**ACM/553**

# ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

## REVIEW OF THE EVIDENCE FOR A LINK BETWEEN EXPOSURE TO *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* (MAP) AND CROHN'S DISEASE IN HUMANS

1. The Food Standards Agency (FSA) commissioned a review of the evidence for a link between exposure to MAP and Crohn's Disease in humans for the stakeholder conference held in London Docklands on 23-24 May 2001.

2. A copy of the Executive Summary from the report of the review is attached for the information of Members. The full report is available from <www.foodstandards.gov.uk/events/contro_map.htm>

**Secretariat**
**November 2001**

**A Review of the Evidence for a Link between Exposure to Mycobacterium Paratuberculosis (MAP) and Crohn's Disease (CD) in Humans**

**A Report for the Food Standards Agency: June 2001**

## EXECUTIVE SUMMARY

### A. The Issue

1. This report was produced at the request of the Food Standards Agency as the introductory paper for a meeting of stakeholders (largely from the dairy and agricultural communities) held on 23/24 May 2001. It has now been updated following that meeting. The Advisory Committee on the Microbiological Safety of Food (ACMSF) at its meeting on 19 September 2000 recommended that the Food Standards Agency should convene such a meeting. They said 'a group of stakeholders with an appropriate level of seniority and practical experience [should] consider all aspects of control of this organism, including longer term options for control in primary production and developments in dairy technology, taking due account of consumer concerns, such as the risk of exposure to children'.
2. This report concentrates on looking at the issue of MAP and CD pragmatically to inform the discussion of 'What should be done now in the UK and in terms of UK policy about exposures to MAP?' It considers the issues from the human population point of view.
3. ***Crohn's Disease*** (CD) affects the digestive system, most commonly the small and large intestine. It causes the wall of the digestive system (gut) to become thickened, inflamed and swollen. Sometimes the thickening leads to narrowing of the lumen of the gut – this can be so severe that obstruction to the flow of food being digested can occur. Sometimes the inflammation progresses to ulceration, fistula formation and even perforation. In addition the disease can be associated with more remote pathology, in particular arthritis and anaemia. Diagnosis can be difficult, especially differentiating CD from the other Inflammatory Bowel Diseases (IBD) such as Ulcerative Colitis (UC).
4. At present there is no cure for the disease, which tends to pursue a variable course, characterised by periods of activity interspersed with remissions, when the disease is either absent or relatively quiescent. A range of factors is thought to increase the risk of relapse of quiescent disease, including stress and dietary factors. Many therapies have been tried to induce or maintain remissions.
5. It is important to realise that several causes for the pathological entity known as Crohn's Disease are quite likely. That being the case, assessment of the results of the various studies linking Crohn's Disease to environmental agents needs to be made in the light of the possibility that only a sub group of CD cases may give positive results for features thought to relate to any particular causative hypothesis.
6. Crohn's Disease most commonly starts between the ages of 15-40, although it can occur earlier. According to the National Association for Colitis and Crohn's Disease, the disease affects (i.e. has a prevalence of) about one in every 1600 people. Other reports found higher prevalences, up to 1 in 690 in one regional study, a considerably higher figure than that from NACC. Perhaps a reasonable ballpark figure is a prevalence of around 1:1 000.
7. 5 000 children have IBD in Britain. The rate for new diagnoses of IBD each year in children under 16 in the UK is 5.2 per 100 000. Figures from the US report that 20% of cases of Crohn's disease are diagnosed before the age of 20; and 40% between 20-29, so that 60% are under 30 at the time of diagnosis.
8. The active disease is characterised by reduced appetite, abdominal pain, bloody diarrhoea and tiredness. During childhood the disease can cause slowing in growth rate due to problems with ensuring an adequate nutritional intake.

9. Treatment is by a combination of medical therapies (anti-inflammatory drugs to reduce the inflammation; drugs to treat the symptoms; and antibiotics to reduce infections associated with the disease), attention to diet to control weight loss, and surgery to deal with the structural effects of the inflamed intestines.

