because confident detection is difficult, and secondly because detection is unlikely before the animal has progressed to the later stages of the disease. During the early stages of the disease animals are clinically normal and current diagnostic methods are unable to detect an immune response or intermittent shedding of the organism. Because of the limited sensitivity of diagnostic tools with individual animals, herd testing gives a better performance.

Faecal culture and determination of antibody response by enzyme-linked immunosorbent assays (ELISAs) are the two major means of detecting Johne's disease in a herd. While faecal cultures are effective for the detection of cattle that are excreting MAP, a disadvantage is the slow growth of the organism in laboratory cultures. A polymerase chain reaction (PCR) can detect MAP within three days, but is expensive, and requires more skilled technicians than do culturing methods. Antibodies to MAP can be detected in the serum of infected animals (by complement fixation, agar gel immunodiffusion, and ELISAs), but the slow development of the disease delays detection until its later stages.

Economic losses from Johne's disease are primarily due to premature disposal of animals and reduced milk production (van Schaik *et al.*, 1996). A vaccine that prevents animals from becoming infected would therefore be particularly valuable. Live and heat-killed vaccines have been developed, and have been commercially available for many years. Both types are capable of eliciting both cellular and humoral immune responses, and provide partial protection, reducing faecal shedding in cattle, the number of clinically affected cows, and the number of animals testing positive bacteriologically or histologically. However, vaccines are not yet completely effective in preventing disease, and may allow continued shedding. A really effective vaccine would offer a viable option for control of the disease.

Presently there are no antibiotics that can be routinely used for the treatment of Johne's disease in livestock. Attempts to treat paratuberculosis with antimicrobial agents have been inconsistent or delivered only temporary results, and are expensive and unrewarding.

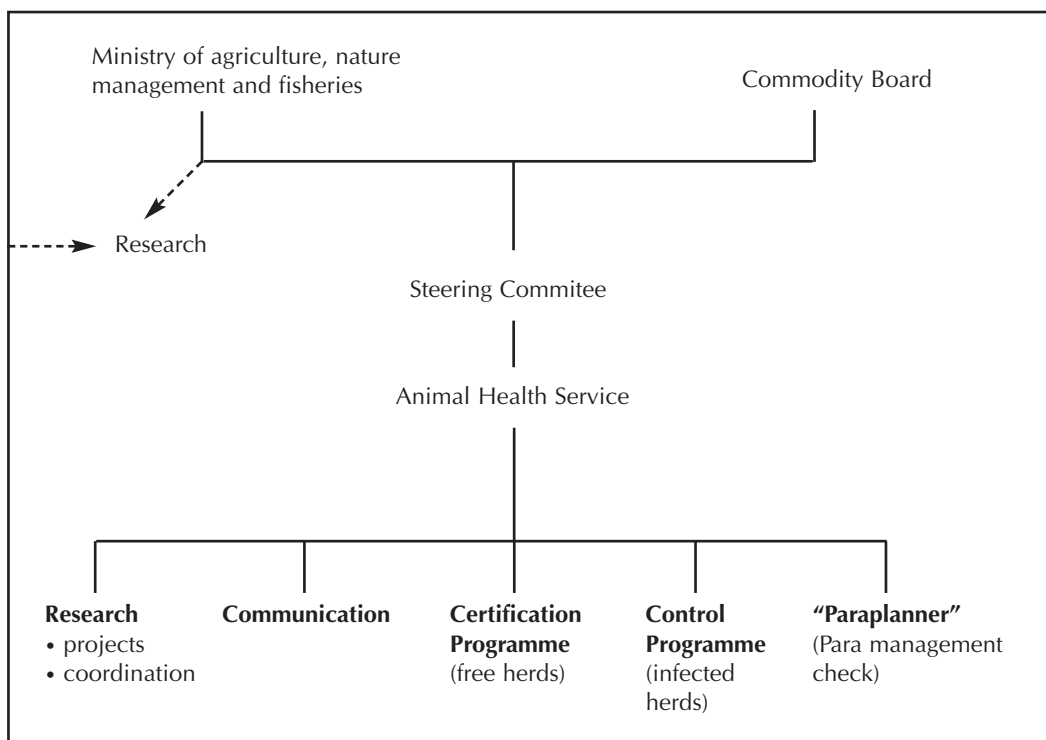
Without effective curative treatments or vaccination procedures, control methods are therefore primarily directed towards the introduction and maintenance of sound management techniques to clear herds of disease. Because single diagnostic tests detect less than half of infections, and only in older animals, long-term, dedicated efforts are required.

The critical importance of calf hygiene was highlighted by a modelling study of control programmes for MAP in mid-sized dairy herds in the USA (Groenendaal & Galligan, 2003). It was concluded that test-and-cull strategies alone do not reduce the prevalence of paratuberculosis in cattle, and they are costly for producers to pursue. Vaccination did not reduce prevalence although it was economically attractive. Improved calf hygiene strategies were found to be critically important and were also economically attractive.

Lack of compliance with herd management recommendations or insufficient time on a programme, hinders success.

Overall, therefore, because control of paratuberculosis is of interest not only to the individual farmer, programmes supported by the cattle industry and its partners and governments are necessary. The structure of such a programme is illustrated in Fig. 1 (Franken, 2002).

