ACM/487

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

MYCOBACTERIUM AVIUM SUBSP. *PARATUBERCULOSIS* : REVIEW OF ADVICE

Introduction

1. The purpose of this paper is to review the information in papers ACM/485 and ACM/486, together with recent expert opinion on the role of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in human Crohn's disease, and to seek the Committee's advice on any implications for the food chain.

National survey of raw and heat treated milk (ACM/485)

2. In December 1999, the Committee was informed that preliminary results from this survey demonstrated that MAP was present in pasteurised milk. A summary of the Committee's views was that:

> "Whilst acknowledging that the relevance of this organism to human illness was unknown, Members expressed some concern that MAP had been detected in pasteurised milk. The Committee agreed that it was important to establish whether MAP had survived the pasteurisation process, or whether there was some other explanation for the positive results. Further work was needed to establish the conditions under which MAP could be eliminated from milk. The ACMSF stood ready to offer further advice in the light of future research results."

3. Results of MAP culture are currently available for 679 samples (81.8% of those tested) and these show that viable MAP was found in 1.9% of raw milk samples and in 2.1% of pasteurised milk samples.

4. The 10 samples of pasteurised milk found to contain the organism came from 8 different dairies, situated throughout the UK. The dairies ranged from small to very large. A variety of pasteurisation times and temperatures had been used and included extended times of up to 25 seconds. Checks were carried out to confirm the accuracy of the information collected by samplers. Dairy records did not reveal any evidence of inadequate pasteurisation or cross contamination following pasteurisation. Molecular typing of the strains showed that cross contamination with laboratory strains could not account for the results.

5. There is thus very compelling evidence that MAP is present in pasteurised milk going into the retail system in the UK. This appears to be the result of survival of the organism through the pasteurisation process, rather than cross contamination, although it was not possible to identify samples from the same batch where both raw and pasteurised samples were positive. MAP was also found in samples subjected to increased pasteurisation temperatures and/or prolonged pasteurisation times.

Heat resistance of MAP in milk at pasteurisation temperatures

6. Laboratory pasteurisation studies have shown that MAP at concentrations above 100 CFU/ml is able to survive high temperature short time (HTST) pasteurisation. The most probable explanation for this was thought to be the clumping of cells in naturally infected raw milk.

7. Laboratory studies suggested that longer pasteurisation times, particularly 25 seconds, were associated with inactivation of high inocula of MAP. Increased temperatures did not have this effect.

8. Further studies on different time/temperature combinations in commercial pasteurisers have not been carried out. Funding for a dairy industry project to identify processing combinations that achieve a 5 log reduction in the count of MAP and to clarify the mechanisms that confer heat resistance on the organism has been agreed under the LINK scheme.

Current views on the relationship between MAP and Crohn's disease

9. Crohn's disease is a chronic inflammatory disease of the bowel which most commonly begins in early adulthood. The severity and course of the disease varies considerably between patients, with symptoms persisting for prolonged periods in some, whilst others have long periods of relatively good health with occasional acute symptomatic episodes. As well as gastrointestinal symptoms such as diarrhoea, abdominal pain, nausea, vomiting and weight loss the disease may also affect the joints, the eye, the lower back and the liver.

10. There is no curative treatment and the disease persists for life. Drug therapy (steroids or salicylic acid derivatives) is used during periods of disease activity. Immunosuppressive therapy may also help to increase the period between relapses. The majority of patients (70 - 80 %) will require at least one operation during the course of their disease. Surgical treatment may be required for strictures, fistulae and other complications, or when a patient fails to respond to medical treatment. Although the disease is not itself fatal, there is evidence that patients have a reduced life expectancy.

11. The claimed link between MAP and Crohn's disease has been the subject of speculation, research and controversy for many years. The UK Advisory Committee on Dangerous Pathogens examined the evidence for and against a link between MAP and Crohn's in 1992 and 1998. On both occasions they concluded that a causal link could not be established on current evidence.

