ANNEX D

Mycobacterium avium subspecies *paratuberculosis*

Mycobacterium avium subspecies *paratuberculosis* (MAP, also called *Mycobacterium paratuberculosis*) known already to cause Johne's disease in livestock, is suspected of being the causative agent of Crohn's disease. Milk, meat and drinking water, but possibly also other foods, may serve as vehicles of transmission. If MAP is proven to be a human pathogen, there is the potential for an enormous impact on human health due to the prevalence of the organism on the farm and in water supplies, and the food safety community will then be faced with a considerable challenge (Käferstein, 2001).

Background

Johne's disease, or paratuberculosis, is a chronic, infectious disease initially described in cattle in 1895. The disease typically manifests itself through diarrhoea, severe weight loss and eventually death. The incubation period may vary from 6 months to 15 years. Generally, cattle show signs of the infection when 3-5 years old. Neonatal and juvenile animals are more susceptible to infection than adult animals. They acquire the infection by ingestion of MAP in feed or milk, or by accidental ingestion of the micro-organism from contaminated surfaces. The disease can also be passed on to foetuses by intrauterine infection. Johne's disease is prevalent world-wide in several domesticated animal species and has significant impact on the global economy. It is considered to be one of the most serious diseases affecting dairy cattle. In many countries, in the range between 10 and 50% of dairy herds may be infected (Harris & Barletta, 2001).
Johne's disease begins in Peyer's patches where MAP are taken up and phagocytised by macrophages. MAP bacilli probably remain in the phagosome, where they multiply intracellularly. Following infection, there are abundant acid-fast organisms present which may be found in the stool of infected animals, in the tissues, or if the disease becomes disseminated via the blood stream, in milk. In the faeces of an infected cow MAP is likely to be present in much higher number (10^8 CFU per g) compared with milk (2-8 CFU/50 ml).
Several factors make the successful control of Johne's disease a challenge, not least of which is its ability to survive in the environment, its long incubation period, and the limited sensitivity of diagnostic tests in individual animals during that period. Although the understanding of the epidemiology of the disease is still incomplete, principles of prevention and control have been developed and accepted to such an extent that several countries have initiated national strategies to tackle the problem (Kennedy et al., 2001).

The clinicopathological features of Johne's disease are very similar to those of Crohn's disease, a severe chronic inflammation of the gastrointestinal tract of humans. Crohn's disease primarily affects young people with an increasing prevalence over the last half century. The aetiology of the disease is complex and a variety of infectious agents have been implicated in its cause, the foremost of which is MAP. The association between MAP and Crohn's disease arose because of similarities between Crohn's disease and Johne's disease and the isolation of MAP from intestinal tissue Crohn's disease patients. However, there are a variety of data both for and against the association of MAP with Crohn's disease (Acheson, 2001). Thus, a causal relationship between MAP and Crohn's disease has not been demonstrated. Finally, it has been hypothesised that only a subset of Crohn's disease cases is directly related to MAP in the context of genetic susceptibility and possibly other factors.

Human exposure to MAP

It is inevitable that humans sharing the same geographic areas with animals which are extensively infected will be exposed to MAP (Hermon-Taylor, 2001). Infected animals excrete MAP onto pastures. Wildlife (deer, rabbits and their predators, and carrion eating birds) sharing habitat with infected domestic livestock can become infected and contribute to environmental contamination. Whether wildlife can become reservoirs of MAP and pass the infection back to domestic animals is not known but of vital concern. MAP can survive for long periods in the environment. Studies have demonstrated that MAP is viable for up to 250 days in water, faeces, and cattle slurry. It appears that moisture, low temperatures and shade favour survival but definitive research on the relative influence of these factors has not been completed. Soil acidity may also affect environmental survival. Uptake of MAP into organisms such as amoebae in which they can survive may provide an ecological niche for the organism outside the infected animal and provide a means of environmental persistence. This may allow them to replicate, to increase their resistance to biocides and potentially acquire a phenotype which is more pathogenic for humans. Rains falling on contaminated land will wash MAP into surface waters and rivers, and consequently water abstracted from these rivers and lakes may convey these organisms to human populations.