Figure 1. Proposed structure for paratuberculosis control programmes (from Franken (2002))



The steering committee in the structure should consist of representatives of the Ministries responsible for agriculture, nature management and the environment, human health, and include representatives of the cattle-related industries (farmers' organisations, the dairy industry, and the relevant animal health organisation). The animal health organisation should run the different activities of the programme. These are the overall management activities of the programme, and also include research projects, and the certification and control programmes.

As the most important preventive tool, the programme should ensure that the dairy herds apply preventive measures as stated in the relevant guidelines. Communication is an important issue and as such should have its own place and budget in the programme.

The costs of the programme for activities on farms, such as certification, control measures and management checks, should most logically be directly charged to the farmer. There is additional funding needed for programme management, communication and research (Franken, 2002).

The importance of costs was emphasised by Dufour *et al.* (2004) and by Pouillot *et al.* (2004). They undertook modelling and cost-benefit studies and concluded that herd-level certification procedures are not economically profitable at present in French cattle herds, but that this could change if certification costs decreased, for example if cheaper and effective diagnostic tests became available. The UK Department for Environmental and Rural Affairs has circulated similar draft guidelines for veterinarians and dairy farmers, aimed at reducing MAP levels in dairy herds and consequently in milk (DEFRA, 2004).

Food

Although it is possible that MAP may gain access to a number of types of food, by far the most attention has been given to milk.

MAP in retail pasteurised milk

Whatever the 'true' heat resistance of MAP cells in milk (see below), while some surveys of retail samples of pasteurised milk for MAP have been negative (e.g. all of 396 samples in a survey in the Republic of Ireland; O'Doherty *et al.*, 2002) others have identified the sporadic presence of viable cells (Grant *et al.*, 2001). Taken alone, such identification is not evidence of exceptional heat resistance. Failure in commercial processing may occur due to inadequate processing (inadequate holding times, leaks in valves, heat exchangers etc) or post-process contamination. Of course, definitive conclusions regarding survival after heat treatment can only be reached if it is also shown that pasteurisation was properly applied, and that no post heat-treatment contamination occurred, i.e. coliforms and other Gram-negative bacteria were absent from the milk after heat treatment. However, from the public health standpoint, the presence of MAP is important rather than the rationale for their occurrence.

Millar *et al.* (1996) reported the presence of MAP in retail pasteurised milk in England and Wales using PCR to detect the MAP-specific IS900 DNA sequence. Over an 18 month period, 7% of 312 samples were found to be positive, though not culturable, so that viability was not proven. Gao *et al.* (2002) found that 15% of retail milk samples from stores and dairy plants in south western Ontario were MAP-positive by PCR, but not by culture. Viability was proven, however, in a study of 827 raw and commercially pasteurised milk samples from 241 dairies in England, Wales, Northern Ireland and Scotland over a 17 month period (Grant *et al.*, 2000). Two percent of samples were culture positive for MAP, and of these, 70% were from samples processed at 72 to 75°C for 25 sec.

MAP was detected, but was not culturable, from one of 104 samples of raw sheep and goat's milk (Grant, 2002c), but Muehlherr *et al.* (2003) found 23.0% of raw goat's tank milk samples and 23.8% of raw ewe's tank milk samples in Switzerland to be PCR-positive for IS900, providing presumptive evidence of MAP.

MAP fate during cheese making

Little work has been done so far to determine the survival of MAP in dairy products other than pasteurised cow's milk. Donaghy, Totton & Rowe (2003) used an improved, laboratory-based method to contaminate 800g blocks of cheddar cheese. MAP survived the cheese making process. Syneresis of the curd caused a 1 log concentration of numbers of cfu of one strain of MAP, though not of another, from milk to cheese. Survival during subsequent storage was not tested. However, data were published by Sung & Collins (2000) about the survival of MAP in soft cheese (Hispanic style, 2% NaCl, pH 6.15). In cheese made from milk spiked with non-heated MAP at a level of 10^4 cfu ml⁻¹ a decimal reduction time of 59.9 days was estimated. Using sub-lethally injured cells (62°C for 240 sec) the decline was faster, resulting in an estimated D-value of 36.5 days. It is obvious that in such types of cheese, with high a_w -values, low contents of NaCl, and pH values close to neutrality, only moderate reductions of MAP can be expected.

Spahr & Schafroth (2001) used raw milk spiked at a level of 10^4 – 10^5 cfu ml⁻¹ (declumped cells) to manufacture hard cheese (Swiss Emmentaler) and semi-hard cheese (Swiss Tilsiter). Calculated D-values for the hard cheese were 27.8 days, for the semi-hard cheese 45.5 days. After 120 days of ripening, MAP at low levels were still detected. A probable 3-4 log reduction was estimated during ripening.

Processing options

Thermal inactivation of MAP

Although heating is the major process that will inactivate any MAP cells that gain entry to food, there remain difficulties in obtaining reliable heat resistance data.