10. Although only carried out as a last resort, a significant proportion of patients end up with a colostomy (the large bowel discharging through the abdominal wall) or an ileostomy (the small bowel discharging through the abdominal wall). Whilst this is compatible with an active and fulfilling life it is an outcome that requires careful management and a lifelong commitment to clinical supervision.

11. Costs to the NHS of treating Crohn's Disease are likely to lie in the region of £200 million-£320 million per year at present. They are likely to rise as increasingly expensive treatments are developed (e.g. the anti-TNF agents which can cost over £1000 per annum). These figures exclude social and economic costs to the country, which could cost a further £600-£960 million per year if the Swedish costs are directly applicable to the UK.

12. Although modern medical and surgical treatments plus the support of the various patient groups can ameliorate the condition considerably, Crohn's Disease remains a serious and debilitating condition that can not only affect adversely the life of the patient but frequently also has serious consequences for the rest of the family.

13. ***Johne's Disease*****:** *M. avium* subspecies *paratuberculosis* (MAP) was originally identified in 1895 as the cause of a chronic inflammatory condition in a German cow. Subsequently the link between this bacillus and Johne's Disease (or paratuberculosis) of cattle and a wide range of other mammals was established.

14. It is an aerobic, non-spore-forming, non-motile, acid-fast bacillus. MAP has a complex cell wall, relatively impermeable and rich in lipids, which confers acid-fast properties and may enhance its survival in the environment. However, some non-acid fast, lightly staining acid-fast, and cell wall deficient types are encountered. The bacilli generally occur in clumps linked together by a network of intercellular filaments. The type strain of MAP is ATCC 19698.More recently using molecular biological techniques 28 strains of MAP have been identified. This organism has also been detected in wild ruminants and deer. Strain typing has been used to trace the spread of the disease from one population to another.

15. Cattle are most susceptible to infection during the first few months of life, frequently being infected whilst taking milk from their mother. The organism is excreted in large numbers in the faeces of animals with the pluri-bacillary form of the disease; colostrum and milk can also be infected systemically. Information on the detection of MAP in animal tissues appears sparse, though clinically symptomatic animals are likely to have MAP in blood and other tissues. It is thought the major source of infection is via faecal excretion followed by contamination of the milk by local spread. Foetal infection also occurs.

16. There is a long and variable incubation period, classically, and clinical symptoms generally only appear at 2-6 years although they can appear as early as 4 months or as late as 15 years. Stress and a variety of challenges to the animal, such as dietary restriction or transportation may precipitate the appearance of the disease in an asymptomatic animal.

17. National studies of the presence of Johne's Disease in herds show it is endemic across Europe, and probably across the world, if one considers the additional data presented at the IDF conference in Brussels in January 2001. The levels of infection cannot be precisely specified, but are clearly significant in those areas tested, and by extrapolation probably in a wide ranger of flocks and herds globally.

(a) There is no consensus as to the best combination of testing, isolation, vaccination and pasture and ground disinfection that is best to assist in the control of the disease and its eventual eradication. The recent IDF meeting in Brussels confirmed international concern about the disease, a general wish to increase the knowledge base with respect to diagnosis and management and control of the disease in animals, but also the absence of any clear consensus on how best to institute and progress control measures. It seems

likely that national programmes will be most effective if they are tailor made for the country, its particular style of farming and the pattern of Johne's Disease in the animals.

(b) Nevertheless, given the increasing mobility of animals between countries, it is also clear that international programmes will have the best long-term chance of achieving lasting reductions in the number of affected countries, and of affected animals in those countries.

(c) MAP infection has also been demonstrated in a range of wild animals, including rabbits.

18. ***Pasteurisation of milk*** has been seen as a safeguard against significant exposure of the public to the live agent. But recent evidence demonstrates that this organism may survive pasteurisation. There is some evidence it can survive in some cheese. There is no information on the ability of the organism to survive roasting and grilling or other ways of serving meat and meat products. It may survive drinking water treatment.

