

**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

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PREFACE

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. 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 set the scene for the stakeholders by summarising the available information on the nature of Crohn's Disease, and the information about its likely causation, especially in relation to possible links to exposure to MAP. It has now been updated following that meeting.
3. It concentrates on looking at the issue of MAP and CD from the entirely pragmatic point of view of informing 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.
4. This is an extremely complex area and one of the major difficulties is facilitating the exchange of information and understanding between the different stakeholders to ensure decisions are fully informed by the available data, appropriately interpreted.
5. There have been numerous reviews of the possible relationship between MAP and CD. All have concluded that the concept of a link is attractive but the evidence for a link not conclusive. This report does not reach a different conclusion.
6. In producing this report, I have drawn extensively on the Report of the Scientific Committee on Animal Health and Animal Welfare from the EU Directorate 8¹, the SAC Veterinary Sciences Division report on Johne's Disease in the UK², Daphne Thompson's review in the Journal of Clinical Microbiology in 1994³, Hermon-Taylor *et al's* review in the Canadian Journal of Gastroenterology in 2000⁴, and the International Dairy Federation meeting in Brussels in January 2001, all of which provide invaluable analyses of highly complex data.
7. I have spoken to as many experts, stakeholders and concerned people and groups as possible in the time, and thank all those who assisted me for their time and help, including the many references and papers to which they referred me. Time and other practical constraints prevented me from consulting as many people as I would have liked, and I apologise to those to whom, for a variety of reasons, I was unable to speak. All those listed at Appendix 1 received a copy of the first and second drafts of the report. The review has benefited greatly from their comments, but responsibility for this final version of the report is mine. The review will be revised in due course, so further comments, corrections and contributions are welcome, and should be sent to Dr Rubery (e.rubery@jims.cam.ac.uk).

8. Support for the hypothesis that there is a link between CD and MAP varied widely amongst those I spoke to. Judgement on the persuasiveness of the evidence to some extent at present appears to depend on one's scientific background. Thus clinicians and immunologists tend to be sceptical about the possible link. Microbiologists and public health specialists are more cautious about discounting the hypothesis. No one discounted the possibility of a link for some cases of CD entirely; neither did anyone suggest it was now proven.
9. Johne's Disease and the issues of its control are dealt with here only insofar as that is necessary to provide the context for the consideration of the role it might play in the aetiology of CD.
10. Similarly, whilst the report touches on the other possible contributory or major causes of CD, it deals with them only in the context of the possible role that MAP might play.
11. Thus ways of assaying for the presence of MAP and other Mycobacteriaceae in tissues and samples are developing fast and at present appear to those other than the *cognoscenti* to have an almost Byzantine complexity. As in any fast-developing area, reports in the journals can be confusing and difficult to interpret. I have tried to avoid getting into the complexities of the assay systems in this paper, since I do not believe this is key to reaching a decision on what should be done about human exposure to the MAP agent. It is sufficient to be aware that the information at present is confusing and difficult to interpret, and to hope that clarity will increase as techniques are refined and new systems developed.
12. Similarly the immunological data has been selected to concentrate on what appeared relevant to the issue of causation.
13. The Executive Summary seeks to condense the paper for those not wishing to return to the underpinning literature. I have also provided a Glossary of Terms at Appendix 2.

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June 2001

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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 range 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 others 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 effects of any changes in the incidence or 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 boy 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 expeditiously as possible. Options such as clarification, bacto-fugation, 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.

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

A. Crohn's Disease

1. The nature of Crohn's Disease

- (a) Dalziel⁵ first described Crohn's Disease in 1923. In 1932 Burrill Crohn and two colleagues⁶ reported a series of cases, and thus the disease got its name.
- (b) Crohn's Disease affects the digestive system, most commonly the small and large intestine (Figure 1). It causes the wall of the digestive system (gut) to become thickened, inflamed and swollen. Sometimes the thickening leads to narrowing of the tube or 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 it from the other Inflammatory Bowel Diseases (IBD) such as Ulcerative Colitis (UC).
- (c) 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.
- (d) Crohn's Disease itself may not be a single entity but could well include pathology caused by a variety of means. Lennard-Jones⁷ carried out a discriminant analysis^{8,9} of CD and UC and found CD to be a heterogenous disorder, in contrast to UC which was clinically and pathologically relatively homogeneous. However, neither disease had any specific defining feature present in every case and absent in all other conditions.
- (e) It is important to be clear that agents which cause the disease to appear (initiators) maybe quite different from agents which cause relapses of quiescent disease. So far no 'cure' for CD has been proposed. Indeed, because the disease pursues a chronic, intermittently relapsing course the definition of a cure is difficult anyway.
- (f) Greenstein and Greenstein have postulated that there are two forms of Crohn's Disease, an aggressive fistulising form and an indolent obstructive form¹⁰. They compare these to the tuberculoid and lepromatous forms of leprosy. Such a division has obvious attractions in relation to the postulated link between Crohn's Disease and MAP. However, the evidence for the two types is not generally accepted at present.
- (g) Whether the proposal of Greenstein and Greenstein is right or not, the likelihood that the pathological entity known as Crohn's Disease includes a spectrum of diseases means that 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.

2. The burden of disease

- (a) When trying to get a feel for the burden of disease in a population due to Crohn's Disease, two measurements are helpful – the **prevalence** of the disease in the population and the **incidence** or rate of diagnosis of the disease in the population.

- (b) The **prevalence** looks at the total numbers of people in the population with the diagnosis at any given time, including those who are symptomatic and asymptomatic at the time of study. With a chronic disease like Crohn's Disease, this figure will consist largely of cases diagnosed years before the date of measurement of the prevalence and, in a disease with early onset and prolonged course like Crohn's Disease, will change slowly with time.
- (c) Prevalence helps greatly in assessing demand for clinical and social services to support patients and by providing a measure of disease and suffering. However, if some factor suddenly makes the disease appear less or more frequently in a population, or if something suddenly makes people live longer or shorter periods after diagnosis, there will be a relatively small effect on the prevalence initially.
- (d) In contrast, **incidence** relates to newly diagnosed cases occurring in a defined period of time, frequently within any one year. If a disease is caused by an environmental agent then altering the exposure to the agent is likely to be picked up first in changes in the incidence of the disease.
- (e) Even here, though, if the disease takes several years to appear after exposure to the agent (i.e. has a long incubation period) then incidence rates will not change immediately, rather there will be a 'lag' period related to the duration of the incubation period, before any changes occur.
- (f) Nevertheless, measuring annual incidence rates and looking at the trend or change in these rates over time does provide the most sensitive way of exploring a possible relationship between an agent and a disease.
- (g) Caution is also necessary, however, before assuming that correlation between exposure to an agent and change in incidence rate of a disease is due to a **causal** relationship. It is also possible that the disease and the agent are both changing due to some third undetected factor, which affects them both.

3. Prevalence and incidence of Crohn's Disease

- (a) 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 people¹¹. Rubin *et al*¹² recently surveyed GP records in the North of England and found an incidence of 8.3 per 100 000 for Crohn's Disease leading to a calculated point prevalence of Crohn's Disease of 145 per 100 000 on January 1st 1995 or 1 in 690, a considerably higher figure than that from NACC, but based on a regional survey. Only 30% of the patients identified with inflammatory bowel disease were definitely under specialist care.
- (b) Ragnath and Tsai, in a smaller review of a practice of 7000 patients in a large city centre found a somewhat lower prevalence of 84 per 100 000. Of the 6 patients identified with Crohn's Disease, three had had surgery and one was on medication.¹³
- (c) There is increasingly persuasive evidence that the rate of diagnosis (i.e. incidence) of new cases is increasing. For example, the recent Tripartite Study of inflammatory bowel disease in children identified 700 new cases of IBD during the year, when only 300 were expected. Although direct comparisons are not possible due to differences in the way the study data were collected and some of the excess could be due to better ascertainment of cases in the recent study¹⁴, this is unlikely to be the full explanation.
- (d) Crohn's Disease most commonly starts between the ages of 15-40, although a significant number of cases tracked by the National Paediatric Infectious Bowel Disease Register are younger than 15 at diagnosis¹⁵. 5 000 children have IBD in Britain, and a rate of 5.2 new diagnoses per 100 000 was reported in the year of study. Figures from the US report that 20% of cases are diagnosed before the age of 20; and 40% between 20-29, so that 60% are under 30 at the time of diagnosis¹⁶.
- (e) The active disease is characterised by reduced appetite, abdominal pain, bloody diarrhoea and tiredness. During childhood the disease can result in a reduced growth rate due to difficulties in ensuring adequate nutritional intake.

- (f) 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.
- (g) Although only carried out as a last resort, a significant proportion of patients (see para A3(h) and (i) below) 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.
- (h) For example, Lapidus *et al*¹⁷, reporting on follow-up of patients with colorectal Crohn's Disease, found that 37% had peri-anal/rectal fistulae, about 50% needed major surgery over a 10 year period and 25% ended up with a permanent ileostomy by 10 years after diagnosis.
- (i) Michener *et al*¹⁸, in a follow-up study over 30 years, found 75% of patients underwent surgery at some stage in their lives, 50% experienced two surgical episodes and 11% three or more. In addition there is considerable loss of quality of life physically, emotionally and socially¹⁹. The patient groups provided ample information to support this.
- (j) In Rees and Levison's series of 67 children with Crohn's Disease, 38 were well with no recurrence after 15 years, 14 had no residual disease, and only 6 were symptomatic. However, the effect on growth was significant, with 21 showing growth retardation, although this was only permanent in 10.
- (k) Therefore, *in conclusion*, 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.

4. Resource consequences of Crohn's disease

- (a) Setting aside the cost to the sufferer and their family and friends from disruption of normal life, the cost of managing this disease is considerable.
- (b) From the figures in Rubin *et al* above one can estimate that there are over 90 000 people with Crohn's disease in the country at present²⁰. Assuming an annual average cost for treating Crohn's Disease patients of £2500, this leads to a calculated direct cost to the NHS per year for treating and managing this disease of over £200 million.
- (c) An American study of lifetime costs of Crohn's Disease estimated an average lifetime cost of \$39 906 dollars in 1993, when discounted at 5% per annum. It suggested that relapse and treatment needs declined over time from diagnosis, and suggested that surgery generally resulted in better value for money than prolonged medical treatment with salicylates²¹. Kennedy, using this cost data as the basis of his calculations, reaches an estimate of £320 million for treating these patients in the UK in 2001²².
- (d) A Swedish study²³ looked at the total cost to society in Sweden of CD, including hospital admissions, ambulatory care, drugs, sickness leave and early retirement. Sweden has a population of 8.8 million, and one of the highest incidences of CD at 6 per 100 000, giving an annual rate of new cases of about 500 on top of the 13 000 already affected.
- (e) They estimated a total cost of \$43.1 million in 1991, of which \$12.7 million (27%) were direct costs (i.e. in-patient care, ambulatory care and drugs). 2% of all patients were responsible for 10% of admissions during 1994. Drugs constituted on 6% of the total cost. Indirect costs largely due to sickness leave and early retirement made up 74% of the total.
- (f) Apart from the Swedish study, none of these calculations take any account of the costs of social support for the patients, loss of productivity due to inability to work; damage to family life and other members of the family from their illness, or human suffering. These

indirect costs can be considerable, as is evidenced by the Swedish estimates, where they were three times greater than the direct costs.

- (g) New drugs are becoming available to treat the disease, such as Remicade (Infliximat), the new mono-clonal antibody against tumour necrosis factor, which costs about £1200 per annum per patient. A rise in costs per patient is therefore likely in the absence of any advance in understanding the nature of the disease and how it can be prevented or cured.
- (h) **In summary**, costs to the NHS of treating Crohn's disease are likely to lie in the region of £200-£320 million per year at present; they are likely to rise as increasingly expensive treatments are developed. 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. New therapies may cause these costs to rise.

5. Pathology

- (a) Crohn's Disease was initially described as a low-grade inflammation of the terminal ileum²⁴. Subsequently it was realised that the disease could affect other parts of the gastro-intestinal tract including the eye,²⁵ mouth, larynx, oesophagus, stomach, colon and anus, and that it could also cause lesions in the skin, muscle, synovial tissues and bone²⁶.
- (b) Microscopically the ileum is infiltrated with mononuclear inflammatory cells, macrophages and lymphocytes. Often macrophages fuse together to form giant cells and a diffuse granulomatous inflammation results. The pathologic lesion in the intestines closely resembles those of intestinal tuberculosis, but acid-fast bacilli (AFBs) are not detected, as would be the case in intestinal pulmonary tuberculosis (TB). Dalziel drew attention to this similarity with TB before going on to link the pathology with Johne's Disease in cattle²⁷. In Johne's Disease, however, AFBs are usually present in large numbers and are readily observed in stained tissue sections. However forms of JD do occur in which no Z-N positive Mycobacteria can be seen in the tissues. Intestinal tuberculosis in man has also been reported to exist on occasions without detectable AFBs^{28, 29}.
- (c) Although there is considerable similarity between JD and CD, there are also significant differences in the pattern of the disease³⁰:
- there is no fissuring ulceration, fistula formation or fibrosis in JD, whereas these are common complications of CD;
 - there is no caseation in the granulomata in CD;
 - the course of the disease is progressive once established in JD, but intermittent in CD;
 - the organism is easy to recover in many cases of JD but has rarely been recovered and then only with difficulty in CD;
 - there is no evidence of the organism on histochemical, immunostaining or in situ hybridisation to demonstrate the organism in CD;
 - Steroids exacerbate TB and other mycobacterial infections in AIDS patients, but produce remissions in CD.
- (d) **In conclusion**, therefore, 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.