MAP may accumulate in biofilms lining water supply systems. Biofilms may be an important replication site for mycobacteria found in water. Bacteria in such locations are more resistant to chemical stress than suspended bacteria.

It has long been known that MAP can be cultured from milk of clinically infected cows with Johne's disease. More recent work has shown that MAP can also be cultured from milk of apparently healthy subclinically infected cows. MAP gets into milk both by transport from the primary site of infection, the small intestine, to the udders via the blood stream and by faecal contamination at collection. The destruction of all viable MAP by exposure to current pasteurisation conditions of 72°C for 15 s is not assured. In a recent study, MAP could be cultured from 1.7% of samples of retail pasteurised cows' milk widely obtained throughout Britain. MAP-positive samples included some exposed to the longer holding time of 25 s during pasteurisation. Other dairy products, as well as raw and processed meats, are also at risk.

In addition to milk ante-mortem contamination of meat products is likely to occur, at least in animals with clinical signs of Johne's disease. Besides, as with any organism found in faeces, post-mortem contamination of the carcass and products made from the carcass, in particular ground beef, is possible. However so far, no reports on survival characteristics of MAP in ground beef or any other meat products have been published.

Research needs / future directions

Ecological characteristics of MAP (Collins et al., 2001):

- Development and standardisation of methods for the isolation and enumeration of MAP from milk, other dairy and meat products, water and soil in order to perform studies on survival in these matrices.
- Definition of the mechanisms for resistance of MAP to stress in the environment and food systems, being relevant both to on-farm infection control among animals and to destruction of the organism in animal products being made into food.
- Characterisation of factors in dairy foods that have a protective or deleterious affect on MAP survival, in particular lactic acid.
- Determination of the frequency with which MAP occurs in aerosols and biofilms and the role free-living amoeba may play in its replication or persistence in the environment.
- Measurement of the survival rates of MAP in faeces handled by various modern storage and processing methods.
- Measure survival rates of MAP when applied to soils of various types and by various methods.

Detection and enumeration of viable MAP from milk and milk products (Grant & Rowe, 2001):

- A consensus is needed on the optimum method for the isolation of MAP from milk and milk products, that is, a decision on the best decontaminant and recovery culture medium, or combination of recovery media, to use to give maximum recovery of cells and maximum detection sensitivity.
- It must be recognised that methods currently applied to milk which include chemical decontamination, in any shape or form, do not permit accurate enumeration of MAP cells present in a milk sample.
- Aids to the sedimentation of MAP from milk, such as CB-18™ should be further investigated in order to improve % recovery figures.
- Research into novel methods for detecting viable MAP in milk that eliminate chemical decontamination should be encouraged so that more accurate enumeration is possible.
- Further work should be carried out to develop a culture medium that would allow detection of MAP from pasteurised milk where the organism could be expected to be present in an injured physiological state.
- The effect of inclusion of antibiotics in culture media for MAP should be assessed in relation to recovery of stressed cells.
- Individual research groups should determine the sensitivity of their particular detection method by a standardised protocol so that any minimum detection limit reported can be easily compared with previously published methods. It is recognised that the tendency of the organism to form clumps complicated this process.

Destruction of MAP by heat in milk and milk products (Stabel et al., 2001):

- Studies using continuous flow pilot-scale pasteuriser units to evaluate heat inactivation of MAP in raw milk.
- Studies to provide definitive data on D- and z-values for MAP in milk need to be performed.
- Improvement of sensitivity of detection of viable MAP cells after heat treatment, including a pre-incubation step in liquid medium to resuscitate injured cells.
- Studies to evaluate the effects of decontamination protocols of milk containing MAP on survivability of sub-lethally injured cells.
- Studies evaluating preparative methods of bacterial inocula on survivability of MAP after heat treatment.
- Determine the level of MAP contamination in bulk tank samples on-farm and in holding tanks at the dairy processing plant.

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