19. In addition there is evidence that the incidence and prevalence of MAP infection (a significant proportion of which will be sub-clinical but still potentially transmissible), and of Johne's Disease in ruminants is increasing globally. At present data on infection with MAP and clinical infection with JD in the UK suggest an upward trend, although the data are not of high quality.

20. It follows that the human population is likely to be being exposed constantly to MAP via food and probably water.

21. Although the ***cause of Crohn's Disease*** remains unknown, it is likely to be due to a combination of:

    - a genetic predisposition;
    - an abnormal immune response;
    - environmental factors probably relating to a response to microorganisms in the bowel but also possibly related to other dietary factors (which are certainly important in the management of the disease once present).

Whilst there is now a fairly good consensus on the nature of the immunological lesions that result in the pathological expression of CD, controversy remains concerning the nature of the precipitating environmental factors. Some feel it is the normal gut flora that initiate the abnormal response; others that MAP can be a precipitating factor, while other propose other infectious agents or certain chemical or physical exposures.

22. Mycobacterium avium subsp. paratuberculosis (MAP) causes Johne's Disease (JD) in animals up to and including some primates. The pathology of the bowel lesion in JD has some similarities to that of the bowel lesion in Crohn's Disease(CD).

23. Those who are sceptical about a link between CD and MAP point out that:

    - the agent cannot be reliably grown in culture from affected tissues;
    - a MAP-specific immune response cannot be detected in the host.

24. However, the situation with respect to leprosy and *M. leprae* is not much different, and recent work may have identified more specific antigens worth further study. Therefore, the evidence remains inconclusive.

25. ***In summary*****,** therefore, we have a possible human pathogen in ruminants and other farm animals. Exposure of the human population to MAP is likely to be increasing. A judgement needs to be made as to what, if anything, can and should reasonably be done to reduce exposure to the agent on grounds of prudence whilst further research is put in hand to clarify whether or not it is pathogenic for man.

26. The resemblance of the two diseases is sufficient to make a common causative agent a plausible and attractive hypothesis. However, there are areas of differences too, and the gross pathology and clinical data alone are insufficient to either confirm or reject the hypothesis.

27. Although a multi-factorial cause for CD is likely, the possibility that an infectious agent or any other agent can play a key role in the causation of even a sub-set of CD patients clearly needs to be taken seriously. It offers the most promising way of preventing at least

a sub-set of the disease, and might also offer possible ways of treating the disease, and even of curing some of those suffering from the disease.

## B. Overview of Key Knowledge

### 1. Epidemiological Data

1.1 There have been many small epidemiological studies in various parts of Europe and the USA. These overall show an increase in the incidence of CD over time. There is evidence of increased risk of developing the disease in members of the family, probably partly related to a genetic predisposition, but also likely to be in part due to environmental factors.

1.2 Ethnic and geographically based studies show variations (climate, diet, water supply or other environmental agent) in rates that are strongly suggestive of a disease with a large etiologic component related to environmental fact(s) which could be infections – chemical, physical or social. The considerable changes in rates observed during the last 50 years cannot really be attributed to genetic variability in susceptibility to the disease alone.

1.3 JD is widely distributed in the food animal populations of Europe and North America. The high proportion of subclinical infections make it impossible to prevent infected by asymptomatic animals from entering the food chain by inspection at the abattoir. The disease has also been demonstrated in many wild populations including rabbits.

1.4 Links between CD and any environmental or genetic predisposing factor are likely to be complex. In particular exposure to environmental agents are likely to precede symptoms by years (5-15) making correlations difficult to demonstrate.

***Recommendations***

1.5 The present epidemiological data need carefully assessing and interpreting by an expert group to get the most information out of it. This group should also be asked to devise a strategy for future studies.