Accurate counts of numbers of MAP cells surviving particular heat treatments have not been easy to obtain for three principal reasons: (i) the growth rate of the organism in media is very slow, so that incubation times up to as many as 20 weeks, or even up to one year, are necessary to record positive growth; (ii) hydrophobic mycobacterial cells tend to clump (Grange, 1996), so that groups of adhering organisms containing hundreds, thousands, or even millions of individuals may give rise to single colony forming units (cfu) and then greatly affect the outcome of experiments (Klijn *et al.*, 2001); (iii) hydrophobic cells congregate at liquid surfaces and in films on the sides of tubes and pipettes resulting in erratic transfer of cells down dilution series, with consequent inaccurate estimation of true viable numbers (Gould, personal communication).

A further difficulty in interpreting published data has been that different groups of workers have employed different heating and recovery techniques, so that laboratory-to-laboratory comparisons are sometimes difficult to make (Lund *et al.*, 2002). While this is of obvious importance, it is unfortunate that there is no single accepted protocol covering the recovery, culture and identification of MAP (consideration of conditions such as those summarised in Table 2 may help in the future). Nevertheless, a number of research groups have obtained MAP heat resistance data in the laboratory. Some of the most relevant log reductions reported following heating at 63°C for 30 min, or at 72°C for 15 sec, are summarised in Table 3. The results were obtained using cultured cells inoculated into raw milk or (Keswani & Frank, 1998) into UHT milk. Different methodologies employed are summarised in Table 3 (Lund *et al.*, 2002).

Table 2. Conditions for a uniform experimental design for heating experiments with milk¹.

Inoculum	Heating technique	Resuscitation	Decontamination	Incubation period
Type and origin of strains. Growth conditions ² . Homogenisation & sonication ³ .	Laboratory batch processes (capillaries, tubes, plastic bags etc). Commercial continuous processes ⁴ .	Heated MAP will be injured, so some form of resuscitation should be employed ⁵ .	If raw milk is used, overgrowth by components of the normal milk flora, particularly bacilli, can be expected. Chemical inhibitors may further injure MAP ⁶ .	Growth of non-injured MAP on standard media requires 8-12 weeks at 37°C. Reported incubation times range from 12 to 52 weeks ⁷ .

1 Considerations at the joint Federal Dairy Research Center/IDF Workshop, May 2003, Kiel, summarised by Hammer (personal communication). The proceedings of the workshop have been published in the IDF bulletin (IDF, 2004).

2 Resistance factors, such as acid tolerance can depend on growth conditions (Sung & Collins, 2003).

3 After disruption of clumps, survival is reduced. The mechanism of this phenomenon is not known, but is not a result of poor heat penetration into a clump, which is estimated to take 2-3 hundredths of a second (Davey, 1990).

4 Applicability of laboratory batch-derived D-values for the design of commercial processes may be questioned. Lethality in continuous processes is strongly dependent on uniform heating and turbulent flow. Knowledge of residence time distribution are necessary for full evaluation.

5 Grant *et al.* (2002b) allowed recovery at 4°C overnight before evaluating survival in heated milk samples. Hammer *et al.* (2002) performed resuscitation in a modified Dubos medium for up to 6 months.

6 It is still under discussion which chemical agent is the most appropriate, and whether heat injured MAP might be further injured by its use (Grant *et al.*, 2002; Hammer *et al.*, 2002; Pearce *et al.*, 2001).

7 It is likely that the shorter the incubation time, the lower the probability of detecting surviving MAP cells.

Table 3. Laboratory determinations of MAP heat resistance

Heating at 63°C for 30 min			Heating at 72°C for 15 sec			Reference
Inoculum (cfu ml ⁻¹)	Decimal reduction	Methodology employed in MAP heat resistance studies	Inoculum (cfu ml ⁻¹)	Decimal reduction	Methodology employed in MAP heat resistance studies	
10 ⁴	<2	10ml volumes in tubes in a water bath	10 ⁴	<2	Double boiler with continuous mixing	Chiodini and Hermon-Taylor (1993)
10 ⁶⁻⁷	5-6	5ml volumes in stoppered tubes immersed in a water bath	10 ⁶⁻⁷	4.3-6	250 ml heated in a batch pasteurising unit	Grant <i>et al.</i> (1996)
10 ³⁻⁴	2-3.7		10 ³⁻⁴	2-3.7		
			10 ⁵⁻⁶	5.6-6	250 ml heated in a batch pasteurising unit	Grant <i>et al.</i> (1999)
			10 ⁵	5	Small scale flow-through pasteurising unit, laminar flow	Hope <i>et al.</i> (1996)
			10 ⁶⁻⁷	0.5-3	Small scale flow-through pasteurising unit, laminar flow	Stabel <i>et al.</i> (1997)
			10 ^{5.5-6}	>4.5-5	Small scale flow-through pasteurising unit, laminar flow	Stabel <i>et al.</i> (1997)
10 ⁵⁻⁶	>6	0.1 ml suspension added to 1.5 ml preheated milk in vials, sealed and immersed in water bath	10 ⁵⁻⁶	1-2	0.1ml suspension added to 1.5 ml preheated milk in vials, sealed and immersed in water bath	Sung and Collins (1998)
10 ⁶⁻⁷	>6	0.05 ml volumes in capillary tubes immersed in water bath	10 ⁵	4	0.05 ml volumes in capillary tubes immersed in water bath	Keswani and Frank (1998)
			10 ⁶	>6	Pilot scale flow-through pasteurising unit	Rademaker <i>et al.</i> (2002)

In addition, Gao *et al.* (2002) heated MAP at levels of 10³, 10⁵ and 10⁷ cfu ml⁻¹ in 2 ml samples of raw and UHT milk, in tubes in water baths, at 63°C for 30 min and at 72°C for 15 sec (Canadian Dairy Code). No survivors were detected from the batch treatment, but MAP was detected in two of 11 HTST simulations, from inocula of 10⁵ and 10⁷ cfu ml⁻¹.