6. Possible causes

- (a) The cause of the disease is not known. In general possibilities fall into three categories:
- genetic predisposition;
 - an abnormal immune response;

- environmental factors.
- (b) These three categories are not mutually exclusive (Figure 4); indeed, the likelihood that there are a number of contributory factors is probably one of the more firmly supported conclusions one can draw from the data.
 - (c) Clinical and epidemiological evidence strongly supports the presence of **genetic factors** that are important determinants of susceptibility and disease behaviour. In particular, there are concordance rates in siblings and in monozygotic twin pairs, suggesting a genetic component at least as strong as that in other immune-mediated diseases such as multiple sclerosis and insulin-dependent diabetes mellitus. Epidemiological studies demonstrate that if one member of a family has Crohn's Disease there is an increased likelihood that others will develop it. Furthermore the site of disease appears to also run in families to some extent³¹. The risk of a child of an affected parent developing Crohn's Disease is around 1 in 100. This is likely to be due at least in part to a genetic predisposition.
 - (d) There is not a simple Mendelian pattern of inheritance as occurs in haemophilia, rather the evidence suggests a polygenic pattern of disease, with the possibility that some of the loci are also involved in ulcerative colitis susceptibility. Efforts to identify the loci are now in hand. A possible link with a region on Chromosome 12 has been suggested. In addition, NOD2, a gene that encodes for a protein with homology to plant resistance gene products, that is located on Chromosome 16, has been shown to possess a frameshift mutation due to a cytosine insertion in Crohn's Disease patients. This mutation results in the production of a truncated NOD2 protein. Wild-type NOD2 activates nuclear factor NF- κ B, making it responsive to bacterial lipopolysaccharides. The mutant form lacks this ability. Other work has associated NOD2 leucine-rich repeat variants with susceptibility to CD. This suggests the nature of the innate immune response to bacterial components is relevant to the development of the disease.^{32,33}
 - (e) These findings are consistent with work exploring the nature of the genetic predisposition suggests this is most likely to be due to a defective mucosal barrier (see Section E.2 and other sections in E below), which allows uptake of bacterial, dietary and other immunogenic macro-molecules³⁴.
 - (f) Another possible component of the pathology of the disease is an **immune hypersensitivity** due to the immune system over-reacting to stimuli in the gut (such as certain foodstuffs and normal gut bacteria) that are dealt with uneventfully by most people. A modification of this theory is that of Roediger, who proposed that an amalgam of speroplasts of mycobacteria, possibly MAP, plus cholesterol esters or phospholipids could produce reactant bodies in the area of the distal ileum where they could act as the antigenic stimulus to cause the disease³⁵.
 - (g) The third possibility is that it is caused by an **infectious agent** such as a virus or bacterium. A range of possible candidates have been proposed over the years as well as MAP, including the measles virus, listeria species, and bacteroides³⁶. The chronic inflammatory nature of the disease bears some resemblance to the changes observed in leprosy and tuberculosis. This has lead support to the hypothesis that a mycobacterium could be the cause of the disease.
 - (h) More recently the possibility of that small **particles of metallic material** ingested, perhaps in certain food additives, has been suggested as a predisposing factor³⁷. The suggestion is that the microparticles of, for example oxides of titanium, aluminium and silicon used as food additives, may rapidly absorb antigen and then fundamentally change the intestinal immune response to these antigens by presenting them in a more accessible way. This could increase their immunogenicity, and so generate an immune hypersensitivity type disorder. This hypothesis is dealt with further at D.7.
 - (i) 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 could also offer possible ways of treating the disease, and even of curing some of those suffering from the disease.

- (j) The rest of this paper concentrates on an analysis of the evidence linking MAP to CD while putting that evidence into the context of other theories and/or possible contributory factors.

7. The MAP hypothesis

- (a) The hypothesis that Crohn's Disease is caused in part by exposures of the human population to *Mycobacterium paratuberculosis* is perhaps most clearly expounded in Hermon-Taylor's editorial in *Gastroenterology* in February 1993³⁸, which is briefly paraphrased below.
- (b) MAP causes chronic inflammatory disease in the intestines of a wide range of ruminants, monogastrics such as dogs and pigs and at least four sub-human primates – Macaques³⁹, Baboons, Gibbon and cotton-top Tamarins. It has been shown to fulfil Koch's postulates in animals, i.e. MAP is isolated from animals with the Johne's Disease; the AFB-staining organism can be identified in the affected tissues in the host; and injection of MAP organisms into the susceptible host produces the disease⁴⁰. The organism has a marked tissue tropism for the gut even if administered sub-cutaneously or intravenously. It can persist in the gastrointestinal tract for years without causing disease. The organism can exist inside macrophages in both clinically and sub-clinically infected animals. It can exist in the reproductive organs of both sexes and can cross the placenta and enter the fetus.
- (c) Infection can be expressed in several ways. There is often a pluri-bacillary type, with abundant Ziehl-Neelsen-positive (ZN) AFB organisms present in the intestines (see para B:1:a). Alternatively the pauci-bacillary form can occur, with chronic granulomatous inflammation analogous to leprosy lesions in humans. In the pauci-bacillary form in ruminants the standard tests for the organism can be negative.
- (d) The two forms of the disease can co-exist in the same animal in ruminants, with granulomatous disease in the liver co-existing with pluri-bacillary lesions in the gut.
- (e) Johne's animals infected with the pluri-bacillary form are known to secrete large amounts of the organism in their faeces. Milk from such animals is likely to be significantly contaminated with the organism largely via faecal contamination but also sometimes due to secretion of the organism directly into the milk by the affected animal. While milk is likely to be the major route for the infection of young animals other tissues in the infected animal have also been shown to contain the organism, including the mucosa of the ileum⁴¹, fetus⁴², genital organs (except the testes) and semen^{43 44}, tissues and blood (using PCR-q.v.below)⁴⁵ and milk⁴⁶. Some of these studies detected the organism in asymptomatic animals.
- (f) Pasteurisation of milk has been seen as a safeguard against significant exposure of the public to the live agent. But recent evidence that this organism may survive pasteurisation has meant that the possibility that the public is exposed to significant levels of the organism in milk and dairy products cannot be discounted (see Section F.1 below).
- (g) 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. The limited present data on infection with MAP and clinical infection with JD in the UK suggest an upward trend, but are of poor quality.
- (h) Consideration of the economic cost of this disease to farmers is not for this paper. However, the National Association for Colitis and Crohn's Disease (NACC) provides some calculations for those who are interested on their website at www.crohns.org, and the International Dairy Federation publication on MAP gives a valuable industry overview of the issue⁴⁷.

8. Summary

- (a) Therefore, MAP is a possible human pathogen in ruminants and other farm animals. Exposure of the human population to the agent 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.
- (b) Whilst this paper will concentrate on evidence linked to the 'MAP' hypothesis, it is important to recognise that a range of other potential candidates for the causative agent for CD⁴⁸ have been proposed over the years including:
 - toxic responses to other microbial pathogens such as measles virus, *Listeria spp*, *Bacteriodes vulgaris*, and many others;
 - a genetically defective mucosal barrier;
 - dysregulation of the hosts immune response to normal intestinal microflora;
 - an amalgam of spheroplasts of Mycobacteria with cholesterol esters or phospholipids to produce reactant bodies that acted as an antigenic stimulus to CD;
 - some combination of two or more of these factors, and a range of other less well documented factors.

B. The Myco-bacteriaceae

1. The general characteristics of the species and what it means for their pathogenicity

- (a) The Mycobacteriaceae include numerous species and are widely distributed in the environment. They are aerobic, non-spore-forming bacilli whose cell walls contain a large proportion of high molecular weight lipid. This is responsible for the characteristic pink coloured envelope on Ziehl-Neelsen (ZN) staining. For this reason they are often called 'acid-fast bacilli' (AFBs)
- (b) Many of these organisms multiply very slowly, (although there is a group that multiply rapidly), commonly with a doubling time of 15-24 hours, in comparison to the 15-30 minutes of organisms like the Salmonellae. They are difficult to culture, requiring complex media and usually taking several months to produce colonies.

2. The diseases they cause in man

- (a) There are around 15 species known to be pathogenic for man, of which *Mycobacterium tuberculosis* (*M.tuberculosis*), which is the major cause of pulmonary tuberculosis, is probably the best known.
- (b) Tuberculosis was shown to be due to an infectious agent by Francoise Villemin in 1865, when affected tissue was injected into guinea pigs, who subsequently developed a similar disease. In 1882, Koch identified the tubercle bacillus as *M tuberculosis* and demonstrated its pathogenicity⁴⁹.
- (c) *M leprae* causes leprosy, which, according to WHO now has a global prevalence of around 1 in a million⁵⁰. So far it has not proved possible to culture this organism in bacterial media or cell culture^{51, 52}.
- (d) *M.bovis* is a zoonosis, able to pass from infected cattle to man, usually via milk. It produces, most commonly, infected lymph nodes in the neck. It had been eliminated from the UK for all practical purposes by a combination of control of the primary disease in cattle plus pasteurisation of the milk, but more recently there is concern about a persistent and possibly increasing problem with the disease in the south west.⁵³
- (e) *M. ulcerans* causes Buruli ulcer, and is an increasing problem in West Africa, where it is now the second most important mycobacterial disease in some populations⁵⁴.

- (f) *M. avium* (MAC) causes infections in man, most commonly in immunosuppressed patients, such as those with HIV/AIDS.
- (g) Other species can cause asymptomatic colonisation of the body⁵⁵, especially in the immunocompromised.
- (h) The diseases caused by the Mycobacteriaceae are in general characterised by an indolent course, with chronic granulomatous lesions, sometimes leading on to caseation. Infection may occur a long time before symptoms appear. While sometimes the disease is expressed by pathology that includes the release of large quantities of the causative organism (pluri-bacillary forms) other forms of the disease may release little or no organisms (pauci-bacillary forms).

C. Mycobacterium Paratuberculosis (MAP)

1. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in animals

- (a) *M. avium* subspecies *paratuberculosis* (MAP) was originally identified in 1895 as the cause of a chronic inflammatory condition in a German cow⁵⁶. Subsequently the link between this bacillus and Johne's Disease (or paratuberculosis) of cattle and a wide range of other mammals was established⁵⁷.
- (b) It is an aerobic, non-spore-forming, non-motile, acid-fast bacillus⁵⁸. 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 19698⁵⁹. More recently, using molecular biological techniques, 28 strains of MAP have been identified⁶⁰. This organism has also been detected in the wild in wild ruminants and deer and strain typing was used to trace the spread of the disease from one population to another.⁶¹
- (c) 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. A major constituent of the cell wall is lipoarabinomannan (LAM), which may play a large part in the formation of the granulomatous lesions found in the disease. LAM differs immunogenically in bovine and ovine isolates, is highly immunogenic and forms the basis of the enzyme-linked immunoassays (ELISA) developed to strain-type the species⁶².
- (d) Cattle are most susceptible to infection during the first few months of life, frequently being infected whilst taking milk from their mother⁶³. 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 is sparse, but JD is a systemic disease and the organism is therefore likely to be present at least intermittently in blood and other tissues of the infected animal. It is thought the major source of infection is via faecal excretion followed by contamination of the milk by local spread. Foetal infection also occurs⁶⁴.
- (e) 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. The duration of the incubation period may be dose-related but, in addition, 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 animal⁶⁵.

2. Infection with *M. avium* subspecies *paratuberculosis* (MAP) in man

- (a) In 1913 it was suggested that this organism might also cause chronic inflammatory disease in the human intestine. Since then an extensive literature on the possible link has appeared, especially in relation to the possibility of a link with Crohn's Disease in man. So far it has proved difficult to reach a definitive conclusion on this issue.

3. Characteristics of the agent from molecular biological studies and what it means for its possible pathogenicity

- (a) Hermon-Taylor *et al*⁶⁶ review the molecular biological information relating to the nature of the MAP organism in considerable detail. The work is of considerable interest in demonstrating the contribution such studies are now able to make to understanding of the etiology and pathogenicity of an organism. This greatly aids the possible focus of attempts to produce diagnostic agents and to develop strategies for the control and treatment of any diseases caused by it.
- (b) It is probable that MAP derived from the relatively non-pathogenic *M avium* (MAC), which is widely distributed in the environment and in the intestines of healthy animals and man, and is not usually pathogenic unless there is immunosuppression. However, the acquisition of three new sequences of DNA, confer on it pathogenic properties.
- (c) The three new sequences are:
 - There are between 14-18 copies of a DNA insertion sequence called IS900 which is 1451-1453 base-pairs and encodes a 43 kdalton DNA binding protein, that is thought to be a transposase, p43, made from the positive strand of DNA. IS900 has been shown to be uniquely specific for MAP, and may be responsible for the activation of the 'GS' complex (see next section). It is therefore a useful basis for the development of specific diagnostic tests for MAP. It is expressed in MAP cultured *in vitro* and has been detected in the diseased intestine of some humans with inflammatory bowel disease.
 - There is a single copy of a further DNA sequence with an unusually low percentage of 'GC' doublets, called 'GS'. This was discovered by subtracting the DNA of non-pathogenic MAC from MAP. It has a similar structure to 'pathogenicity islands' in other bacteria⁶⁷ and codes for 6 genes that produce enzymes involved in the modification of glycosyl residues, in particular, fucose moieties. Hermon-Taylor proposes that this sequence is responsible for the biosynthesis and modification of fucose on the surface of the bacteria, where these enzymes attach fucose to the terminal oligosaccharide moiety of the surface glycopeptidolipid. Hermon-Taylor suggests that this gives MAP a 'Teflon coat' that enables it to successfully survive inside the host and to evade recognition by the immune system.
 - There is a further region which has incorporated the gene 'hspX'.
 - Hermon-Taylor *et al* postulate that IS900 and GS are 'foreign' DNA that have become incorporated into an *M avium* background species and led to the development of a pathogenic phenotype. Work on another pathogenic strain of MAP also attributes its pathogenicity to changes in the GS region resulting in alterations to the glycolipid coat, thus providing further support for the hypothesis.
- (d) The total genome sequence of a bovine MAP isolate is nearing completion at the University of Minnesota⁶⁸.

4. Culturability of MAP

- (a) It is difficult to culture MAP. 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.
- (b) 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.

- (c) 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^{69,70}). In addition, of course, many infected cattle will be asymptomatic (a herd infectivity rate of 25 times the symptomatic rate has been quoted⁷¹).
- (d) More recently work by Grant *et al* at Belfast on the development of immuno-magnetic separation (IMS) techniques offer the possibility for much increased sensitivity of the separation techniques available before the organism is cultured⁷².
- (e) The interpretation of research in this area has been be-devilled by difficulties in both carrying out the experiments and in interpreting the results. Recent developments in techniques hold out considerable hope that clarification of some of the uncertainties will shortly be possible.