1.6 A carefully conceived strategy for monitoring and surveillance and further epidemiological studies needs to be devised in the UK, in Europe and globally to ensure as much information as possible is gathered from the field as quickly and as efficiently as possible on both CD and JD.

1.7 These studies need to include studies on geographical distribution of Johne's Disease in the past and present.

1.8 Regular monitoring of the incidence of CD in children and adults in the UK, the rest of Europe and globally needs to be set up urgently by establishing an effective reporting system so that a baseline against which changes in the incidence of this disease and the possible affects of any changes in the incidence of prevalence of JD and exposure to MAP in the human population can be assessed.

***Conclusion***

1.9 For the present all that can be said with certainty is that there is not enough data available on the incidence and prevalence of the two diseases both in time and geographically to enable any conclusions on correlations or causality to be made. While such studies are urgently needed they will not be easy to develop or to interpret, and they will take several years to produce results. They need to be internationally co-ordinated if they are to be as informative as possible.

### 2. Culture evidence to date

2.1 It is difficult to culture MAP. One can say that it is easier to culture than *M. leprae*, but harder than *M. tuberculosis.* When taking specimens from biological samples (such as faeces) it is necessary to subject the sample to extreme treatments to reduce the risk of overgrowth by the many contaminating organisms. This will frequently result in severe reduction in the load of viable MAP that remains in the specimen for culture, and reduce the sensitivity of the assay.

2.2 Media used to culture MAP must include Mycobactin J as a supplement. Purification of specimens prior to culture by the use of a range of techniques including biochemical markers is necessary.

2.3 Because of these facts, it is generally accepted that all methods of culture result in a significant underestimate of the number of infected samples. Figures as low as 1 in 5 false negatives in cattle and 4 out of 5 false negatives in sheep have been quoted although recent improvements have considerably increased the sensitivity of the assays. In addition, of course, many infected cattle will be asymptomatic (a herd infectivity rate of 25 times the symptomatic rate has been quoted.

2.4 Cell wall defective microorganisms have been isolated from the tissues of three patients with Crohn's Disease. Initially it was difficult to be certain that the cell-wall deficient organism was the same as the acid-fast staining bacillus with a cell wall. However once the MAP-unique sequence IS900 was confirmed in the defective organism by polymerase chain reaction (PCR) and DNA hybridisation, organisms were accepted to be MAP.

2.5 Further reports isolating MAP from the tissues of CD patients have followed. All isolates have proved to be extremely fastidious organisms to culture; all required mycobactin for their growth and all were cell-wall defective at least initially.

2.6 The significance of these organisms in the etiology of CD has remained unclear. However, while some workers found MAP more frequently in CD patients than other IBD and UC patients, other workers believe their results only support a "bystander role" for MAP, the organism simply finding a friendly niche in the inflamed environment of the damaged bowel.

## 3. A Possible Human Disease Model

3.1 Hermon-Taylor has recently identified MAP by IS900 PCR in the cervical lymph nodes of a 7-year by with scrofula. Five years later he developed classical CD. A resected portion of his bowel, removed following treatment with clarithromycin and rifabutin was also positive for MAP on IS900 PCR.

3.2 This case history is tantalising, providing an attractive model for a pre-intestinal stage of CD, caused by MAP, that is similar to that followed by infections with other tubercle-type organisms. This pattern would also provide an age of onset similar to that found in the population. It also mimics the long relatively asymptomatic incubation period found in JD. However, there is at present no evidence that it is a common presentation of CD in the population.

3.3 Naser has now cultured MAP from the milk of lactating mothers with CD. Five controls from healthy mothers were negative for IS900 PCR.

## 4. Immunopathology

4.1 There is general agreement that CD is a response to over-stimulation of the mucosal and systemic immune systems that perpetuates an inflammatory cascade that leads to the gut lesions. It is likely that the chronic inflammatory response observed results from interaction of persistent stimuli, most likely from microbial antigens contained in the bowel, with genetically determined host susceptibility factors. It is likely that the specific immunopathological pathway is a T-lymphocyte helper type of response.