Three of the reports summarised in Table 3 showed >10⁵ fold kills following heating at 63°C for 30 min, whereas two reports showed only <10² to 10^{3.7} fold kills. It is not clear why such small kills were recorded by Chiodini & Hermon-Taylor (1993). Technical problems are known often to result in erroneously high survivor estimations, sometimes enormously so (Donnelly *et al.*, 1987), particularly for hydrophobic cells. The low kill obtained from a small inoculum, but high kill obtained from a large inoculum (Grant *et al.*, 1996), are difficult to reconcile. Grant *et al.* (1996)

demonstrated severe 'tailing' of MAP survivor curves, with 1 in 10^6 or so cfu ml⁻¹ apparently hardly reducing between 10 and 30 min of heating time at 63.5°C. If such tailing were due to clumping, the same fraction of survivors would be expected from high and from low inocula.

In 6 of the 10 studies involving heating at 72°C for 15 sec, the process delivered >10⁴ fold reductions in MAP cfu, but in four of the studies reductions were only 10¹ to 10^{3.7} fold (Table 3). Stabel *et al.* (1997), recording low kills, used a laboratory scale flow-through pasteurising unit in which calculations by Hasting *et al.* (2001) concluded that laminar flow would occur such that the fastest moving particles would be at the desired target temperature for only 7.5 sec. Keswani & Frank (1998), obtaining a 10⁴ fold kill, heated 0.05 ml volumes in capillary tubes immersed in a water bath, which should have delivered rapid heat-up and satisfactory total treatment.

Stabel *et al.* (2001) summarised the various heating and culturing methodologies that have been employed, pointing out the difficulties in comparing data from different research groups and the sometimes questionable methods used, but concluded that overall the data suggested that HTST pasteurisation should result in a 5 to 6 log kill of MAP. However, this conclusion is not easily reconcilable with the reported survival of MAP in HTST pilot plant studies and its occurrence in retail pasteurised milk.

Several studies have evaluated the efficacy of pilot plant or commercial pasteurisers in the inactivation of MAP. The major elements of four key ones are summarised in Table 4.

The conclusions of the authors from the studies summarised in the table were:

- Properly maintained and operated equipment should ensure the absence of viable MAP in retail milk and other pasteurised dairy products. An additional safeguard is the widespread commercial practice of pasteurising 1.5°C to 2.0°C above 72°C (Pearce *et al.*, 2001).
- Pasteurisation conditions applied in the dairy industry seem sufficient to inactivate MAP (Rademaker *et al.*, 2002). Results support the conclusions of Pearce *et al.* (2001).
- Low level survival of MAP during conventional pasteurisation is possible (Hammer *et al.*, 2002).
- There is clear evidence that MAP bacteria in naturally infected milk are capable of surviving commercial high temperature, short time pasteurisation if they are present in raw milk in high numbers (Grant *et al.*, 2002a,c). Grant *et al.* claimed their study to be particularly significant because, in contrast to earlier inoculated milk studies, it was the first using naturally infected milk processed in a commercial-scale pasteuriser under confirmed turbulent flow conditions. The possibility of post process contamination was suggested by the occasional isolation of *E. coli*.

Taken altogether, the disparities between these various heat resistance studies is obvious. In some instances there are likely explanations. In other instances, reasons for the disparities are not clear. Overall, from the studies in which reductions of 10⁵ fold or more were obtained following heating at 72°C for 15 sec, a D-value of about 3 sec would be indicated if one assumes simple exponential kinetics over that part of the inactivation curve that is of relevance. But the well established examples of survival after heating for 25 sec, and even after heating at greatly raised temperatures (e.g. 90°C; Grant *et al.*, 1999; Rowe *et al.*, 2000; Hammer *et al.*, 2002) suggest that simple exponential kinetics are inadequate to explain the inactivation of MAP in milk.

Rationalisation of these disparities is urgently needed so that practical, sensible decisions can be made. To this end, an approach to develop guidelines for uniform experimental designs for heating experiments with milk were undertaken at a joint Federal Dairy Research Center/IDF workshop, May 2003 in Kiel.

MAP-specific items that should be considered in heating experiments are summarised in Table 2.