D. Epidemiology

1. General

- (a) Epidemiological data is usually most valuable in demonstrating correlations. It is a powerful tool in assisting in identifying promising leads that can be further investigated by other means. Its use in support of arguments that any environmental exposure or observation is causally related to the occurrence of a condition is more problematical.
- (b) Before considering the results of the various studies, one needs to consider the likely time course of exposure, infection and expression of disease. If MAP causes CD the relationship is clearly not a straightforward one of infection followed a few days later by disease. Infection in ruminants is followed by a latent period of 2-5 years before symptoms occur. In ruminants, the calf is thought to be most frequently infected before weaning while feeding from its infected mother. But the disease is not usually symptomatic for several years.
- (c) It is likely that calves have a susceptible period during infancy and are relatively resistant to infection if exposed to the agent in later life (see section C.1 above). If a similar situation obtains in humans vis-à-vis CD then one would anticipate great difficulties in linking conclusively exposure to the agent to clinical disease.
- (d) Then there is the issue of the detection of the organism in infected tissues. Because we know there is a pauci-bacillary type of disease, and indeed this is the type that most clearly resembles CD in humans, one might not expect the organism to be easy to detect in CD. Conversely, even if found in low levels, as has indeed been the case on a number of occasions, the organism may be present merely as a passenger (bystander organism), and have nothing to do with the causation or persistence of the disease, since chronic inflamed tissues are readily colonised by other microbial agents.
- (e) A spheroplast form of the organism, which has no AFB-staining/ZN-positive envelope, can occur in the intestinal tissues and further complicate the picture.
- (f) Furthermore, probably many infected animals do not show symptoms of the disease, and expression may be exacerbated or precipitated by stress or other inter-current events⁷³. Then CD itself is likely to be a 'portfolio' disease including pathology caused by a variety of stimuli which result in similar pathology.

2. Population studies

- (a) The greatest prevalence of Crohn's Disease is found in Western Europe and North America, suggesting a possible association with the developed world. Isolated areas of Greece, in contrast, have very low prevalence and incidence rates; in contrast, their rates of UC are the same as the rest of Europe.⁷⁴

- (b) In Stockholm a fourfold increase in diagnosed cases was reported over the last 20 years by Rees and Levison in 1987. While some of this is likely to be due to better diagnosis and greater awareness,⁷⁵ it is likely that some of the increase is real.
- (c) In the UK, good quality trend data are not available, but an increase in cases may be occurring. In Scotland, Barton *et al* in 1989 report an increase in the number of new cases of Crohn's Disease diagnosed in children under 16 from 6.6 per million in 1968 to 22.9 per million in 1983, an over threefold increase. This is consistent with a review of 17 large, well-conducted epidemiological studies by Calkins *et al* (Table 1) in 1984, which found increasing levels in the 1960s and 70s, but possible plateauing in some studies in the more recent years examined⁷⁶. Other retrospective studies have also suggested a trend upwards in the incidence of IBD^{77, 78}. However, these findings could be due in part or possibly entirely to changes in diagnostic criteria or ascertainment.
- (d) Sawczenko *et al* have just completed a prospective study of childhood inflammatory bowel disease (IBD) in the British Isles⁷⁹, which suggests that the incidence may be rising. They reported an incidence of IBD of 5.2/100 000 per year in children under 16 years in the UK and Republic of Ireland between 1998-9. The incidence of Crohn's Disease was 3.1/100 000 in the UK and England; 4.2/100 000 in Scotland, 3.2/100 000 in Wales, 2.4/100 000 in Northern Ireland and 2.3/100 000 in the Republic of Ireland. Crohn's Disease was overall about twice as common as ulcerative colitis.
- (e) The authors comment that the higher overall rate in Scotland is also twice as high as the rates earlier reported in the retrospective 1983 survey, and also greater than the 1990-1992 rate. The authors plan to repeat the survey in three years time, and this will provide invaluable trend data for the UK. The upward trend in Scotland is mirrored by the increase in Sweden reported over the last decade⁸⁰. There is also evidence that the disease is appearing at an increasingly early age in Scotland, and CICRA are intending to fund some studies on possible environmental or other triggers that might be responsible⁸¹.
- (f) Asian and West Indian children living in the UK appear to be developing a similar rate to UK-born children, while the disease is rarer in their native lands^{82,83,84,85}.
- (g) A pan-European study of the incidence of Inflammatory Bowel Diseases published in 1996⁸⁶, confirmed an 80% higher rate of CD in the north compared to the south of Europe. The lowest rate of CD was in northwest Greece. The magnitude of the difference was less than in previously reported studies and the authors postulated that this was due to an increase in rates in the south while rates in the north had stabilised. These results are discussed more fully in the EC Review¹, where they are tabulated for clarity. Other reports have suggested incidence in the north of Europe is plateauing. It seemed unlikely that the differences were due to ascertainment problems, since the rates for ulcerative colitis did not vary in a similar way.
- (h) The 80% higher incidence of CD in the North compared with the South could not be explained by differences in tobacco consumption or education. The age-specific incidence rates show that the 15-24 year age group was at the greatest risk of being diagnosed with the disease, and that the risk in the two sexes was similar.
- (i) In the USA a study of the prevalence of Crohn's Disease in Olmsted County on 1 January 1991 found 133 cases/100 000, a rise of 46% from 1980. This study is valuable as it covers over 50 years, uses the Mayo high quality medical records, and dealing with a population with low migration rates and a mixed urban/rural lifestyle⁸⁷. A further US study showed a decrease in tuberculosis in black populations accompanied by a corresponding increase in CD; in the early 1990s the incidence of CD declined and that of tuberculosis increased^{88,89}. After 1995, however, TB declined again to levels nearer to the pre-HIV era⁹⁰.
- (j) It appears that a similar North-South gradient exists in North America, since the highest reported incidence of CD in the world is in Manitoba, Canada, with a rate of 14.6 per 100 000 population per year^{91, 92}; in Northern Alberta the incidence rate was 10 per 100 000⁹³ and 7 per 100 000 in Olmsted County, Minnesota, 400 miles south of Manitoba⁹⁴.

- (k) In South Africa, CD was absent or rare in black people before 1980 and between 1980-85 only 25% of that of the white population⁹⁵.
- (l) The incidence of Crohn's Disease in the Jewish population in central Israel increased ten-fold between 1970 and 1980. In 1970 there was around a ten-fold difference between the prevalence in Europe-American immigrants compared to Asia-African immigrants, which had decreased to a two-fold difference by 1980 (Table 2). Worldwide, studies on the incidence of CD in Jews shows an increased incidence with marked geographical variation. Such data are again compatible with the possibility of an environmental agent contributing to the causation of the disease. The authors suggest these results are best explained by ascribing a mixture of genetic and environmental factors to the causation of the disease⁹⁶. A chemical or nutritionally related agent would also produce such a picture.
- (m) In contrast CD is rare amongst Bedouin Arabs living in the same areas as Jews (Beer-sheva)⁹⁷. The Bedouins are a rural population and show no evidence of increase in incidence of CD, levels remaining similar to the incidence in Arabs in Kuwait and Egypt. The incidence of CD in Jews in Beer-sheva varied with ethnic origin.
- (n) The EC funded a concerted action programme on the incidence of CD, which is summarised in Table 5 of their report⁹⁸. The EC review supports the proposal that such gradients make an effect of climate, diet, water supply or other environmental factors an attractive hypothesis.
- (o) Tamboli has suggested that there is considerable overlap of geographic regions with high prevalence of Johne's Disease and Crohn's Disease⁹⁹. However, a rigorous analysis of the geographical and temporal distribution of animal and human epidemiological data does not appear to have been carried out and there may be difficulties due to availability of data. Such a study, or analysis by an expert group, would seem well worth doing if it were possible.
- (p) **In conclusion**, therefore, these ethnic and geographically based studies show variations in rates that are strongly suggestive of a disease with a large etiologic component related to environmental factor(s) which could be infectious, chemical, physical or social. The considerable changes in rates observed during the last 50 years cannot readily be attributed to genetic variability in susceptibility to the disease alone.

3. Studies linking CD to an environmental agent

- (a) Clusters of cases can indicate an underlying infectious cause although they can also occur simply by chance.
- (b) Hermon-Taylor¹⁰⁰ reports a cluster of 13 cases in two families in northern France occurring in two periods: 7 in 1970-1973 and 4 in 1982-84 investigated by van Kruiningan¹⁰¹. The cluster was investigated for a wide range of micro-organisms including MAP, the only significant finding was raised antibodies to Breda viruses in over half of the affected cases and in nearly half of the rest of the family. However, both families had had a prolonged exposure to unpasteurised milk and so the possibility of exposure to MAP organisms if the animal source was infected did exist. Van Kruiningen *et al* suggests this may be due to an infectious agent with a considerable latency period between the exposures and the appearance of the disease.
- (c) Non-familial clustering of CD has been reported from a Cotswold village in England¹⁰² and among three college contemporaries, two of whom had a family history of IBD, and one of whom developed ulcerative colitis¹⁰³. Lennard-Jones reports studying 65 families each with three or more relatives having IBD¹⁰⁴. There were widely varying intervals between successive diagnoses, and also wide separation geographically between successive diagnoses. The paper concludes that the data does not support a suggestion of an infective agent with a long but relatively constant latent interval as a factor in Crohn's disease. However, a constant latent interval is not a necessary component of CD related to MAP infection. Stress and other precipitators may affect the time of appearance of symptoms. JD, for example, is known to have a tendency to appear in

response to stress. More details of the cases and the criteria upon which the study was based are needed to assess these results.

- (d) Mayberry reports an example of Crohn's disease clustering on the coastal plain of South Wales at Cardiff.¹⁰⁵, which had a level of Crohn's disease of 2.39 per 100 000 in 1972-4. Mayberry and Hitchens¹⁰⁶ reported highly significant ($p < 0.001$) clusters of cases in of Crohn's disease, but not of ulcerative colitis, in 11 Cardiff city wards. Of these 8 directly bordered the river Taff, and the remaining three were adjacent to the north and east; the direction that aerosol would be carried by the prevailing winds from the southwest. Hermon-Taylor points out that the Brecons, steep upland pastures heavily grazed by sheep and cattle lie to the North of Cardiff, and that it can be hypothesised that MAP organisms that they might excrete could pass from the topsoil into the Taff, which runs through Cardiff. Further investigation of the significance of these data would be helped by information on the source of the water supply to the different wards in Cardiff, as a water-borne exposure route might be expected to produce a higher exposure of the population than an aerosol route. MAP can survive for long periods in water. The SAC report, chapter 2, p.8, quotes five months' survival in pond water and 47 months' survival in soil. Information on the effectiveness of water treatments to produce drinking water in eliminating MAP has not been obtained in the UK.
- (e) Perhaps rather surprisingly there have been no studies of rates of CD in farming communities, animal health care workers or others having direct contact with infected animals. No link with rural communities has been established – rather an increased incidence in urban communities has been suggested in some studies.
- (f) **In conclusion**, the epidemiological work on humans and animals to date is sufficiently suggestive of geographically and time-related changes in the incidence of CD to suggest that a further package of carefully constructed studies, linked to better ascertainment systems and monitoring, would be well worthwhile and might result in much clearer information on likely aetiology.
- (g) **Recommendation**: that an epidemiological workshop is convened to look at the animal, human and epidemiological work done to date and to develop a strategy for work for the future so that the data becomes more coherent and so easier to interpret is justified. There is a potential for more information to be got from well-devised and conducted studies.

4. Familial incidence and case histories

- (a) Peeters *et al*¹⁰⁷ found an age-adjusted risk for Crohn's Disease of 1.5 in first-degree relatives of cases. Weterman and Pena¹⁰⁸ found similar effects in families in the Netherlands. Their review and other papers note an increased concordance rate in monozygotic twins, which suggests at least some of this increased risk is related to a genetic predisposition.
- (b) An interesting study by Gent *et al*¹⁰⁹ explored the possibility that early exposure to microbes might be protective against the disease by comparing 133 patients with Crohn's Disease with 231 with ulcerative colitis with respect to whether their first house had a hot tap. They found a positive association between the possession of a hot tap and Crohn's Disease with an odds ratio of 5.0, and no effect in ulcerative colitis, where an environmental cause is not generally thought to be likely.
- (c) The effect of diet on the incidence of Crohn's Disease could also contribute to familial differences in rates but no studies of the effect of diet on the incidence of the disease and whether rates differ in vegetarians and vegans have been found.
- (d) An interesting case history is reported by Hermon-Taylor *et al*¹¹⁰ in 1998 in which an 8-year-old boy presented with cervical lymphadenopathy that was histologically suggestive of mycobacterial infection. MAP was later demonstrated in these nodes¹¹¹. Cultures then and at relapse were negative, and there was no response to a short trial of rifampicin and isoniazid. Five years later he developed anaemia and arthritis. Abdominal palpation detected an enlarged loop of bowel and a white cell scan was consistent with CD. He

was treated with rifabutin and clarithromycin and improved after an initial exacerbation of symptoms. However he required resection of a narrowed piece of bowel after 6 months therapy when he was otherwise asymptomatic. Although no diagnostic link with MAP was demonstrated the authors speculate that this was a case of MAP infection developing into CD, possibly linked to drinking infected milk. Chamberlin¹¹², more recently, reports the presence of DNA from MAP in the resected surgical specimens when PCR tests were carried out.

- (e) This case is of interest in identifying a possible disease pattern for the prodromal period in young infected people. It needs following up with further work including, perhaps, a case control study to see if other Crohn's Disease cases had a similar prodromal phase to their illness. No other publications on possible early symptoms have appeared in the literature.

5. Geographical distribution of Johne's Disease

- (a) The prevalence of JD in the UK is not known; there has been no national study to date. Passive submissions to veterinary investigation centres lack a denominator but have shown an upward trend in the number of laboratory diagnoses over the last ten years¹¹³. An average of around 350 cases between 1980-1992 has risen to around 600 in 1995-99. Cetinkaya *et al* carried out a survey of sub-clinical infection in animals at the abattoir in the south west of England in 1994¹¹⁴ using a PCR assay based on the detection of IS900. They found 3.5% of animals had positive intestinal lymph nodes on PCR and 2.6% were positive on culture. The rates varied (non-significantly) during the year from 1.6% in April to 4.6% in November. In young cattle the prevalence was 2.0%. These levels are lower than those reported in studies of abattoir cattle in the England in the 1950s, when prevalence rates between 11 and 17% were found¹¹⁵. They are similar to figures from a similar survey in the USA, suggesting that the prevalence of MAP in cattle in southwest England and the USA may be comparable.
- (b) Referral surveys in southwest Britain using postal and telephone questionnaires surveyed dairy farms and veterinary practices respectively and estimated herd prevalences of 17% and 1%¹¹⁶.
- (c) The Netherlands had one of the highest prevalence rates of JD in the world, but has put in place energetic procedures to get the disease under control¹¹⁷. Prevalence studies in Belgium and, more recently, the Netherlands using ELISA-type tests found a seroprevalence of 1.2% in individual animals and 17.4% of beef herds containing affected animals in Belgium¹¹⁸, and 2.5% in individual animals and 54.7% in dairy herds¹¹⁹ in the Netherlands. Whilst there are a variety of ways of interpreting these data due to the relative insensitivity of the test these results do show that subclinical disease due to MAP is a widespread problem in Europe.
- (d) There have been studies in sheep; in Greece 9.8% of ovines tested positive in a delayed-type hypersensitivity test¹²⁰; in Spain in the region of Aragon serological, bacteriological and pathological techniques were used to identify 46.7% of flocks to be infected¹²¹ with a within flock prevalence of 33-46%. Similar results have been reported in the Basque country¹²².
- (e) Outside of Europe the situation appears similar. In the USA a national survey in the 1980s using bacteriological assay of over 7000 samples found infection in 1.6% of cattle and 2.9% of cull cows¹²³.
- (f) MAP species can also cause disease in other domestic and wild ruminants¹²⁴. This paper demonstrates the transfer of MAP from imported Charolais heifers to local wild ruminants including wild red deer, and roe-deer. However the study also demonstrated that other strains of MAP were already present in deer remote geographically from the imported heifers. Further studies linked other strains to imports of Holstein and Limousine cattle. Overall the study identified three strain types by the use of restriction fragment length polymorphism (RFLP). In all over 28 strains of MAP can be identified using this discriminator¹²⁵.