12. Recently, this view has been echoed by the EU Scientific Committee on Animal Health and Animal Welfare, and in a report published by the Food Safety Authority of Ireland (FSAI). The summary and conclusions from the EU Committee's report are appended (Annex A), and Members can obtain the full report either from the ACMSF Secretariat or from the EU website (http://europa.eu.int/comm/food/fs/sc/scah/out38_en.pdf). The FSAI report has already been circulated to members (ACM/493).

13. The consensus of medical and scientific opinion is that the case for a link has not been proved. At the same time, the possibility of an association, at least with some cases, cannot be dismissed out of hand. There is evidence of MAP in at least some patients with Crohn's and, on balance, it appears to be found more commonly in those with Crohn's than in those who do not have the disease. On the basis of similarities between Johne's disease in ruminants and Crohn's disease in humans, the association is also biologically plausible.

14. On the other hand, if there is an association, it is by no means clear that this is a causal one and the results of treatment studies to date do not provide consistent evidence of an effect.

15. Whilst studies continue to be carried out in an attempt to resolve the issue, there appears to be a general view that this is not a controversy that will be resolved in the near future, if at all.

Other studies

16. A number of countries have introduced control programmes for Johne's disease, the disease in ruminants caused by MAP. MAFF is currently funding a review of these programmes and their effectiveness, and a report will be presented to the ACMSF later this year or early next year.

17. Milk may not be the only source of MAP to which humans are exposed. Studies of drinking water treatment are currently being carried out to look at the epidemiology of Crohn's disease in relation to ground and surface waters and to see if *M. avium* complex survives water treatment processes. Reports of both studies are likely to be available later this year.

18. As mentioned above, the dairy industry is currently considering work to examine different time/temperature combinations for the heat treatment of milk under commercial conditions.

Discussion

19. It seems unlikely that the question of MAP's involvement in the aetiology of Crohn's disease will be resolved in the near future. On the basis of the precautionary principle, it could be considered that all possible measures should be taken to eliminate this possible hazard. However, such measures need to be proportionate to the risk. In making a judgement about proportionality, consideration should be given not only to the balance of evidence for and against MAP as a cause of Crohn's disease but to the severity of the disease in question.

20. At present it is not clear whether it is possible to eliminate MAP by any heat treatment process that does not affect the organoleptic qualities of milk. Further work within the industry may or may not provide a solution to this.

21. If treatment of milk cannot guarantee removal of the organism, the question arises as to whether contamination of the milk can be prevented by programmes to eradicate Johne's disease, or by measures to ensure that milk from animals shedding MAP does not enter the food chain. In recent years there has been an increasing amount of work on Johne's disease in animals. From this work there are indications that control measures can be applied to reduce the incidence in national herds.

22. In the immediate future, however, it appears that the only measure that would remove viable MAP from milk would be heat treatments that affect the organoleptic qualities of the milk. The heat treatment studies described in ACM/486 do not provide specific data on the efficacy of processes such as ultra heat treatment. However the response to the heat treatments that have been studied suggests that such treatment should eliminate viable MAP, and no MAP was found in the admittedly very small number of UHT samples tested in the survey.

23. When pilot work carried out in preparation for the milk survey suggested that MAP might survive pasteurisation, a view was sought from the Department of Health on the possible public health implications. The advice given at the time (August 1998) was that there was no need to change dietary habits. In subsequent advice, it has been suggested that members of the public who are concerned about a possible risk, could change to UHT milk.

24. In reaching a view as to whether this advice remains appropriate, members may wish to consider:

- the balance of evidence for and against a causal link between MAP and Crohn's

- the nature and severity of Crohn's disease

- the possibility that milk is not the only source of exposure to this organism

- the possibility, raised in the FSAI paper, that, since the disease process is generally considered to be of an immunological nature, it might equally well be triggered by non-viable bacteria.

25. Members may consider that, in the light of these considerations, it is appropriate to underline the benefits of milk as a food and to endorse existing advice. They may also consider that, whilst consumers should be offered advice on how to minimise their exposure to this organism in milk, the weight of evidence does not justify recommending that consumers need to take such steps in order to safeguard their health.

Action

26. Members are asked:

- whether the results now available confirm, to their satisfaction, the ability of MAP to survive pasteurisation, including pasteurisation at time/temperature combinations that go beyond the legal minimum (72°C for 15 secs) .