Table 4. Inactivation of MAP in pilot plant and commercial pasteurisers

Heating system	Inoculum	Process	Result	Reference
Pilot scale HTST pasteuriser.	Type strain & 3 bovine isolates (0.7-13 x10 ³ cfu ml ⁻¹). Clumps dispersed prior to heating. Faeces from "moderate shedder" cow (20-32 cfu ml ⁻¹).	15 sec at 63, 66, 69 & 72°C.	No survivors at 72°C. One strain survived 69°C. No survivors at 72°C. Low recovery (0.4 cfu ml ⁻¹) in 1 of 2 trials at 69°C.	Pearce <i>et al.</i> (2001)
Pilot scale HTST pasteuriser.	5 bovine isolates	18 sec at 68.1-79.1°C. 15-30 sec at 72-75°C ¹ 18-19 sec at 80-90°C. 40-60 sec at 72-90°C	Survivors in 77 of 282 trials. Survivors in all 45 trials Survivors in all 53 trials Survivors in all 48 trials. Low level survival in all trials, est. 4-6 log reductions ² .	Hammer <i>et al.</i> (2002)
Commercial scale pasteuriser.	Naturally MAP-infected milk.	15 or 25 sec at 73°C, +/- prior homogenisation	Viable cells cultured from 6.7% of 60 raw, and 6.9% of 144 pasteurised samples. On one occasion, high initial levels resulted in survival after all four processes ³ .	Grant <i>et al.</i> (2002b)
HTST pilot plant	Cultured cells. Raw & UHT milk inoculated with 10 ⁶ cfu ml ⁻¹	68, 72 & 78°C for 10, 20 & 30 sec	2 log kill (68°C 10 sec); 4 log kill (68°C 20 sec); No recovery from higher temperatures or longer times.	Rademaker <i>et al.</i> (2002)

1 German Milk Ordinance conditions (≅72°C for 15 sec equal and above)

2 Cerf & Griffiths (2000) re-emphasised that, based on conventional microbial inactivation kinetics, it is always more efficient to increase temperature than time of heating, so that 90°C survival remains surprising.

3 It is significant that in some instances other supposedly heat-sensitive bacteria (*E. coli*) were detected as well.

A further problem is that, after heating, low numbers of surviving MAP cells must be expected. Any concentration step will enhance detection. The same effect can be expected with respect to the sample size processed. The most frequently applied concentration method is centrifugation. Grant *et al.* (2000) introduced immunomagnetic separation for concentration purposes. If centrifugation is used, it must be considered that it depends on the g value whether MAP may also be concentrated in the cream layer, and not only in the sediment.

The survival of MAP in pasteurised milk is clearly exacerbated by the high numbers of cells, many in clumps that may irregularly be present. Grant *et al.* (1996) had already reasoned that clumping played a key role in survival, and used a novel vital staining method to demonstrate viable cells in cell clumps, a conclusion supported by Sung & Collins (1998), Keswani & Frank (1998) and Hammer (2000). However, low numbers can, of course, be satisfactorily killed. This was demonstrated when the sensitivity of MAP detection in milk was greatly increased by the use of an immunomagnetic separation technique coupled to PCR (Grant *et al.*, 1998a,b; 2000), such as to detect the equivalent of about 20 cfu ml⁻¹. Use of this technique enabled the demonstration that levels of MAP <10 cfu ml⁻¹ were satisfactorily eradicated from milk during standard HTST pasteurisation processes.