- (g) There are problems in all of these studies (Table 5) with types and sensitivities of the assays, with what is being sampled etc., nevertheless, what they do show is a significant epidemic of MAP-related infection across Europe, and probably across the world, if one considers the additional data presented at the IDF conference in Brussels in January 2001¹²⁶. The levels of infection cannot be precisely specified, but are clearly significant in those areas tested, and by extrapolation probably in a wide range of flocks and herds globally.
- (h) It follows from this that the world population is being exposed constantly and to a substantial extent to the MAP agent via food and probably water.
- (i) 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. A 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 measures¹²⁷. 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.
- (j) Nevertheless, given the increasing mobility of animal 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.

6. Strain types in Johne's Disease and Crohn's Disease

- (a) The strains of MAP that have been identified in humans with Crohn's Disease are close to those strains most commonly identified in cattle^{128,129}. The availability of these powerful strain-typing technologies (RFLP, DNA analysis, Representational Difference Analysis, etc.) should provide the opportunity for some more focussed studies to explore further possible links between the animal and human diseases.

7. Diet and Crohn's Disease

- (a) Crohn's disease itself will perturb the diet, as the patient seeks to consume foods that do not increase symptoms, and also to maintain weight and nutritional status in the face of intestinal malfunction. Therefore studies that show increased carbohydrate intake, or decreased fibre and fruit intake could well be due to secondary changes in the diet rather than features that relate to causation¹³⁰.
- (b) More recently it has been suggested that a cell mediated hypersensitivity due to zirconium (present in tooth paste) or titanium (present in a white colour used as a food additive) and perhaps also following exposure to other small particulate agents such as silicon and aluminium, might generate granulomatous responses and perhaps induce CD^{131, 132}. While this is an interesting and novel hypothesis there is to date limited data to support it. However, a trial of the efficacy and tolerability of a low particle diet in a double blind control pilot study in Crohn's Disease has been reported recently by Lomer *et al* at the Rayne Institute¹³³.

8. Other factors

- (a) Cosnes *et al* looked at the effect of smoking on the diagnosis and source of the disease¹³⁴. Although smoking increased the severity of the disease and the need for surgical intervention, there was no evidence that it affected the incidence of the disease.
- (b) The risk of cancer in patients with CD has in the past sometimes been reported as slightly increased. However, recent studies have failed to confirm this, and it is possible the earlier results were due to ascertainment bias¹³⁵.

- (c) There has been considerable interest in the possibility that measles virus may be associated with an increased risk of Crohn's Disease¹³⁶. Daszek *et al* summarise the evidence for measles virus being detected in CD-affected tissues in the gut, and suggest a pathologic similarity to sub-acute sclerosing para-encephalitis (SSPE), a persistent measles virus infection that occurs in the brain as a rare complication of measles. This lead to concerns that the use of live attenuated measles vaccine to prevent infection could also increase the risk of CD.
- (d) However, a case control study in 1997 using a variety of sources of live monovalent measles vaccine¹³⁷ did not demonstrate any increased risk. Subsequent increasing public concern lead to a Chief Medical Officer letter in March 1998, reporting the results of consultations with the Joint Committee on Vaccination and Immunisation in the UK (JCVI) and a special committee convened by the Medical Research Council. They concluded that there was no link between measles, measles vaccine or MMR (Measles, Mumps and Rubella combined vaccine) and CD. In particular data from the immunisation of 7 million children in 1994 with the combined vaccine did not show any subsequent increase in new cases or exacerbation of existing cases of CD. Further details of the basis of these conclusions can be found in the CMO letter¹³⁸.
- (e) Similarly the WHO has concluded that measles vaccines are not associated with CD on the basis that:
- the incidence of SSPE has fallen dramatically since measles vaccine has been introduced, while that of CD has increased;
 - that the published reports of measles virus in the CD - affected gut are inconclusive;
 - that the three key epidemiological studies related to the hypothesis all show significant weaknesses; the steady rise in CD started in 1940, long before the measles vaccine was introduced.
- (f) It can therefore be concluded that for the present the weight of expert opinion on the evidence does not support a link between CD and measles vaccines. This conclusion has recently received further support from the results of the case control study carried out by the Vaccine Safety Datalink Project based in Seattle. This concluded that vaccination with MMR or other Measles containing vaccine, or the time of vaccination did not increase the risk for IBD¹³⁹

9. Conclusions

- (a) 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.
- (b) 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 demonstrate¹⁴⁰.
- (c) 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.
- (d) 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.
- (e) These studies need to include studies on geographical distribution of Johne's Disease in the past and present.
- (f) 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.

- (g) 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.

E. Investigation of the Affected Tissues

1. The nature of the intestinal tract

- (a) The digestive tract is suspended in the abdominal cavity from the back wall of the abdomen by the mesentery. Blood and lymphatic supplies reach it via the fatty layer enclosed in the mesenteric membrane. The intestine is essentially a long tube of varying width, which consists of an outer muscular layer, a sub-mucosal, connective tissue layer containing the nerves and some lymphatic tissue, and an inner, mucosal layer that lines the lumen. (See Figures 1-3).
- (b) The mucosal layer consists of an epithelial layer that lines the gut lumen and comes into direct contact with food and other material ingested or secreted into the gut. It is supported on the lamina propria, a layer of connective tissue which contains lymphoid nodules and many lymphocytes. Beneath this is the muscularis mucosa which consists of muscle and nerves and rests directly on the submucosa. The surface area of the epithelial layer is enormously increased by large folds to produce villi or finger-like projections from the surface, and indentations or crypts and glands which secrete mucus and other lubricants or active agents into the lumen. Some of the glands leading off from the epithelium pierce the muscle layer and extend into the submucosal layer, but they empty into the lumen¹⁴¹.
- (c) The lamina propria of adults is heavily populated with B and T lymphocytes, plasma cells, macrophages, mast cells, eosinophils and basophils. The majority of the B-cells secrete IgA and is organised into dense groups of lymphatic tissue called Peyer's patches, as well as being distributed more diffusely in the lamina propria where they, together with the intact mucosal layer provide an effective barrier against the antigens contained in the food and other material passing through the bowel. Figure 5 outlines the path for a normal inflammatory response.
- (d) Small amounts of antigen can enter the body either by endocytosis or pinocytosis or via the tight junction complex. Antigens that do penetrate in this way usually give rise to systemic tolerance of the antigen. If there is a change in the barrier such that normally excluded antigens are able to be presented to the immune system, or normally presented antigens are responded to in an abnormal fashion, then pathological processes can result.
- (e) 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 (Figure 4). However it is still not clear whether these changes are a primary disorder of the immune system or are secondary to the inflammatory process. Nor is there any consensus on the initiating process that leads to the inflammatory response. Possibilities include abnormal permeability of the gut wall to antigenic agents, abnormal response to normal gut flora, or an abnormal immune response to normal stimulators.
- (f) 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 symptoms¹⁴².

- (g) It is probably fair to say that no one can say which of these alternatives truly reflects the situation. Much of the work carried out in this area relates to examining the nature the deranged immune system in the inflamed bowel in established cases, and while relevant to symptomatic treatment of the disease is of little relevance in terms of a consideration of the possible causation of the disease. Aspects of this work will be briefly outlined here to provide context, but is not central to a consideration of causality.

2. Immune changes in the gut wall

- (a) In inflammatory bowel disease (IBD) there is an excessive and tissue-damaging chronic inflammatory response in the gut wall. Treatments, which reduce this immune response, allow the mucosa to heal and the gut function to approach or return to normal. Corticosteroids are very effective at either inhibiting this immune response or possibly at down regulating local inflammation, and will improve around 70% of IBD sufferers¹⁴³. An extensive literature on the immune response of the gut wall in IBD exists, but most of it is irrelevant to a consideration of the underlying cause of the disease.
- (b) Studies using intracolonic application of trinitrobenzene sulfonic acid, a contact sensitising agent in rats and mice has produced a disease very similar to Crohn's disease in rats, and a less persistent and milder disease in mice¹⁴⁴. This response is a T cell helper type 1 (Th 1) response in the colonic wall. Macdonald concludes that from the range of studies carried out using these models most of the immunoregulatory events in the mucosal inflammation are controlled by CD4+ T-lymphocytes. He also says that the results suggest mucosal inflammation may result from both CD4 Th 1-biased or Th 2 biased T-lymphocyte differentiation and that mucosal T-lymphocyte activation is antigen dependent, and originates from intestinal bacteria.
- (c) While direct evidence for a role for CD4+ T-lymphocytes in CD (as opposed to this mouse/rat model system) is lacking Macdonald feels the circumstantial evidence supports the possibility that CD in man resembles the induced disease in rats and mice in its immune response.
- (d) Supporting evidence is:
- active CD is characterised by an increased number of activated mucosal T-lymphocytes secreting interferon-gamma (IFN-gamma), and by increased mucosal production of cytokines that activate Th1 -lymphocytes (IL-12 and IL-18);
 - CD may disappear during the development of AIDS, or after bone marrow transplantation when the patient's cellular immune response is being suppressed;
 - treatment with depleting anti-CD4 antibodies may induce remissions in CD^{145,146};
 - CD, unlike UC, is classically associated with non-caseating granulomata, the hallmark of cell-mediated immunity;
 - antibiotic therapy can be a useful adjunct for distal CD and may extend remission times;
 - some studies have shown that diverting the faecal stream from the lumen after surgical resection prevents recurrence.
- (e) Antigen-dependent T-cell activity is regulated normally by the immune system by the following mechanisms:
- if the T-cell receptor is blocked in the absence of a second signal (for example co-stimulation with B7 or CD28) then anergy results;
 - most active T-cells are programmed to undergo cell death (apoptosis) through fas/fas ligand interactions;
 - counter-regulatory cytokines (i.e. IL-10) can interfere with T-lymphocyte activation.
- (f) In this connection one study has shown that mucosal lymphocytes from patients with CD are relatively resistant to Fas –mediated apoptosis, which could explain the uncontrolled activation of the immune system that is seen in this condition¹⁴⁷.

- (g) In the mouse model referred to above, it has been shown that mucosal inflammation can be prevented by neutralising IL-12 antibodies, and similar results have been reported in monocytes from the lamina propria of CD patients whether the tissue is inflamed or normal in appearance, suggesting that this ability is not directly related to the inflammatory response but rather a constitutive capacity of the patient's mononuclear cells¹⁴⁸.
- (h) IL-12 is produced by antigen presenting cells mostly in response to bacteria or bacterial products¹⁴⁹. It has therefore been postulated that the inflammation may be driven by microbial stimulation of IL-12 production. Normal intestinal macrophages fail to produce detectable levels of IL-12 when stimulated with bacterial products, but in CD mucosa, IL-12 –producing cells are positively stained for the CD14 marker, and may be activated.¹⁵⁰
- (i) Figure 6 provides a diagrammatic illustration of the mechanism for IL-12 induction that leads to chronic inflammation¹⁵¹.
- (j) These studies are interesting (if complex to understand and interpret), but have to be treated with caution, given the likelihood that much of the immune response in established CD will be determined by factors quite different from those related to the initiation of the disease process, Most of what is observed here could be due to secondary features.
- (k) Even allowing for this a critical question that remains to be addressed is what induces the IL-12 in CD gut? The factor postulated to be most likely by immunologists is normal bacterial flora in the lumen of the gut. Therefore, this work would support a microbiological initiator for CD, but postulates that it is the genetic background and abnormal immune response that initiates the disease, not MAP.
- (l) Extensive work has also been carried out on 'downstream' changes in immune factors and cytokines in CD, but these are less relevant to the cause of the disease, but rather to control of the symptoms.

3. Increased intestinal permeability

- (a) Several studies have suggested that abnormalities in the intestinal wall can make a subset of the population resulting in increased susceptibility to Crohn's Disease. In particular, the possibility of increased intestinal permeability has been studied by a number of workers.
- (b) If the mucosal barrier were excessively leaky then it could allow unrestrained uptake of antigens and proinflammatory molecules, including luminal bacteria and bacterial products, and this could generate an abnormal immune response and initiate the inflammation¹⁵². Soderheim *et al*¹⁵³ found non-inflamed ileal mucosa from patients with Crohn's Disease had increased epithelial permeability to ovalbumin probably due to augmented transcytosis. They postulate this could increase the antigen load to the *lamina propria* and this could be a significant initiating pathologic event.
- (c) In other studies¹⁵⁴ they found baseline permeability was higher in patients with CD and their spouses than in controls, but that after ingestion of acetylsalicylic acid the permeability increased significantly in all groups, with relatives and patients both demonstrating a sensitive group with a greater increase in permeability than controls, while spouses were more comparable to controls in their changes. The proportions with abnormal responses were 32% in patients, 14% in spouses, 41% in relatives and 3% in controls. The authors concluded that baseline permeability was determined by environmental factors, but that acetylsalicylic acid permeability was related to a genetic factor. They concluded that both environmental factors and genetic factors played a part in the genesis of CD.
- (d) Peeters *et al*¹⁵⁵ have shown that this increased permeability can also be demonstrated in 25% of healthy first-degree relatives of CD patients. The effect was linked to increased circulating CD45RO lymphocytes.
- (e) Examination of family trees did not suggest that these findings were due to a genetic predisposition, indeed 5 spouses of CD patients were found to have the increased

permeability. The authors therefore suggest that a shared environmental agent may be responsible for the changes. Similar abnormalities were not found in parents or the relatives of sufferers from ulcerative colitis.