4.2 Recent work that shows that some people have a genetic predisposition to 'leaky bowel' i.e. to a bowel wall that is more permeable to antigens, provides an attractive possible explanation for a mechanism for linking the immunological observations (many in mice and rats) with the epidemiological data on clustering of cases in families and occasionally geographically.

4.3 There is not yet agreement whether the abnormal response generated is to normal gut flora or to specific organisms such as MAP.

4.4 Work on the cytokine response to T-cell mediated stimulation suggests that a second lesion may be a lack of anti-inflammatory cytokines, which results in relative over-production of pro-inflammatory cytokines locally and so increases that local inflammatory reaction.

## 5. Demonstration of an Immune Response

5.1 Failure to demonstrate a specific immune response to the organism has been another reason for caution in linking MAP to CD. One of the difficulties was the high level of cross-reacting antigens with other mycobacteriae. More recently recombinant clones expressing more discriminating *M paratuberculosis*- specific antigens have enabled the existence of specific humoral immune responses to be demonstrated in 77% of 66 CD sera compared with 8% of 12 sera from controls ($p<0.0001$). Further work is needed to explore the relevance of these findings.

## 6. Effects of Antibiotic Therapy

6.1 Given the indolent nature of the disease, the difficulty in identifying the organism in the tissues and the general resistance of mycobacteria to any but carefully tailored combinations of therapy, the relative success or failure of different small trials of therapy on the progress of the disease cannot be accorded much weight when addressing the issue of causality.

6.2 On the other hand, for patients suffering from the disease the possibility that in due course an effective combination of drugs can be devised is of great interest. To be effective against this organism antibiotics will have to have intra-cellular activity. Therefore the new macrolides, azithromycin and clarithromycin might be effective.

6.3 Small ad hoc trials of a range of combinations have produced remissions in some cases, and no effect in others, certainly overall sufficient to justify a properly constructed trial.

6.4 So far a controlled drug trial using macrolide antibiotics in combination with other anti tuberculous drugs has not been completed, but one is in progress. This is an urgent need. Given the toxicity of the drugs it is important that studies are well constructed to give interpretable results.

## 7. Is MAP a Food or Water-borne Pathogen?

7.1 MAP is a common infection of food animals, causing Johne's Disease. In the US up to 18% of cattle have been found to be infected in specific studies. The disease is of low prevalence almost absent in Austria, Norway, and Sweden; its herd prevalence exceeds 15% in the USA, Denmark, Belgium and Costa Rica. In Australia the incidence is 11%.

7.2 In the EC no national surveys have been carried out in the UK, France and Germany. Data from Belgium found 17% of herds infected based on serology. In Denmark bulk milk testing for antibody showed 70% of herds had evidence of infection. In the Netherlands 55% of dairy herds had serological evidence of infection.

7.3 Passive testing of submitted samples in the UK shows an upward trend in positive samples since 1993. Regional surveys suggest a herd prevalence that varies between 1% and 17.5% depending on the area of the country. A single survey in the south west of England, on individual cattle, produced figures that suggest the prevalence could be of the same order as that in the US.

7.4 Therefore, in the UK there is undoubtedly the potential for exposure to MAP via milk, meat and water contaminated from run off from fields,

7.5 The work of Grant and colleagues has demonstrated that pasteurisation cannot be relied upon to sterilise contaminated milk. It is not yet clear what modification of the pasteurisation process, if any could improve or remove this source of exposure.

7.6 There are also uncertainties relating to unpasteurised milk, UHT and sterilised milk (where there is as yet inadequate evidence of firm conclusions) cheese, meat after cooking

and direct contamination of children and workers with this agent. Information on whether MAP can survive drinking water treatments is not available, but studies in the US have demonstrated survival.