Possible resistance mechanisms

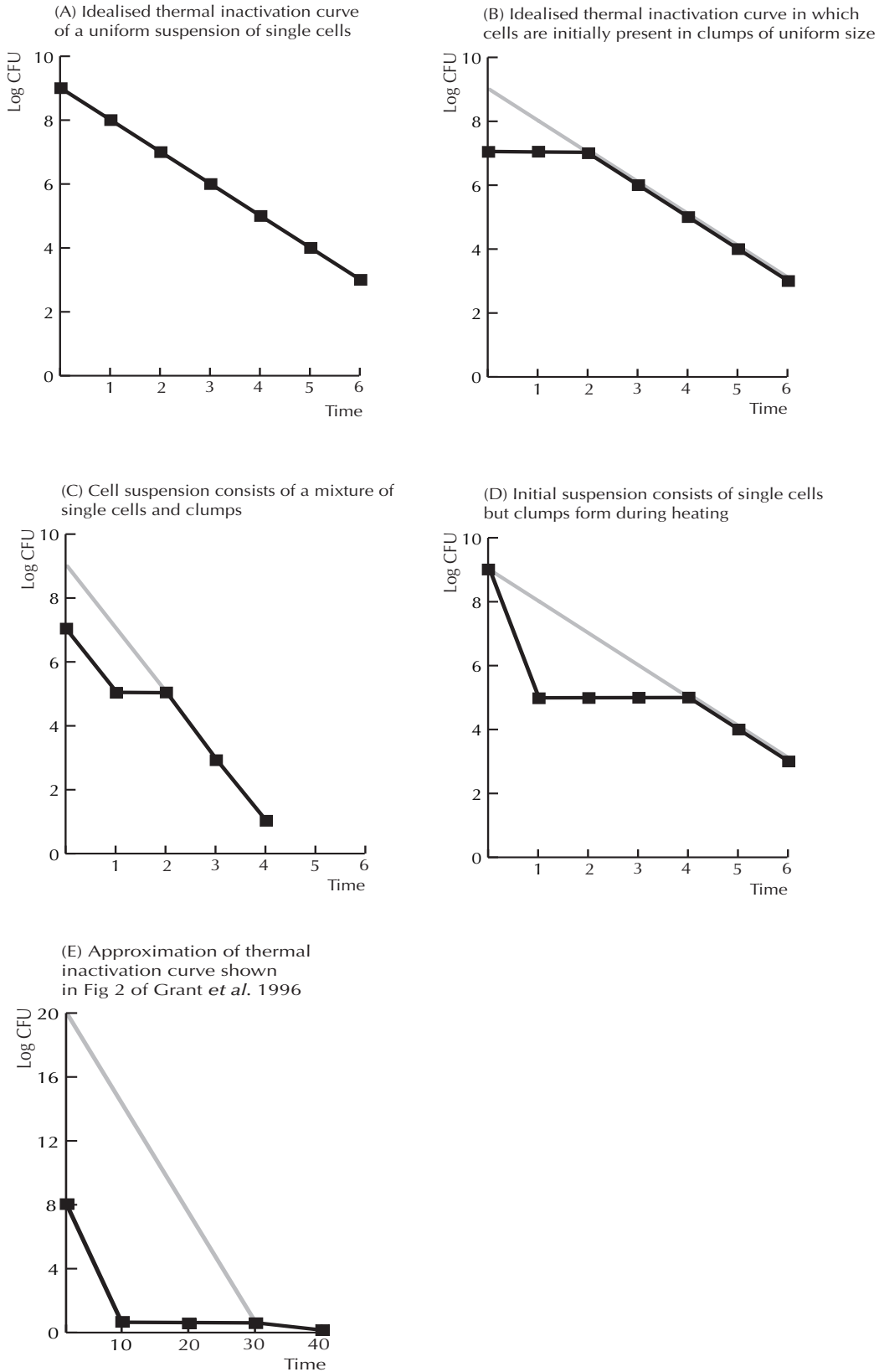
The unexpectedly high apparent heat resistance and irregular inactivation kinetics of MAP is often attributed to the presence of clumps in bacterial suspensions and/or to the acquisition of thermotolerance by a physiological adaptation mechanism. The presence of clumps of cells in a bacterial suspension can certainly give rise to deviations from the classic exponential order of death (Fig 2A) for purely statistical reasons arising from the discrepancy between the number of colony forming units and the actual number of viable bacteria present. A clump of bacteria gives rise to a single colony forming unit (CFU) but all cells within a clump have to be inactivated before a decrease in CFU occurs. Depending on the number of clumps and whether they are present initially or are formed during heating, this can give rise to 'shoulders', 'plateaus', or 'tails' on survival curves (Stumbo, 1965; Fig 2A-D).

It is notoriously difficult to obtain uniform suspensions of MAP, and large clumps in suspensions are readily visible under the microscope (Keswani & Frank, 1998; Sung & Collins, 1998). Grant *et al.* (1996) suggested that such clumps might explain the very long tails on survivor curves, and this idea was reinforced by the mathematical treatment of Klijn *et al.* (2001). However, the maximum length of tail that can occur during heating is dependent on the total number of cells initially present and the number and size of clumps; and the relationship between these variables places a limit on the length of tail that is possible in practice. With this in mind it can be concluded that the extreme case of tailing reported in Grant *et al.* (1996) would require an initial count of about 10¹⁹ cells per ml, which is impossible (Fig 2E).

Tails can also occur if clumping takes place during heating because, when cells aggregate into clumps, the colony count declines and the subsequent inactivation of bacteria in the clumps leads to a plateau or tail in the inactivation curve. For an appreciable tail to occur by this mechanism would require an increase in clump size of several orders of magnitude and a requirement that almost all single cells aggregate into clumps. If, for example, only 90% of single cells aggregated this would result in only a 1 log decrease in CFU and a rather short plateau on the survival curve (Fig 2D). It is thus unlikely that, even with substantial aggregation during heating, the purely statistical effects of clump formation are sufficient to explain extensive tailing, though non-statistical artefacts due to hydrophobic attachment of cells to tubes, pipettes etc. should not be ruled out. Three groups of workers found that disruption or removal of clumps by sonication,

Fig 2. Idealised thermal inactivation curves.

The solid line represents the number of colony forming units whereas the fainter line shows the estimated total viable number that would be obtained in the absence of any clumping effects.



repeated passage through a thin syringe needle or filtration did not have significant effects on the shape of survivor curves or estimates of D values (Keswani & Frank, 1998; Sung & Collins, 1998; Stabel *et al.*, 2001). Rowe *et al.* (2000) found that declumping cells by vortexing with glass beads reduced D values by a factor of two but survival curves were log-linear in both cases. MAP grown on different media by Sung *et al.* (2004) had different heat resistances, but all showed log-linear survival over at least 6 logs when heated at 65°C.

An alternative possibility is that cells within clumps undergo a physiological adaptation leading to an increase in heat resistance. Evidence that cells in a clump may be more resistant to heat than when present singly was obtained by using a novel double-staining procedure to identify metabolically active cells in heat-treated milk samples. When samples taken from the 'tail' region were examined by this method, metabolically active cells were always located within clumps (Grant *et al.*, 1997).