4. Hypersensitivity to external stimuli

- (a) Other studies¹⁵⁶ have shown that the mast cell in CD patients is more sensitive to bacterial toxin stimuli to release its inflammatory burst of chemicals, thus increasing the inflammatory effect of any bacterial infection on the mucosa of the intestinal wall of CD patients.

5 Cytokine responses in the gut

- (a) Work on the cytokine response to the T-cell mediated stimulation suggests that a second lesion may be a relative lack of anti-inflammatory cytokines which results in relative over-production of pro-inflammatory cytokines locally and so increases the local inflammatory reaction^{157, 158} (see Fig. 7). There have been encouraging studies using agents to redress this balance, for example, treatment with agents which block tumour necrosis factor alpha in the gut¹⁵⁹.
- (b) Remicade, a proprietary anti-TNF antibody therapy, has now been licensed (since August 1998 in the US and EU) for use in the treatment of Crohn's Disease resistant to conventional therapies. In responsive patients (82% of one series of 108 patients) significant improvement in signs and symptoms have occurred, including healing of fistulae, and almost half had complete disappearance of their symptoms at one month after treatment.
- (c) An important side-effect of treatment in some patients has been the onset or re-activation of potentially life-threatening tuberculosis infections. Many of the reports of these cases have come from countries with a high incidence of TB, and/or from patients previously treated with immunising pressants and/or cortico steroids.
- (d) It is too soon, probably, to interpret these data, but it has been argued¹⁶⁰ that MAP would also be expected to grow out in a similar fashion if it was the cause of CD.
- (e) It would certainly seem worthwhile exploring the microbiological status vis-à-vis MAP of some of the patients on Remicade in more detail.

6. Presence of MAP by culture of the organism and the possible role of spheroplasts

- (a) AFBs are not observed in pathological specimens, but Chiodini *et al* were the first to isolate MAP from resected terminal ileum of 3 young patients with CD¹⁶¹. These organisms required Mycobactin J for growth and had cultural characteristics similar to *M. paratuberculosis*. Chiodini *et al* later reported that all three strains had initially been isolated as non-acid fast coccobacillary forms that had the ultrastructural appearance of spheroplasts (cell wall-deficient forms) which, after several months in culture, transformed into the characteristic mycobacterial forms.
- (b) Further workers subsequently isolated the organism from CD patients in the US¹⁶², the Netherlands, Australia, France and the Czech Republic¹⁶³. More recently Chiodini's group have proved conclusively that the spheroplasts and the AFB-positive forms were the same organism by showing identical restriction patterns of the rDNA genes¹⁶⁴. They found spheroplasts in 4 patients with CD and unidentified spheroplasts were found in a further 12 CD patients, but not in samples from UC or other IBD.
- (c) Spheroplasts may persist in the body for longer than parent forms, have poor chemotactic activity and resist phagocytosis to a greater extent¹⁶⁵. Stable spheroplasts may replicate slowly over long periods of time thereby maintaining infection at subclinical or chronic levels.
- (d) Where MAP has been isolated from CD patients it has commonly been the case that they were isolated initially as spheroplasts, and since these will lack the protective cell

wall of the complete organism they may be more likely to be destroyed during the harsh decontamination procedures commonly used before cultures are set up. Absence of a cell wall could also explain the inability to detect an AFB organism in the tissues.

- (e) However, the difficulties in interpreting this data is underlined by the series of papers and letters between Kobayashi *et al*¹⁶⁶ and Graham *et al*^{167 168} over the interpretation of studies of spheroplast cultures of mycobacterium species, and immunocytochemical studies and DNA hybridisation studies on biopsies and tissue samples from the gut. In some studies detection of these micro-organisms is confined to CD cases; in other studies they are found in CD and UC and IBD patients and controls (Table 4). Chamberlin *et al* have recently concluded that the case is now persuasively in favour of a link between CD and the presence of MAP spheroplasts. However, he presents no new data. Certainly the data are entirely consistent with a link in some cases of CD, but this is not conclusive proof. All that can perhaps be clearly stated at this stage is that the technology in the field is advancing rapidly, and that it is likely that these apparently conflicting results will become more easy to understand in time as the preparation of samples becomes more standardised and the underlying meaning of the different tests becomes clearer.
- (f) Finally, in 2000 Naser, Schwartz and Shafran¹⁶⁹ reported that they had isolated MAP from the milk of two lactating women with CD and none of 5 milk samples from normal controls. The colonies identified were confirmed to be Mycobactin J-dependent and AFB staining, and to be MAP DNA by PCR. If these results can be confirmed there is the possibility of exposure of the offspring to MAP during breast-feeding as a possible route of transmission of the agent in a similar fashion to that found in Johne's Disease (providing MAP is an etiologic agent for CD of course).

7. Presence of MAP by identification of PCR of IS900

- (a) The Polymerase Chain Reaction (PCR) amplification of IS900 (see C.3.c above) provides a highly specific and sensitive method for detecting MAP independent of *in vitro* culture.
- (b) Whilst it is difficult to culture the organism from affected tissue the exquisite sensitivity of the PCR technique, which is able to detect as few as two copies of the MAP genome, has resulted in a much higher detection rate for the organism in CD tissues. However, great care needs to be taken to avoid contamination of samples and in including negative controls in the assays because the great sensitivity means inadvertent contamination can easily give rise to false positions. The assay will not discriminate between living and dead cells. Interpretation of the results has been complicated by the fact that MAP was also frequently detected in other IBD tissues with a similar frequency in the earlier experiments, possibly due to a lack of standardisation of the technique.
- (c) Thus Mishina *et al*¹⁷⁰ examined excised ileal mucosal tissue from perforating and non-perforating CD sub-populations using reverse transcriptase Polymerase Chain Reaction amplification to look for the presence of MAP-specific IS900 DNA sequences. All patients with IBD (8 with CD – four of each of the two proposed sub-types – and two cases of UC) had MAP specific sequences detectable in the tested material, which migrated with MAP control material. In addition, an MAC control isolated from the water supply of a major city in the USA also had the MAP specific sequence and so was identified as a MAP strain.
- (d) On the other hand McFadden and Fidler¹⁷¹ failed to detect MAP DNA. Fidler *et al*¹⁷² detected the DNA in 4 of 31 samples that included granulomatous lesions and not from the rest of the CD samples or from the UC and control samples. Sanderson *et al*¹⁷³ found MAP DNA in 65% of 40 samples of Crohn's Disease, 4.3% of 23 ulcerative colitis patients' tissues and 12.5% of 40 control tissues.
- (e) Clearly the picture is complex at present. Reviews of the literature to date have generally concluded that more careful studies and a greater understanding of the way the organism behaves in tissues, including whether it is merely a bystander or a pathogenic

agent, and the optimum methods for fragmenting the organism to release the DNA are needed before the data so far obtained can be interpreted meaningfully¹⁷⁴. The Report of the EU Scientific Committee has some useful detailed tables that summarises the results published to date¹⁷⁵.

- (f) Molecular epidemiology has shown that the strains of MAP which are found in humans with Crohn's Disease are closest to the strains of MAP which originate in cattle and other animals with JD, using restriction endonuclease analysis, restriction fragment length polymorphism, IS 1311 polymorphism analyses, and DNA hybridisation using IS900^{176,177}.

8. Immunocytochemical studies

- (a) Cartun *et al*¹⁷⁸ investigated 36 formalin fixed tissues for micro-organisms using immunocytochemical (ICC) assays. 20 bacterial assays tested were negative, including studies for Mycobacteria. Positive results were found for E coli and some streptococci. The significance of these results is difficult to assess.

9. Demonstration of a MAP-specific immune response in CD patients

- (a) If, as has been postulated, CD is an immunologic hyper-responsive reaction to an exogenous and/or endogenous agent, one might expect an immune response to MAP to be detected. Until recently it has proved difficult to demonstrate this^{179,180}. However, intracellular bacteria like the mycobacteria do not readily elicit a humoral (antibody) response during the early stages of infection; this only develops when the infected macrophages lyse and release the bacteria¹⁸¹. The tuberculoid, or paucibacillary stage equates with this early cell-mediated stage of the disease. It is the pluribacillary form that should demonstrate a strong humoral response. Furthermore, the severity of clinical signs of disease need not correlate with changes in the immune response¹⁸². If the hypothesis is that CD is the paucibacillary form of MAP infection in humans then the best immunologic test would be a delayed-type skin hypersensitivity reaction. No reports of efforts to demonstrate such responses have been found in the literature. Several other possible explanations have been proposed for difficulties in detecting antibodies in MAP, such as differences in methodology or assay systems. Hermon-Taylor suggests that the presence of the fucose outer coating (see para C.3(c) above) renders the bacteria less visible to the immune system of the host, which therefore fails to mount an adequate immune response to the organism¹⁸³.
- (b) Another possible reason is the high number of cross-reacting antigens *M.paratuberculosis* has in common with other mycobacteria or acid-fast micro-organisms, making it almost impossible to demonstrate an MAP-specific humoral immune response in CD patients.
- (c) More weight has been given to this latter suggestion recently by El-Zaatari *et al*, who have used recombinant clones expressing MAP antigens (from a genomic library from strain Linda) as a more sensitive and discriminating test than those using crude antigens^{184,185,186}. 24 recombinant clones expressing antigen(s) or epitope(s) were purified and analysed, and used to seek an MAP-specific humoral immune response in CD patients compared with those with other microbial diseases and controls.
- (d) Two putative recombinant clones encoding MAP-specific antigens – 35k (p35) and 36k (p36) – were identified, and specific reactivity to them found in CD patients as compared to controls^{187 188}. Of 77% of 61 sera from CD patients reacted with both antigens, compared to 8% of 12 sera from UC patients and 0% of 35 samples from normal controls (p<0.001). These results demonstrate specific sero reactivity against MAP p35 and p36 antigens in most CD patients' sera, and are consistent with a causal role for MAP in the development of CD.
- (e) Similar work by the Oslo group, Olsen *et al*¹⁸⁹ using a 14 kDa secreted antigen and PPD-J demonstrated significantly elevated levels of the antibody in 10 CD patients compared

with 10 UC patients that, in the CD patients, correlated negatively with duration of disease.

- (f) Work is now needed to see if such assays have any predictive value, and whether the absence of antibodies identifies sub-populations with different aetiology(ies) for their CD and perhaps a different disease pattern once closer examination of the clinical course in the different groups¹⁹⁰.

10. Other immune aspects of CD and MAP

- (a) Baldassano's group has shown that during active CD, endotoxin penetrates the mucosal barrier and upregulates the mononuclear phagocyte. When these 'primed' mononuclear phagocytes come in contact with bacterial chemotactic products including micro-organisms in the lumen they release excessive amounts of inflammatory mediators and so can in susceptible patients generate a heightened inflammatory response¹⁹¹.

11. Overall consideration of the possible pathogenesis of CD

- (a) Sartor¹⁹² summarises the consideration of the immune-aspects of CD by identifying a series of five steps in the pathway to clinical symptoms (Table 6). This helps to focus on the sequence of events that occurs before clinical symptoms appear, and helps to explain the complexity of the picture, the variability of results from individual studies and to underline the likelihood that this is going to turn out to be a disease caused by a variety of factors. Therefore while the case for MAP being the causal agent for CD in some people is arguably getting more persuasive, as assays are refined and results are published, it is likely that there will remain cases not caused by MAP. The difficult question is probably going to be what *proportion* of cases of CD are caused by MAP, and is it a sufficient proportion for us to spend large sums of money on dealing with the agent?

12. Transmission of Crohn's Disease to animals

- (a) Seven-day-old goats were fed cream containing 10^7 CFU of the organism isolated from CD patients per 100 ml. They developed a granulomatous ileo-colitis, or Crohn's Disease-like infection within 5-6 months of infection^{193,194}. Bacillary organisms were isolated from all infected animals and none of the controls. However, no AFBs were found on histological examination. One of the human strains, Linda, has also produced Crohn's Disease on injection into BALB/C mice, but not when injected into other laboratory species.

13 Other circumstantial evidence

- (a) Molecular typing of human and animal strains of MAP by restriction fragment length polymorphism (RFLP) analysis provides some evidence that cattle may be the source of the organism in humans with Crohn's Disease. Thompson has reviewed this literature¹⁹⁵.
- (b) Tamboli has suggested that there is considerable overlap of geographic regions with high prevalence of Johne's Disease and Crohn's Disease¹⁹⁶ (see para D.3. e).

F. Possible Routes of Exposure

1. Food-borne: Pasteurised milk

- (a) The most likely routes of exposure, if MAP were implicated in CD, would be via food with water as the second possibility. As with all microbial contaminations of food, there is an

initial requirement to devise a satisfactory method to isolate the organism from the food product before meaningful studies can be undertaken. The difficulties in culturing MAP from any source are compounded when dealing with foods. Nevertheless, MAP has been cultured from milk of cattle with Johne's Disease, and probably will occur in the milk of other infected ruminants if looked for¹⁹⁷. Johne's Disease (JD) in cattle is a systemic infection, so beef and beef products from infected animals could potentially contain MAP bacilli¹⁹⁸. Abattoir practices, which risk contamination of carcasses with intestinal contents, are also likely to spread the organism onto the meat. Data on the sensitivity of the organism to cooking temperatures is not available.