7.7 A MAFF-LINK project currently being carried forward by Grant and Rowe at Belfast, with Donald Muir and Dr Alan Williams at the Hannah Research Institute, Ayr, to establish practical conditions under which viable MAP can be eliminated from the final pasteurised product, is important and needs to be carried forward as expeditionably as possible. Options such as clarification, bactofugation, fat separation, recombination and homogenisation in addition to heat treatment, will be investigated. The need to retain the organoleptic qualities of the final milk product will also be born in mind. This work offers the most promising short-term way to reduce human exposure to viable MAP via milk and milk products.

## C. Conclusions and Recommendations

1. MAP is a bacterium from the same family as that which cause tuberculosis (TB). It does ***not*** cause TB. In cattle and sheep it can cause a chronic infection of the gut called Johne's Disease. Ever since Crohn's Disease – a chronic inflammatory disease of the gut in humans – was recognised, the similarity of this disease and Johne's Disease has been noted, and the possibility that MAP infection is important in CD has been considered. However, isolation of MAP from CD patients is rare.

2. The FSA survey of MAP in milk has shown that MAP can survive pasteurisation in a small proportion of cases.

3. It is likely that a significant part of the abnormalities observed in CD are related to disturbance in the normal immune process in the diseased bowel. However, it is still not clear how this is initiated. It is clear a genetic predisposition to an abnormal response to gut molecules is part of the picture in at least some cases.

4. MAP can be hypothesised as playing a part by damaging the bowel in a 'normal' person and so permitting an abnormal response to develop; by taking advantage of abnormal permeability in a susceptible sub-set and so causing a disease in a susceptible sub-set; or by being normally an 'innocent bystander' that takes advantage of a diseased bowel caused by inflammation caused by other factors, and then multiplies and exists in the damaged bowel. In the latter case MAP could either exacerbate symptoms from the basic pathological process, or could merely grow in the favourable environment without causing any symptoms.

5. The possible link between MAP and Crohn's Disease has been extensively investigated. There continues to be insufficient evidence for a link at present, and it is clear that if MAP is causally linked to CD it forms only one strand in the picture; genetic and immunologic factors also playing a significant part. Improved methodologies are needed and are being developed to clarify the picture. It is likely that considerable progress might be made in the near future in the understanding of the issue.

6. Consistent with the FSA and the Government's commitment to the adoption of a precautionary approach to food safety wherever possible, the FSA is working with the industry to explore ways of improving the efficacy of the pasteurisation process with respect to MAP, and MAFF has recently published a review of the infection of ruminants with MAP in England and Wales, putting it in the context of the global situation (the report is on the MAFF web site www.maff.gov.uk).

7. Some of the key areas for further action include:

    - improving information on the prevalence of the infection in ruminants;
    - improving information on the extent of Crohn's Disease in humans;
      (both these are essential and need to be collected over time so that the efficacy of any measures to control the diseases in humans and animals can be assessed properly over time).
    - a rigorous analysis of the geographical and temporal distribution of JD and CD by a suitably constructed expert group so that evidence of possible causality can be better assessed;
    - pursuing the work already being funded on improving the efficacy of pasteurisation and other related treatments of raw milk via the MAFF-LINK project and other related studies;
    - increasing education and training in all groups involved in dairy food production on ways of minimising the spread of this infection during the production of milk and dairy products, and possibly other ruminant food products, at all stages in the food chain from the food animal on the farm to the dairy and other food on the plate;
    - further work on the characterisation of the biological response of humans to MAP exposure and the nature of the early factors initiating Crohn's Disease.

8. The various epidemiological recommendations might best be taken forward by convening a supra-national epidemiological workshop to consider the animal and human data, to

interpret what is available as well as possible, and to advise on the most fruitful further studies.

9. A meeting of clinicians, immunologists and other experts on Johne's Disease and Crohn's Disease to seek agreement on the evidential basis for a link between the two diseases from a human disease management point of view.

10. An open meeting on the issue will take place later in the year (probably in the Autumn) which is intended to inform the public as fully as possible about this rather complex issue, and also to ensure that the FSA takes account of any views the public may have on the best way to manage the issue.