Stress response regulons affecting resistance to heat, acid, oxidative and osmotic stresses have been described in many species of bacteria (Yura *et al.*, 2000; Hengge-Aronis, 2000) and it is therefore likely that MAP has mechanisms for adapting to heat stress. Although ostensibly attractive, this explanation is very unlikely to account for the ability of cells to survive temperatures as high as 90°C (Hammer *et al.*, 2002) unless, somehow, induction of spore-like resistance can occur. Studies of the heat-shock response in a wide range of bacteria have shown that the increase in heat resistance, expressed in terms of D values, is typically two to five fold (Doyle & Mazzotta, 2000). To allow survival of MAP at 90°C would require an increase in its reported resistance (measured as D values) of at least a thousand fold and probably much more. None of the stress responses that have been characterised in other bacteria would result in resistance increases of this order and it is extremely unlikely that any physiological mechanism (apart from spore formation) could achieve this degree of stabilisation of cellular macromolecules. Growth on different media effected MAP heat resistance (Sung *et al.*, 2004), though not sufficiently to explain the extreme resistances that have been reported.

The importance of resuscitation and/or post pasteurisation holding on the recovery of MAP following heat treatment have been pointed out by Hammer *et al.* (2002) and Grant *et al.* (2002b). Whilst resuscitation is important to reduce experimental variation and improve recovery of injured cells, its use has never been reported to allow recovery of mesophilic vegetative cells exposed to heat treatments as high as 90°C. It is not a factor likely to explain the reported survival of MAP after extreme heat challenge (though, of course, inadequate resuscitation might explain failure to recover any MAP after treatment at 72°C for 15 sec).

In the absence of a physiological explanation, the only other obvious mechanism that could account for such extreme heat resistance would be a change in the physicochemical environment of the organisms. Vegetative bacteria are protected from heat when present in foods that are low in moisture or high in fat, and at low water activity, and under these conditions they can resist temperatures of 100°C or higher (Olsen & Nottingham, 1980). The protection afforded to bacteria present within lipid material is due to an increased solubility of water in the lipid phase as temperature rises and a consequent reduction in the local water activity (Senhaji, 1977; Senhaji & Loncin, 1977). For MAP to be protected by this mechanism would require the cells to be located within a fatty matrix and the amount of cellular water present not to exceed that necessary to saturate the lipid phase. If such mechanisms do not withstand experimental scrutiny we would be forced to reconsider the possibility that, despite the great care taken by experimentalists, there is some unknown property of MAP that prevents some cells receiving the intended heat treatment in pasteurisers or submerged ampoules.

Other options for control

In the evaluation of control options, the increased milk stream (quantities) from the collection at individual farm level, through collection centres and finally up to the processing facility needs to be considered. Taking this situation into account, the fate of MAP can be considered. On the one hand, milk originating from a single infected animal will be diluted all along the process flow by clean milk but on the other, this mixing process will lead to larger quantities of milk being contaminated with low levels of MAP.

Although the thermal tolerance data indicate that MAP is more thermoresistant than many other vegetative bacteria, during HTST treatment at even the lowest legally defined conditions (15 sec, 72°C) most studies have indicated that a 5 log reduction is achieved. Further reduction might be possible by application of other processing options, such as homogenisation or milk purification, bactofugation and filtration. Data on the efficacy of these processing options, besides heat treatment, are rare. Grant *et al.* (2002b) reported some evidence that homogenisation may enhance the lethality of the heating process during commercial-scale pasteurisation. In general during milk purification by centrifugation, 50-60% of the bacterial load attached to particles such as faeces or straw will be removed. More effective is centrifugation in a bactofuge. This process is capable of removing 90-95% of spores and vegetative bacteria. Microfiltration leads to a more than 99% reduction of bacterial load. It is likely that similar effects can be expected with respect to a reduction of MAP by application of these processes. However, more sound scientific data generation is desirable.

As proper UHT treatment inactivates most bacterial spores, survival of MAP would seem to be unlikely. In addition, often UHT treatment is a subsequent step after pasteurisation. However, in view of the uncertainties associated with survival of MAP after heating, with recovery even after treatment at 90°C, the behaviour of MAP during UHT treatment should not be assumed and would bear investigation.

Few data are reported on the effects of cheese ripening (see above), and covering only a very small segment of cheese manufacturing. However, a wide variety of cheeses are produced from heat treated milk, and bactofugation is already often used for cheese milk so that a cumulative reduction of MAP can be expected.

Further important processes in milk manufacturing include production of milk powders, butter, yoghurt and other fermented products, but for these no information on the behaviour of MAP is available. Whether reliable data can be generated here may be doubtful because new or improved methodology would be necessary to detect extremely low numbers. A considerable reduction should be expected by the heat treatment. Concentration of MAP in cream resulting from its hydrophobic character may be important, and needs further investigation. Fermentation processes generally commence after an initial pasteurisation. Surviving MAP may be affected by the decrease in pH during fermentation, but the high acid tolerance of the organism should be considered (Collins *et al.* 1984). Possible effects of starter cultures and their products are possible future research items.

Few data on the effects on MAP of processing of meat and meat products are available at present. It is likely that, comparable to milk, heating will be most effective in minimising survival.