- (b) A number of recent studies by Grant and colleagues at Queen's University Belfast have shown that MAP in milk is more heat-resistant than other mycobacteria and may survive heat treatments simulating heat-pasteurisation at 72 degrees for 15 seconds (the standard commercial pasteurisation procedure) in the laboratory^{199,200}. Grant *et al* summarise the studies using laboratory pasteurisation procedures²⁰¹. One group reported no survival of MAP after high temperature short time pasteurisation (HTST) but they used frozen MAP inocula, whose viability could have been affected by the freezing process.
- (c) Subsequently Grant *et al* have shown that even at levels of MAP as low as 100 CFU/ml, it is possible to detect viable MAP after HTST pasteurisation procedures. Further studies by the same group suggested that 72 degrees for 25 seconds did kill MAP.²⁰² The retail industry responded to this information by voluntarily extending the period of sterilisation for milk to 25 seconds in 1998 in an effort to increase the lethality of the process²⁰³.
- (d) However, another way of addressing the problem is look for MAP DNA and viable MAP in milk on sale at retail outlets. Field studies of pasteurised milk on sale in cartons and bottles in England have been shown to contain MAP at levels between 2000 and 3000 MAP organisms per ml, and MAP-specific IS900 by PCR.^{204,205} During the peak periods for finding MAP in unpasteurised milk, in January-March and September-November, up to 25% of samples test positive. Pasteurised samples were positive in 7% of cases (22 of 312 samples) over an 18-month period. The authors failed to demonstrate viable organisms in the milk, in spite of using prolonged incubation periods. However the workers suggested that the organism might still be alive, since the PCR-positive material was detected in the cream and pellet portions of the milk, which is where the intact organisms are found in spiking experiments²⁰⁶. Given the recognised difficulties in culturing this agent, and the fact that following heat shock, increased difficulty in culture is likely, and may not necessarily reflect lack of viability *in vivo*, these studies gave cause for some concern.
- (e) More recently Grant *et al* carried out a survey of bulk milk and commercially pasteurised milk from 241 approved dairies in England, Wales and Scotland²⁰⁷. A total of 827 raw and commercially pasteurised milk samples from 241 approved dairy processing establishments throughout England, Scotland Northern Ireland and Wales were tested over a 17-month period (March 1999-July 2000). Overall 2% of both raw and pasteurised milk samples tested culture-positive for MAP. 70% of the positives had received heat treatment at 72-75 C for 25-seconds. All phosphatase tests were negative, strongly suggesting that the pasteurisation procedure was carried out satisfactorily. These findings provide convincing evidence that MAP can survive standard pasteurisation procedures, or at least that the UK population is being exposed to viable MAP in its milk supply, whatever the explanation for the source of the agent.
- (f) Further careful work by the Belfast group²⁰⁸ has explored the reason for this resistance²⁰⁹. MAP is present in milk in clumps of cells, unlike *M. tuberculosis* and *M. bovis*, which exists in filaments in milk. It seems likely that it is these clumps that are resistant to heating, since, using a novel acid-fast viability staining technique, the workers were able to show the remaining viable organisms were in the centre of the clumps of cells. Other workers have now corroborated this work^{210,211,212}. It seems inherently unlikely that any temperature gradient is responsible for the extended survival of cells in the centre of the clumps. It is more likely to be due to the surrounding cells

providing a more favourable milieu for heat-damaged cells so that they are more likely to recover.

- (g) Grant has also explored variations of the pasteurisation process²¹³. While reduction in the surviving fraction was achieved by prolongations of time and /or elevations of temperature, none of the combinations reliably achieved a complete sterilisation of the milk.
- (h) A MAFF-LINK project is currently being carried forward by Grant and Rowe at Belfast, with Professor 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 expeditiously as possible. Options such as clarification, bacto-fugation, 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.
- (i) These results naturally lead one to ask what the situation is with respect to other dairy products. Only a little work has been done so far; this suggests that MAP may survive in cheeses too. However, further work is needed and some well-constructed surveys to provide more information on the situation in the field²¹⁴.
- (j) More recently Grant and Rowe devised a sensitive technique, immunomagnetic separation (IMS), technique for assaying MAP in milk, which greatly increases the sensitivity of the available PCR techniques^{215 216}. Before carrying out the assay for IS900 they remove the MAP from the milk with an antibody to MAP attached to Dynabeads (paramagnetic beads to which the specific antibody can be attached). Once the antigen (i.e. MAP) binds to these the antigen and MAP organisms can be removed from the biological material by centrifugation, thus purifying and concentrating the antigen-containing material swiftly and simply. The beads are then heated to 100% for 15 minutes to release the DNA of the MAP organisms-, which is then assayed for IS900 by PCR.
- (k) Using this test and spiked milk samples Grant correctly identified 97.5% of 40 samples containing MAP compared with 72.5% using the standard assay. The IMS-PCR assay has a specificity of 95% and a sensitivity of 100% at an estimated concentration of 20 colony forming units (CFU) per ml (compared to a detection level of 2000 CFU/ml by standard IS900 PCR assay) a hundred-fold increase in the sensitivity of the assay.
- (l) Using the radiometric test Grant has now devised a radiometric culture technique which is more sensitive than the conventional HEYM culture method. This has enabled her to examine the efficiency of pasteurisation variations on low levels of milk spiked with MAP²¹⁷. She found sterilisation was achieved with contamination levels below 10 CFU/ml. While this is encouraging, it is clear that pasteurisation is not providing the same margin of safety for MAP as it does for *M. tuberculosis* and *M. bovis*.
- (m) Grant also detected natural MAP in raw sheep's milk, and raw and commercially pasteurised cow's milk²¹⁸. In this study 104 raw sheep and goats milk were sampled. Overall, one sample of raw goats milk tested positive for MAP on IMS-PCR but did not grow on culture. These results suggest sheep and goats milk from the regions tested may not represent a significant vehicle for exposure of the population to MAP.
- (n) Grant is also examining the nature of the clumping process in more detail to see if methods can be devised to prevent it or produce separation of the clumps prior to pasteurisation. Possible strategies would be dispersal of the clumps prior to sterilisation by homogenisation (but information on the rate of reformation of the clumps is not available), or the clumps could be physically removed by processes such as bacto-fugation, designed to remove bacterial endospores. More work is needed to assess these possibilities²¹⁹.

2. Food-borne: Unpasteurised milk and cheese

- (a) The sale of unpasteurised milk for human consumption is permitted under controlled conditions in England and Wales. It is not permitted in Scotland and Northern Ireland, although the legislation has been in place for less than ten years in both places. Consumption had been a low proportion of total milk intake in all parts of the UK for some time. The possibility that the cell walls of dead MAP could be equally effective in initiating CD was raised by the Northern Ireland Food Standards Agency Committee. However, immunologists consulted²²⁰ have advised that this can be discounted, the protein load from dead MAP organisms in milk being likely to be completely swamped by the 10^{12} - 10^{13} live gut flora in the colon, such that the “noise to signal” ratio would obliterate the likely immunogenicity of any MAP protein.
- (b) Other possible sources of exposure that have not been specifically investigated so far are cheese and unpasteurised cheeses.
- (c) In this context, it is relevant to consider the following points:
 - Crohn’s Disease is more common in developed than in undeveloped countries. Some of this difference may be due to differences in the level of health care or in the completeness of surveillance.
 - Crohn’s Disease may be more common in urban than rural areas, whereas unpasteurised milk tends to be consumed more commonly in rural areas and contact with infected animal will be greater in rural areas.

3. Food-borne: Meat and meat products

- (a) The studies outlined at A.7 demonstrate that the organism is widely distributed in the body. They also mean that the agent is likely to be in animal food products from infected but undiagnosed animals, and that there is a potential for human exposure via meat and other animal products as well as via milk and dairy products. It is likely that the level of exposure via dairy products is significantly greater than via other food sources, given likely numbers of bacteria in the different tissues and normal intakes of milk and meat.
- (b) Meat is not always eaten well cooked, so, if present in meat and meat products it is likely viable organisms will be consumed from time to time. No information on the ability of the organism to survive roasting and grilling or other ways of serving meat and meat products has been obtained.

4. Water-borne

- (a) Given the relatively long survival of the organism in the environment (ref. SAC report), it is also important to consider the possibility that water run-off from fields grazed by infected animals may also contain viable organisms, that they will survive for significant periods in the environment and that they could survive water-treatment procedures and be present in some potable water supplies²²¹.
- (b) Cattle with severe JD shed 5×10^{12} organisms per day in their faeces. This will normally fall onto pastures²²². Water may be contaminated by grazing animals and their excreta, and it is possible that in the UK the agent survives water treatment procedures before domestic consumptions²²³, since mycobacteria have been shown as capable of surviving municipal water treatments in the United States²²⁴.
- (c) The Medical Research Council and National Environmental Research Council have jointly funded a study of MAP in environmental and municipal water supplies in South Wales, which began in September 2000²²⁵ and is expected to report in 2003.
- (d) There is good evidence that MAP also infects non-ruminant wildlife in Scotland (and therefore possibly elsewhere in the UK) . Animals affected include rabbits, foxes, stoats, badgers, wood mice and birds such as jackdaws and rooks²²⁶. This means that eradication of MAP from food animals would not, by itself, necessarily eliminate human

exposure to the agent from water sources. A vaccine for domestic livestock therefore appears a more promising way forward (see G.2 below).

G. Other Mechanisms to Control Exposure to MAP and/or its Effects

1. Effect of antibiotic treatment on MAP

- (a) Members of the Mycobacterium Avium Complex (MAC) generally have a tendency to develop resistance to those anti-biotics known to be effective against them quite quickly²²⁷.
- (b) On theoretical grounds most of the anti-biotic treatments tried have been inhibitory rather than bactericidal, therefore, relapse after prolonged therapy, or recurrence once therapy is stopped is likely²²⁸. So far this has frequently proved to be the case²²⁹.
- (c) Spontaneous remissions occur in about 20% of cases, and a placebo effect from treatment is also possible in patients undergoing short-term therapy.
- (d) These factors necessarily complicate an assessment of any antibiotic therapy for the treatment of Crohn's Disease.
- (e) Furthermore, given the rather indolent nature of the disease and the lack of evidence of rapid multiplication of any organism in the tissues, it might be anticipated that any effects of treatment dependent on the presence of cell multiplication would not be rapid or easy to determine.
- (f) This indeed proves to be the case. Reports of effects of treatment of CD with standard antimycobacterial therapy exist but they are mostly difficult to assess, being based on small numbers, treatment for short periods and in uncontrolled conditions. While some studies show transient benefits, results overall are not conclusive²³⁰.
- (g) Rastogi and Labrousse have shown that clarithromycin was bactericidal for all 10 strains of *M. avium* tested, and that activity was enhanced by the addition of ethambutol in 8 of 9 strains tested and of rifampicin (in 3 of 9 strains tested)²³¹. Hermon-Taylor believes that treatment with Clarithromycin and Rifabutin is promising²³².
- (h) A double blind controlled trial on carefully standardised and managed patients to examine the hypothesis further is desirable since these drugs are toxic and should be avoided where benefit is unlikely to occur. A trial started in September 1999, funded by Pharmacia-Upjohn, and based in 20 medical centres throughout Australia. It is hoped that the results will be available by 2003.
- (i) Meanwhile, four open studies have reported in abstracts that a substantial proportion of active CD will heal on Rifabutin and clarithromycin, the 2 drugs that are known to be more active against MAC²³³.
- (j) ***In summary***, the effect of antibiotics is clearly of great interest and relevance to clinicians and those suffering from CD. However, the response or resistance of *established* CD to various combinations of antibiotics, at the present state of our knowledge about the sensitivity and growth characteristics of the *organism in vivo*, has limited implications in connection with establishing the validity of the hypothesised link between MAP and CD.
- (k) It is quite possible that MAP is causally related to CD, yet therapeutic regimes with existing antibiotics could be ineffective. The regimes chosen or the antibiotics used could be inappropriate, or the organism could establish a damaged mucosa that then developed CD in the absence of persistent viable MAP in the tissues. This makes establishing the nature of the symptoms and signs in the early prodromal phase of the disease (if this exists) an important aim.

2. Vaccines

- (a) Attempts are being made to develop vaccines using DNA based technologies. The University of Minnesota is believed to be near to publishing the genome sequence of the

K-10 strain of MAP. This should provide potential targets for new diagnostics and for vaccine development. Sequences for the related Mycobacterium spp. *leprae*, *tuberculosis* and *avium* will enable differential comparison between the organisms and so aid in developing specificity in the vaccine²³⁴.

- (b) Recent work in El-Zaatari's laboratory that has identified 35 000 molecular weight antigen specific to MAP and JD. This may open the way to a more specific diagnostic test and the development of a vaccine²³⁵.

H. Summing up the Evidence and Drawing some Tentative Conclusions

1. The management of uncertainty and risk

- (a) The precise relationship between MAP and CD remains uncertain in spite of a large amount of research and consideration of the available information.
- (b) Whilst it is relatively easy to identify a significant body of further work to attempt to clarify the relationship, there remains a need to take a decision immediately on what should be done-even if that decision is to wait for the results of further studies and surveys.
- (c) This is by no means an unusual situation to find oneself in when dealing with public health questions. Indeed it is the rule rather than the exception to be forced into taking decisions before there is persuasive data available when managing public health issues nationally or globally.
- (d) Perhaps the most serious and well known such issue of recent years is that of the risk to humans of exposure to BSE in cattle. Since the management of this issue has been the subject of a recent inquiry²³⁶ and the Government has responded to its recommendations²³⁷, it is sensible to start a consideration of how the uncertainties should be handled by looking at what is recommended there.
- (e) Page 76 of the Government's response²³⁸ lists the conclusions of the Inquiry on 'Risk Management and Communication', and the Governments responses. Points of relevance to this report and the decisions that might flow from it are paraphrased here. To give maximum clarity, since the Government has generally accepted the recommendations of Phillips, recommendations from Phillips and responses by Government have not been differentiated below:
- The Government is committed to applying the precautionary principle where appropriate;
 - Where the likelihood of a risk to human life may appear remote, where there is uncertainty, all reasonably practical precautions should be taken;
 - Where precautionary measures are put in place to address a potential hazard they should be enforced strictly even if the risk they address appears to be remote. Care should be taken to ensure the implications and consequences of all aspects of the measure are considered, and where necessary enforced;
 - Complex and uncertain issues should be dealt with openly and transparently as only in this way will trust and credibility in Government and its actions be generated;
 - Where there is uncertainty this should be made clear both in the evidence published and in the explanation of the rationale behind the decisions on action to be taken;
 - The importance of precautionary measures should not be played down on the grounds that the risk is unproved;
 - Where policy decisions turn on risks to human health ,the Department of Health should be involved in formulation of policy from the start;
 - Reference of problems to expert committees should not be permitted to introduce delays where decisions are urgent. Internal expert advice should be used where appropriate and where it assists in reaching a rapid decision;