- to advise on the implications of the findings for the food chain and, in particular, to consider whether existing advice on the consumption of milk remains appropriate.

- to advise on any further work they consider should be put in hand to provide a sounder basis for assessing or managing the risks.

Judith Hilton
Secretariat
September 2000

Annex A

EU Scientific Committee on Animal Health and Welfare: Summary and Conclusions

9. Summary

This report has confined itself to an examination of the possibility that Crohn's disease is caused by *Mycobacterium avium* subsp. *paratuberculosis.* It has not examined other possible causes of the disease in detail.

1. *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) is an organism which can cause chronic inflammation of the intestine (Johne's disease) in cattle, sheep and goats as well as in other animals including rabbits and macaques.

2. *Map* infections are widespread in domestic livestock, including cattle, sheep and goats. Rabbits, deer and other animals have been identified as wildlife hosts of *Map*.

3. Animals can harbour *Map* without showing signs of disease or reacting to serological tests.

4. *Map* is found in large quantities in the intestine of diseased animals and in lesser amounts in lymph nodes, liver, spleen and other tissues.

5. Clinically infected animals may shed up to 10^{12} *Map* per ml in their faeces. Subclinically infected animals also shed the organism though usually in lower amounts. Infected dairy cows and sheep shed *Map* in their milk.

6. *Map* can survive for prolonged periods (up to 9 months) on contaminated pastures and in the environment.

7. *Map* infections in animals are usually acquired at an early age from faeces-contaminated environments and from milk, younger animals being more susceptible than adults.

8. Paratuberculosis in animals presents a range of histopathological types from multibacillary to lymphocytic/paucimicrobial forms.

9. *Map* is a very slow growing organism. Laboratory culture is a time consuming and insensitive means of detecting low levels of contamination by *Map* in environmental and food samples. In animals, faecal culture is regarded as the gold standard for diagnosis but it is slow and dependent on the organism being present in the faeces.

10. Immunological assays for *Map* infection in animals generally have poor sensitivity or poor specificity and can be unreliable, particularly for the early detection of infection.

11. Genetic analyses have revealed different strains of *Map*.

12. *Map* contains 14-18 copies of a specific DNA insertion element (IS900) which can be used to detect the presence of *Map* DNA. These PCR tests can be very sensitive when optimal sample processing procedures and reaction conditions are used.

13. *Map* is more robust than *M. tuberculosis* and *M. bovis*. The complete destruction of all viable *Map* in milk by pasteurisation at 65°C for 30 minutes or 72°C for 15 seconds, may not be assured. Viable *Map* has been identified in pasteurised milk supplies.

14. There is preliminary evidence that *Map* may be present in human milk. If these initial results are confirmed, this would be a significant finding.

15. Crohn's disease is a chronic inflammation of the intestine in humans which presents some clinical and pathological similarities to the lymphocytic/ paucimicrobial form of paratuberculosis in animals.

16. Crohn's disease occurs in Western European countries with an overall incidence of 5.6 per 100,000 individuals per year.

17. Insufficient data are available to compare the incidence and prevalence of Crohn’s disease and paratuberculosis so as to determine epidemiological links between both diseases.

18. *Map* has been detected in approximately 30% of pathological samples from patients with Crohn's disease by IS900 PCR tests and also, at a lower level, in healthy people. *Map* can only be grown in conventional culture in about 5% of cases and then only after months or years of incubation.

19. The results of IS900 PCR tests on DNA extracts of Crohn's disease intestine carried out in many different laboratories have been conflicting. Several studies which succeeded in detecting *Map* in patients with Crohn’s disease also detected the organism in a smaller percentage of healthy subjects. Other studies were unable to detect the organism.

20. Crohn’s disease patients display an increased serological response to many intestinal micro-organisms including *Map*. This may simply reflect an increased immunological response in patients to many micro-organisms perhaps as a consequence of a ‘leaky intestine’.

21. A few Crohn's disease patients show clinical remission when treated with anti-tuberculosis drugs, but relapse usually occurs. New drugs such as rifabutin and macrolide antibiotics active against *M. avium* sp., are reported to be effective in higher numbers of Crohn's disease patients in open studies, though relapse still occurs. Results of large-scale well designed controlled therapeutic trials are not yet available.