CONCLUSIONS

MAP shed by infected cattle and other animals contaminates the environment and, whilst not multiplying there, gains irregular access to a number of foods, of which milk has received the most attention. Small fractions of populations of MAP cells appear, unexpectedly, to show greatly enhanced heat resistance, and MAP may occasionally be recoverable from pasteurised milk, though the reasons for this remain unknown. In contrast to the many studies of MAP contamination of milk that have been undertaken, other potential sources, especially water and meat, have so far received too little attention.

The public health importance of such survival of MAP depends on their possible involvement in human disease, in particular Crohn's disease. At the present time, despite substantial research (see reviews European Commission, 2000; Rubery, 2002) the possible involvement of MAP in human disease remains under discussion. Further studies are needed to clarify the issue.

RECOMMENDATIONS

While further studies are underway, it has been suggested that the food industry should adopt a precautionary approach (ACMSF, 2003; Greenstein, 2003) and support programmes and new initiatives aimed at reducing the chance of MAP contamination of foods so that more effective control measures can be developed. There is a need to learn more about MAP occurrence in meat and water.

The most effective and long lasting actions are likely to be the on-farm management programmes aimed at reducing infection in cattle. While food processing, predominantly by heating, is effective in reducing numbers of any contaminant MAP, normally by approximately 5 log, the occasional occurrence of survivors remains unexplained, and requires further research.

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APPENDIX – FACT SHEET

The organism

The mycobacterial species *M. avium* is subdivided into three subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis* (MAP), and *M. avium* subsp. *silvaticum*. The subspecies designation of MAP is based on DNA-DNA hybridisation and numerical taxonomy. MAP can be differentiated phenotypically from *M. avium* and *M. silvaticum* by its dependence on mycobactin for growth. It can be differentiated genotypically by the presence of multiple copies in its genome of an insertion element, IS900.

Ecology – sources in the food chain

MAP may enter the food chain from a variety of sources. The organism, shed from cattle or from other animals, may contaminate pastures, and therefore water run-off. Faecal contamination may introduce MAP into raw meat and raw milk. Meat may also be contaminated endogenously in infected cattle. Milk may also be contaminated directly via the mammary gland. Contamination by animal effluent has been shown capable of introducing MAP onto food crops.

Disease in animals

MAP is the causative agent of Johne's disease in cattle, sheep and goats, where it causes a fatal chronic intestinal disorder. It occurs also in some non-ruminants.

It has been spreading through domestic livestock for many years, and is endemic in most countries. It is common in dairy herds, where it causes significant financial losses resulting from decreased milk production, animal deaths and replacement costs.

Links to Crohn's disease

The pathogenesis of Crohn's disease is complex and does not appear to involve a simple cause and effect relationship. There are three widely-accepted, well-researched, interacting elements: genetic susceptibility; immune-mediated tissue injury; and enteric environmental modifiers such as the intestinal microbial flora. However, there have been reports of a possible association of MAP with Crohn's disease in humans, though the current evidence is sparse, and it is insufficient thoroughly to confirm or disprove such an association. Different researchers have different opinions about the possibility of a link, so the possibility should not be ignored.

Food materials likely to be contaminated

By far the most studied food is raw milk and, since low level survival of pasteurisation has been demonstrated, there may be the possibility of contamination of products derived from it (pasteurised milk, butter, yoghurt, cheese and other fermented products). Other food materials that might be contaminated at some frequency have been much less studied. They include meat and products derived from it (burgers, sausages, dry-cured and fermented products), products that may become contaminated with cattle effluent (some crop plants) and water.

Survival of MAP in food

Whilst MAP is not expected to be able to multiply in foods, it is a good survivor. It is not inactivated by most food preservatives, and so low levels of contamination are likely to persist in the food chain.

Control in the food chain

Improved control will most likely derive from continuously upgraded intervention procedures on the farm, to reduce the chances of intra- and inter-herd transmission. In foods, heating remains the most effective eradication process, with additional improvements possible from techniques such as milk centrifugation and microfiltration.

Implications for the future

With respect to food, the major implications will derive from future work on the pathogenesis of Crohn's disease, and the availability of new evidence supporting or countering a participatory role for MAP. The complexity of the situation is such that this is not likely to be unambiguously resolved in the short term.

ABBREVIATIONS AND DEFINITIONS

D-value (Decimal reduction value): The time taken for a 90% decrease (10% survival) of numbers of viable cells in a bacterial population under a specified set of environmental conditions.

Cfu (colony forming units): A unit when attempting to estimate the numbers of microorganisms in a sample by inoculating into solid media and counting the numbers of colonies that appear after suitable incubation. Use of “cfu ml⁻¹” is preferable to “numbers of microorganisms ml⁻¹” since an individual colony may grow from a single bacterium or from a clump containing many.

Concordance rate: Level of agreement. In the context of Crohn’s disease, if genetic factors alone are responsible one might expect monozygotic twins, who are genetically identical, to show a high level of concordance.

Infliximab: A monoclonal antibody against TNF (see below) that is therapeutically effective in Crohn’s disease.

IS900: Insertion sequence. A specific base sequence of DNA that is present in the MAP chromosome in multiple copies. Its detection by PCR allows much faster detection of MAP than culture methods.

TNF: Tumour necrosis factor is the major mediator of the inflammatory process in Crohn’s disease.

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