- (f) In addition the Advisory Committee on the Microbiological Safety of Food (ACMSF) considered Microbial Risk Assessment²³⁹ in a Report in 1996. Relevant recommendations from their report are:
- Systems for collecting, collating, analysing and dissemination of information on epidemiological aspects of a potential hazard should be actively conserved and developed in response to particular needs;
 - This should be done in cooperation with relevant public and professional bodies;
 - PHLS has an essential and unique role to play in the surveillance, recognition and prediction of microbiological hazards;
 - Targeted epidemiological studies represent the best quality information for informing certain types of microbiological risk assessment. Therefore assessments with major public health implications should be underpinned by targeted epidemiological studies as far as is appropriate.
- (g) The Rio Declaration, in relation to 'Food Safety', states that '*Where there are threats of irreversible damage, lack of full scientific certainty shall not be used as reason to postpone cost-effective measures to prevent environmental degradation*'. Whilst it is not entirely clear what 'environmental degradation' means in the context of food, the spirit behind this statement would be that efforts to reduce exposure ought to be considered and put in place wherever this is reasonable. The lack of a certain link should not be used as an excuse for inaction. Such advice is consistent with that outlined above from the BSE Inquiry Report.
- (h) Whilst endorsing such an aspiration, in the context of MAP there are real difficulties in deciding what action is reasonable and desirable, and what action is likely to be effective in reducing human exposure.
- (i) Perspective and knowledge base also affect the weight people place on different parts of the evidence, or lack of evidence. This also needs to be borne in mind as the data are considered.
- (j) Thus those more familiar with the literature on the possible links between MAP or other microbiological agents and CD tend to feel the evidence linking MAP and CD, while not in any way conclusive, is sufficient to justify concern and serious consideration of precautionary action.
- (k) On the other hand, clinicians focusing on the best way of treating their patients can be strongly dismissive of the hypothesis, pointing out that antibiotic therapy does not seem to help long-term in alleviating the symptoms. Alan Kennedy²⁴⁰ draws an analogy with the situation with respect to *Helicobacter pylori* and duodenal and gastric ulcers a few years ago. Certainly the concept of infectious organisms and chronic diseases is not one that the majority of clinicians are familiar with. Furthermore the quantity and complexity of the relevant data (as evidenced by this report) is so considerable that it is difficult to make an informed judgement without the investment of more time than most people are prepared to give.
- (l) Immunologists concentrate on the information on leakiness by the bowel, excessive production of specific cytokines, and the benefits now apparent from Tumour Necrosis Factor- α antibody treatment (Remicade), and therefore tend to favour an immunological explanation for the disease.
- (m) Patient groups, anxious not to cause undue alarm or to further complicate the nutritional problems of their clients, have generally adopted an agnostic position. Some are reassessing their position and this seems sensible.
- (n) In fact, the data available appears, to a considerable extent, entirely consistent with all the above points of view. If one tries to integrate the different perspectives the only point of disagreement lies in the postulated nature of the initiating insult in the bowel. The immunologic model postulates that normal gut flora initiate the pathologic process leading to CD, while the MAP hypothesis postulates a specific organism – pathogenic in ruminants.

- (o) But ***the difference between these two viewpoints is not academic in terms of public health***, since if MAP is the causative agent, then efforts to reduce human exposure to the agent are needed, whereas if normal gut flora are the precipitating factor preventative action is more problematic and treatment of the disease is probably the most promising option.
- (p) A meeting of those involved in the clinical management of CD that covered the public health, immunologic, infectious disease and veterinary perspectives, and that enabled all aspects of the aetiology of the disease to be addressed in a forum where the full range of expertise was available to those present, might be timely given recent advances in knowledge in this area, and the importance that patients are properly informed about the present state of knowledge and the nature of the various hypotheses concerning causation, prevention and treatment.
- (q) Something similar in nature to the CIBA Symposium format, with meetings, discussions and papers over a full week, ending with an attempt to arrive at consensus on some issues, would be most valuable in ensuring the best use is made of the considerable information that is available about this disease and its possible aetiology. The Royal Colleges and various gastroenterology societies for adult disease and paediatricians, in collaboration with the key researchers, should consider how best to take this forward, led by the Department of Health and the FSA.
- (r) ***In conclusion***, therefore, for the present one has to say that, while the jury is still out on the ***relationship*** between MAP and CD, there is undoubtedly sufficient cause for concern for further action to be taken urgently to determine what the available data means and how the information needed to reach firmer conclusions can be obtained as rapidly, efficiently and effectively as possible. This question can be divided into two areas:
- What action should be taken to reduce exposure to MAP even though the causal link is not established; and
 - What action can be taken to increase the knowledge base so that future decisions may be based on more information?
- (s) A more detailed list of the scientific questions that need addressing is at Appendix 3.
- (t) It is likely that a significant reduction in terms of reducing human risk of exposure to MAP from food and the environment may take many years to achieve. On the other hand progress with techniques to pursue the link between CD and MAP may be reaching a point at which the answer will become much clearer. Whatever the state of the knowledge base, there is always the risk that public concern will suddenly focus on this issue, and it is important therefore to have a well organised plan to progress it. This makes it important to put in place a long-term programme that will enable new findings to be incorporated as they appear, and that will ensure that action is seen to be well in hand if and when the time arises that the link between MAP and CD is established for certain.

I. 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 symptoms²⁴¹.
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.

APPENDIX 1
List of those Circulated with Draft and/or Consulted

Name	Organisation
Professor John E Lennard-Jones	Digestive Disorders Foundation
Chris Hughan	Crohn's in Childhood Research Association
Richard Driscoll	National Association for Colitis and Crohn's Disease
Professor Collum O'Morain	Consultant Gastroenterologist, Charlemont Clinic, Dublin
Professor Fergus O'Shanahan	Consultant Gastroenterologist, Department of Medicine, Cork University Hospital
Rhona O'Brien	Irish Society for Colitis and Crohn's Disease
Professor Philip Quirke	Department of Pathology, University of Leeds
Dr Diana Walford	Public Health Laboratory Service
Dr Bob Mitchell	Communicable Disease Surveillance Centre, Public Health Laboratory Service
Dr Huw Jenkins	Consultant Paediatric Gastroenterologist, Department of Child Health, University Hospital of Wales
Dr Norman Simmons	Emeritus Consultant in Microbiology to the Guy's and St Thomas' Hospital Trust
Dr Michael Painter	City of Manchester Infection Control Surveillance Unit
Professor Douglas Georgala	Chairman, Advisory Committee on Microbiological Safety of Food
Professor John Hermon-Taylor	Department of Surgery, St George's Hospital Medical School
Alan Kennedy	Paratuberculosis Awareness and Research Association
Professor Cecil McMurray	Department of Agriculture and Rural Development, Belfast, Northern Ireland
Dr Irene Grant	Department of Agriculture and Rural Development, Belfast, Northern Ireland
Professor Paul Fine	Professor of Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine
Dr Alastair Forbes	British Society of Gastroenterology
Professor Tom MacDonald	Professor of Immunology, School of Medicine, University of Southampton

APPENDIX 2

Glossary of Terms

Abattoir	Slaughterhouse
Acetylsalicylic acid	A white powder obtained by action of acetic anhydride on salicylic acid.
ACMSF	Advisory Committee on Microbiological Safety of Food
AFB	Acid-fast bacilli
Antigenic	Capable of causing the production of an antibody
Asymptomatic	Without symptoms
Auto immune disease	Illness where the immune system reacts against the patient's own tissues
Bacillus	A rod shaped microorganism
Bacteroides	A type of bacterium
Biopsy	Excision of small piece of tissue for further examination
Biosynthesis	The formation of complex compounds from simple substance by living organisms.
BSE	Bovine Spongiform Encephalopathy, a slowly progressive and ultimately fatal neurological disorder of adult cattle caused by a prion.
BSE Report	Report on Inquiry set up by the Prime Minister into the emergence and identification of Bovine Spongiform Encephalopathy (BSE) and variant Creutzfeldt Jakob Disease (vCJD) and the action taken in response to it up to 20 March 1996. It was chaired by Lord Phillips of Worth Matravers and reported on 26 October 2000.
Bovine	Of, relating to, or resembling members of the Bovidae group of animals (i.e. cattle).
Bystander organism	Organism found in the body that is not causing illness or damage.
Caseation	Process of converting necrotic tissue into a granular amorphous mass resembling cheese.
Causality	The principle that nothing can happen without being caused.
Cervical lymphadenopathy	Enlarged lymph nodes in the neck.
CFU	Colony forming units – number of colonies of organisms the sample of bacteria gives rise to when grown in the laboratory.
Chemo-tactic/-taxis	Attraction of an organism by a chemical stimulus
Cholesterol	A sterol found in all animal tissues, blood, bile, and animal fats: a precursor of other body steroids.
Chromosome	Any of the microscopic rod shaped structures that appear in a cell nucleus during cell division consisting of nucleoprotein arranged into units (genes). Genes are responsible for the transmission of hereditary characteristics.
Chronic	Developing slowly or lasting for a long time.

CJD	Creutzfeldt Jakob Disease, a human transmissible spongiform encephalopathy caused by a prion.
Clarithromycin	An antibiotic of the erythromycin family
Colon	See figures 1, 2 and 3
Colostomy	Incision of the colon to make a more or less permanent fistula so that bowel contents are emptied onto the abdominal wall, used in treatment of narrowing of lower portion of colon, and in inflammatory diseases such as CD and ulcerative colitis to relieve symptoms.
Colostrum	Secretion from the mammary gland before the onset of true lactation two or three days after delivery. So called "first milk".
Control (sample)	Standard against which observations or conclusions can be checked in order to establish their validity.
Culture	A mass of microorganisms growing in laboratory culture media.
DH/DoH	Department of Health.
Diabetes mellitus	Disease of pancreas when secretion or utilisation of insulin is impaired.
DNA	Deoxyribonucleic Acid. The main constituent of the chromosomes of all bacteria and higher organisms
E coli	Bacteria found in the gut and elsewhere.
Endogenous	Developing or originating within an organism or part of an organism.
Enzyme	Complex proteins or conjugated proteins that are produced by living cells and act as catalysts in specific biochemical reactions.
Epidemiology	Branch of medical science concerned with the considerations of diseases or other features by examining populations and groups.
Epithelial	Relating to surface layer of cells (see Figs. 2 and 3)
Ester	Any of a class of compounds produced by reaction between acids and alcohols with the elimination of water.
Etiology	The causes of a disease or phenomenon.
Exogenous	Developing or originating outside an organism or part of an organism.
EU Scientific Committee	European Union Scientific Committee
Fistula	Abnormal tubelike passage from a normal cavity or tube to a free surface or to another cavity.
FSA	Food Standards Agency
Fucose	Sugar similar to glucose
Gastrointestinal tract	The passage from the mouth to the anus through which food passes.
Genome	The complement of haploid chromosomes contained in a single gamete or nucleus.
Glycopeptidolipid	Molecule consisting of sugar, protein and fatty units.

Granulomatous	Relating to granular tumour or growth, usually of lymphoid and epitheloid cells.
Helicobacter pylori	Bacteria that causes gastric ulceration.
Herd infectivity rate	The number of herds that contain infected cattle.
Histological	Relating to the study, especially the microscopic study, of the tissues of an animal or plant.
HIV/AIDS	Human immunodeficiency virus / Aquired immune deficiency syndrome – a disease caused by HIV, in which certain white blood cells are destroyed, resulting in the body's inability to protect itself against disease.
IBD	Inflammatory Bowel Disease
Ileostomy	Surgical formation of a permanent opening from the ileum onto the abdominal wall.
Ileum / ileal	Part of the small intestine.
Ileocolitis	Inflammation of the mucous membrane of the ileum and colon.
Immunogenic	Causing or producing immunity or an immune response.
Immunocytochemical	Using an immune mechanism to develop an assay for a chemical.
Immunosuppression	Suppression of the body's immune system, which can be done to reduce the likelihood of rejection of a transplanted organ, or as part of the treatment of a disease.
Inoculation	The introduction of material (sometimes a pathogen such as a virus or bacterium) into an animal.
Isoniazid	Drug used to treat tuberculosis
JCVI	Joint Committee on Vaccination and Immunisation (UK)
JD	Johne's Disease: Paratuberculosis of cattle and other animals
Lamina propria	A thin layer of fibrous connective tissue which lies immediately beneath the surface epithelium of mucous membranes.
Lesion	Any structural change in a bodily part resulting from injury or disease.
Lipid	Any of a large group of organic compounds that are esters of fatty acids.
Listeria	A rod-like Gram-positive bacterium of the genus <i>Listeria</i> .
Lymphocyte	Lymph cell or white blood corpuscle without cytoplasmic granules. Produces a significant part of the immune response.
Macrophage	Large phagocytic cell occurring in the blood, lymph, and connective tissue of verterbrates. Takes part in local defences against infection by microbes.
Macromolecule	Any very large molecule, such as a protein, nucleic acid or synthetic polymer.
MAC	Mycobacterium Avium Complex
MAP	Mycobacterium paratuberculosis
MAFF	Ministry of Agriculture, Fisheries and Food

Mast cell	Large round or ovoid cell found in loose connective tissue, involved in the immune process.
Mendelian	Transfer of a characteristic from one generation to the next by a single gene.
MMR	Measles Mumps and Rubella vaccine
MRC	Medical Research Council, a non-departmental public body incorporated by Royal Charter in 1920. Accountable to the Office of Science and Technology (OST), which is now part of the DTI. It funds research at universities and at its own research units.
Multiple sclerosis	Chronic progressive disease of the central nervous system characterised by loss of some of the myelin sheath surrounding certain nerve fibres.
Mycobacteria	Acid fast bacteria belonging to the Mycobacteriaceae which includes the causative organisms of tuberculosis and leprosy.
Mycobactin J	Iron-containing compound essential for the growth of MAP.
NACCD	National Association for Colitis and Crohn's Disease – a patients' support group.
Oesophagus	Part of the alimentary canal between the pharynx and the stomach.
Oligosaccharide moiety	Small molecule containing a few sugar units
Ovalbumin	White of egg
Pasteurisation	Process of heating fluid such as milk to a high temperature for a short period to destroy harmful or undesirable microorganisms without affecting significantly the taste qualities.
Pathogen	Any agent that can cause disease.
Pathology	The study of the causes of, and changes produced in the body by, disease.
PCR	Polymerase Chain Reaction
Phagocyte	Cell which has the ability to ingest and destroy particulate substances such as bacteria, protozoa, cells, dust particles and colloids.
Phenotype	Physical appearance or make up of an individual.
Phospholipid	A lipid substance containing phosphorous, fatty acids and nitrogenous base.
Placebo	Inactive substance or other sham form of therapy administered to a patient, usually as a control to compare its effects with a real drug or treatment.
Prodromal	Early symptoms that precede the development of the full picture of a disease.
Pulmonary tuberculosis	Disease of the lungs caused by Mycobacterium tuberculosis.
Representational difference analysis	Statistical method to see if there are differences within data.
Restriction endonuclease analysis	Using a specific enzyme to look for particular sequences in DNA and breaking DNA at that point.