10. Research

10.1 Crohn's disease

Further research is required to resolve this important issue raised in the request from the Commission. This research should be targeted on the following aspects;

1. Large scale epidemiological studies of Crohn's disease patients to examine risk factors, particularly in early life.

2. Attempts to localise the presence and pathology of *M avium* subsp. *paratuberculosis* in the human gut. The significance of the finding of spheroplasts in this disease needs to be determined

3. Follow up work to fully evaluate recent findings such as the finding of *M avium* subsp. *paratuberculosis* in breast milk and immunological recognition of mycobacterial and *Map* components in man.

4. Large scale multi-centre double blind drug trials using combination therapy of those drugs liable to be active against *Map*, preferably on patients in whom *M paratuberculosis* has been detected.

5. Experiments should be carried out both *in vitro* and *in vivo* to determine possible methods of transmission. Facilitating the creation and maintenance of a network of researchers at EU level combining expertise in chronic inflammatory bowel diseases in humans and in *mycobacterium* sp. infections in animals, would greatly contribute to attaining these goals.

10.2 Paratuberculosis

Aside from any possible link with Crohn's disease, the development of the necessary tools to eradicate paratuberculosis from animals should also be a priority.

1. Improvement diagnostic methods are required and their development should be encouraged. There is a need to validate the methodology of currently used diagnostic methods, particularly sample preparation methods. Comparative testing between various laboratories should be carried out.

2. The development of efficient vaccines and diagnostic tests to distinguish infected and vaccinated animals.

3. All these activities would be greatly accelerated by the availability of the complete genomic sequence of *Map*.

4. The distribution and levels of *Map* in infected animals should be determined.

5. Experiments with various time temperature combinations for pasteurisation should be carried out to determine optimum combinations to inactivate the *Map* bacterium.

6. The survival of *Map* in the environment and the role of natural water and water supplies in the transmission of paratuberculosis needs to be determined.

7. Statistically robust studies on the prevalence of Map in domestic and wild animals should be carried out. These will be of assistance for future eradication programmes and will also allow comparisons to be carried out with the geographical prevalence of Crohns disease.

11. Conclusions

The currently available evidence is insufficient to confirm or disprove that *Mycobacterium paratuberculosis* is a causative agent of at least some cases of Crohn's disease in man.

There are sufficient grounds for concern to warrant increased and urgent research activity to resolve the issue. This research is described in section 10.1 of this report.

Crohn's Disease is most likely a multifactorial condition. Its incidence is more common in the western world, in families where there have been other cases and in homes where hygiene in early life has been good. There are considerable clinical and pathological similarities, though also some significant differences between paratuberculosis in animals and Crohn's Disease in man.

The organism *Mycobacterium avium* subsp *paratuberculosis* (*Map*) is relatively common and it is likely that many people have come into contact with it. The detection of *Map* in a greater proportion of Crohn's disease patients than in controls suggests that the organism may have a role either as a causative agent, as a secondary invader which exacerbates the disease or as a non pathogenic coloniser because of changed bowel conditions.

Exposure to high levels of the organism can arise from direct contact with infected farm animals or by drinking raw milk from infected animals. Exposure to lower levels of the organism could possibly arise from heat treated milk or from

sources such as wildlife hosts. A simple relationship between exposure to high (or low) levels of organisms and the development of Crohn's disease does not appear to exist.

If *Map* is involved in the causation of Crohn's disease, it would also require the presence of other susceptibility factors. It is also possible that its involvement may relate to a sub set of Crohn's disease cases.

The current tools available to control paratuberculosis in animals are inadequate. Using current methods, eradication programmes are likely to be long lasting, expensive and with low probability of success, particularly in endemic areas. Because paratuberculosis is an economically significant disease, the development of tools, such as efficient diagnostic tests, to enable eradication would be extremely beneficial and would encourage eradication programmes throughout the world.

Results from recent drug trials in humans with drugs likely to be active against *Map* have been encouraging but inconclusive. Larger scale double blind studies are in progress to obtain better data.