RFLP	Restriction fragment length polymorphism
Rifabutin	Antibiotic
Rifampicin	Drug used in the treatment of tuberculosis, meningitis and leprosy.
Rio Declaration	Global declaration on the environment agreed at the Rio Conference.
Ruminant	Animal that chews the cud (partly digested food) regurgitated from its rumen, and has a stomach of four compartments.
SAC	Scientific Advisory Committee (of MAFF)
Salicylates	Any salt of salicylic acid
Salmonellae	Any Gram-negative rod-shaped anaerobic bacteria of the genus <i>Salmonella</i> , many species of which cause food poisoning.
Serum	The non-cellular part of the blood after clotting factors are removed.
Spheroplast	Circular microbe derived from a bacillus after its cell wall has been lost.
SSPE	Sub-acute Sclerosing Pan-Encephalitis
Streptococci	Any Gram-positive spherical bacteria of the genus <i>Streptococcus</i> , typically occurring in chains and including many pathogenic species.
Subcutaneous	Situated, used or introduced beneath the skin.
Synovial	Relating to transparent viscid lubricating fluid secreted by the membrane lining joints, tendon sheaths, etc.
(Reverse) Transcriptase	Enzyme present in retroviruses that copies RNA into DNA, thus reversing the usual flow of genetic information.
Tropism	Response of an organism to an external stimulus by growth in a direction determined by the stimulus.
UC	Ulcerative Colitis
UHT milk	Ultra heat treated milk.
Virus	Any of a group of submicroscopic entities consisting of a nucleic acid surrounded by a protein coat and capable of replication only within the cells of animals and plants; may be pathogenic.
ZN	Ziehl-Neelsen: stain to enable mycobacteria to be seen in tissues under the microscope as pink rods.
Zoonosis	Any infection or disease that is transmitted to man from animals.

APPENDIX 3

Some Key Issues for Further Research

1. Milk

- (a) The MAFF-LINK project and the work of the Belfast group on the mechanisms that aid MAP in surviving pasteurisation, clumping, etc., should be progressed as rapidly as possible. *M. tuberculosis* does not clump and is killed by pasteurisation. This offers the hope that declumping effectively would return the sensitivity of the organism to within the parameters that are reasonable for pasteurisation without significant loss of organoleptic qualities.
- (b) Studies across the UK on the levels of contamination of milk with MAP, on whether unpasteurised milk is more contaminated with live MAP, and the geographical distribution of contaminated milk, would provide information on the distribution of infected farms in the UK.
- (c) Studies on other possible ways of rendering the milk MAP-free-by altering the conditions of pasteurisation or by other manipulations to milk consistent with maintaining acceptable organoleptic properties.
- (d) More information on the status of UHT milk is needed. If it were clear that this did eliminate the organism, then this would provide an option for those wishing to avoid exposure to MAP.
- (e) More information is also needed on the relative status of unpasteurised milk, *vis-a-vis* pasteurised milk and MAP organisms.

2. Meat

- (a) Studies of whether MAP can be detected in meat and meat products, including offals where appropriate.

3. Water

- (a) Any further work identified as necessary once the results of the MRC /NERC study of MAP in environmental water supplies is completed.
- (b) Work on the efficacy of water purification procedures for public water supplies.
- (c) Work on the presence of MAP in private water supplies.

4. Studies in humans

- (a) A well designed study using PCR in a multi-centred blinded study to identify the percentage of patients in which PCR to IS900 can be detected and its significance using and an agreed protocol specifying the tissue extraction and PCR procedures that will be used.
- (b) To look at the location of IS900 on the mucosal wall or in the intestinal lesions.
- (c) To determine whether a localised cellular immune response to MAP is occurring within inflamed intestinal tract.
- (d) Further exploration of the p35 antigen and its potential to provide information on exposure to MAP in human populations.
- (f) The result of the Pharmacia study of the effects of antimicrobial treatment in a controlled long-term study²⁴² will be of interest.

5. Epidemiological study in humans

- (a) Baseline and trend data on incidence of disease in children and adults; its natural history in UK and across Europe; also if possible with links to studies internationally. The recent study by Sawczenko et al in children provides a benchmark for what can be done, but a similar method for making adult CD reportable in the UK with a high level of compliance across the country is an essential preliminary to a proper understanding of the extent of the problem, and to being able to measure the efficacy of any efforts to affect the incidence of the disease in the future.
- (b) Consideration of the pathology of Crohn's Disease and whether there are sub-types that would benefit from being studied separately, especially in epidemiological studies. Data from the antibody studies may be relevant here.
- (c) The epidemiological data needs to be reviewed by a working group to consider what programme of epidemiological work should urgently be put in place so that as much knowledge as possible about the aetiology of the disease can be obtained from the relationship between the cases and the distribution of MAP in the environment.
- (d) Consideration should also be given to other possible microbiological associations when constructing these studies.
- (e) The epidemiological work and the surveillance work should be carried out in such a way that the clinicians and the epidemiologists are all closely involved in the process of developing the studies and collecting and analysing the data so that the maximum buy in to the studies occurs.
- (f) Animal epidemiologists also need to be involved to do linkage studies on environmental relationships, and an international focus should obtain.
- (g) Work on the development of vaccines against MAP for humans may need to await the outcome of work on a vaccine for animals. At present ethical issues would constrain the work, given the uncertainty of the causative link.

6. Epidemiology in animals

- (a) Prevalence, incidence trend studies and details of regional changes in the extent of JD in food and other animals in the UK are needed to inform strategies for developing and implementing programmes to control the disease in the national food animals.
- (b) Work on the development of vaccines is a key issue that needs to be pursued vigorously in animal health and farming economic grounds.

7. Understanding the relationship between the different data

- (a) A meeting of the different experts which focused on the issue of causality, and sought to integrate information from the epidemiology, immunology, clinical and animal sources and reach a common interpretation of the data, would be of great value in aiding patients and patient groups in reaching decisions on action they might take, or advise their members to take, based on the present knowledge base.

Table 1
Incidence of CD in population studies

Area	Study period	Incidence
Cardiff (18)	1971-77	4.8
Cardiff (16)	1981-85	8.3
Olmsted County Minnesota	1973-77	6.8
Stockholm County (10)	1970-74	4.5
Copenhagen Coy (11)	1970-78	2.7
Beersheba (Israel)	1976-80	1.8
Northern Ireland	1966-73	1.3
Cape Town	1975-80	1.2
Central Israel (Fireman et al)	1970-80	1.6

Fireman *et al* (1989)

Table 2
Prevalence of CD (per 100 000) in Jewish community groups

Birth	1970			1980		
	no.	prev.	age adj. rate	no.	prev.	age adj. rate
Israel	23	3.95	7.45	90	12.63	15.29
Asia-Africa	7	2.50	1.69	34	12.25	12.37
Euro-America	55	14.3 7	3.27	12 0	30.50	26.05
Total	85			24 4		

Fireman *et al* (1989)

Table 3
Incidence of Crohn's Disease

Place	Years	Persons	Males	Females
Cardiff	(1972-74)	2.39	1.99	2.76
Aberdeen	(1962-68)	2.2	1.6	3.0
Vastmanland	(1962-67)	3.1	3.4	2.8
Nottingham	(1970-72)	3.6		
Western Cape	(1970-74)	0.5		

Mayberry & Hitchens (1978)

Table 4
Isolation of MAP from patients

	Crohn's controls	
Number of patients examined	135	121
Positive cultures (bacillary)	10	1
Positive cultures (IS900)	26	0
Total % positive	38	0.8

Chiodini & Rossiter (1996)

Table 5
Johne's Disease rates

Country	Culture%	Serum %	Herd/Flock
UK SW – 1996	2.5	3.5	
(Abattoirs in 50s)	11-17		
Belgium – beef		1.2	17.4
Netherlands – dairy		2.5	54.7
Greece – ovines		9.8	
Spain – ovines		33-46	46.7
USA – 1980s	1.6-1.9		

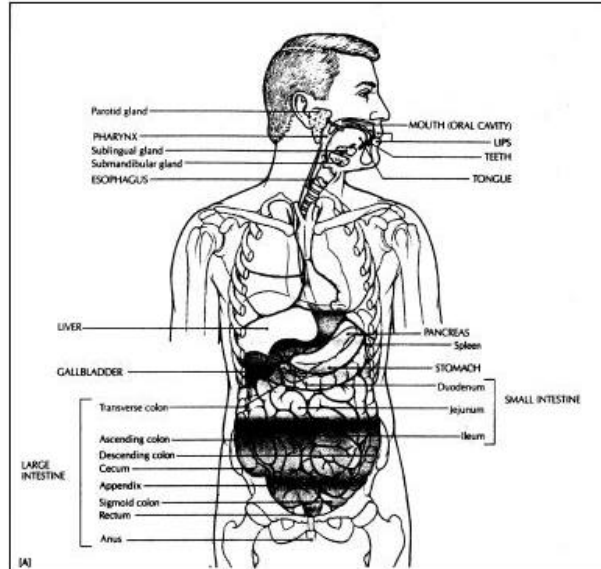
European Commission SANCO/B3/R16 (2000)

Table 6
Stages in the pathogenesis of CD

Initiating events	Perpetuating events	Immunoregulatory abnormalities	Tissue damage	Clinical symptoms
Infections	Luminal bacteria	Genetic susceptibility	PMN	Diarrhoea
Toxins			Macrophage	Bleeding
	Bacterial	T lymphocytes	Tx, LT, PAF	Pain
NSAIDS	Products	>IL-1/IL-1ra	O, NO	>Weight
	Dietary antigens	T/H1 VS. T/H2 HLA-DR?	Proteases Complement	
		Antigen presentation	IRN-gamma, TNF-alpha	

Sartor (1997)

Fig. 1
Anatomy of the abdomen



Figs 2 and 3
Anatomy of the gut and gut wall

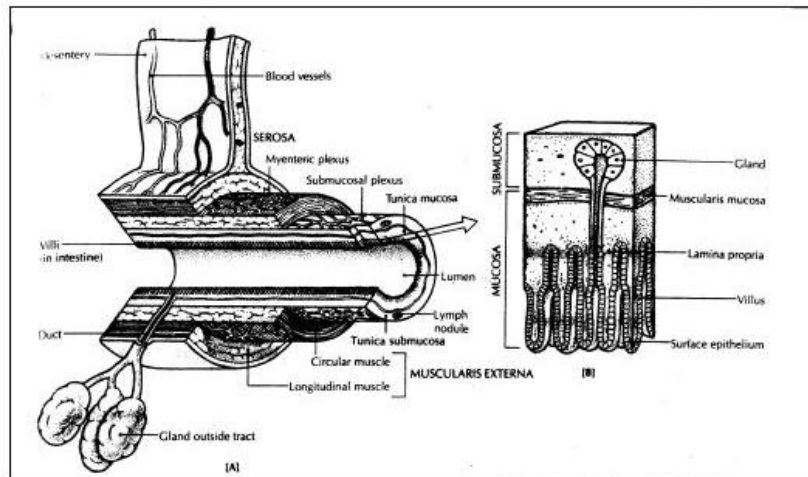
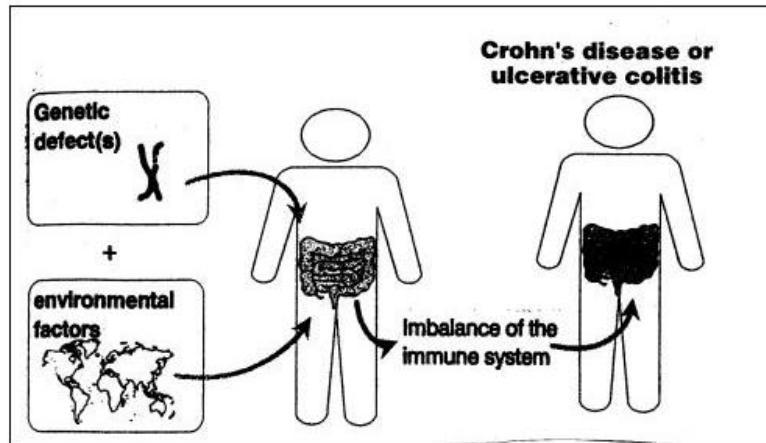
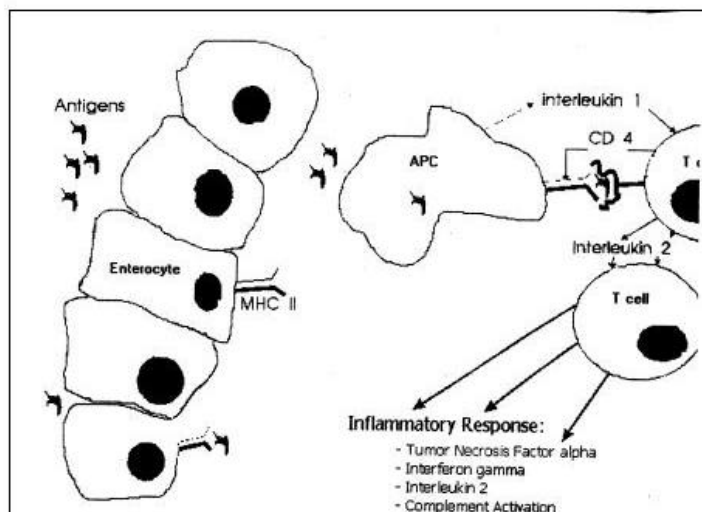


Fig. 4
 Relationship of genetic, environmental and immune factors to Crohn's Disease



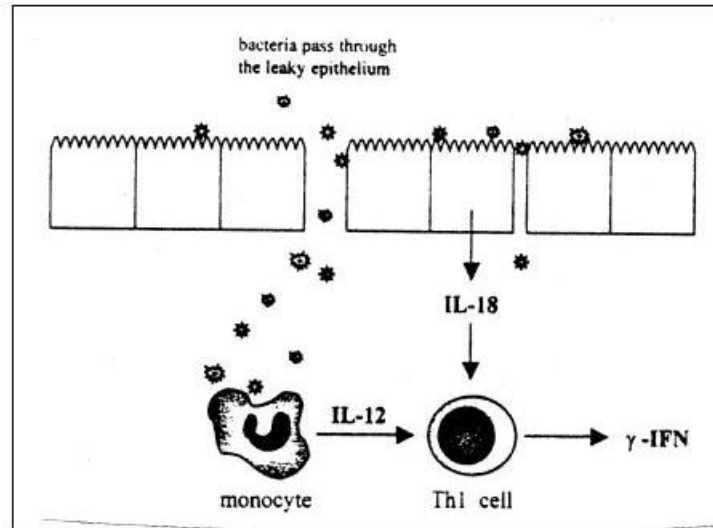
\From Rogler & Andus (1998)

Fig. 5
 Normal inflammatory response



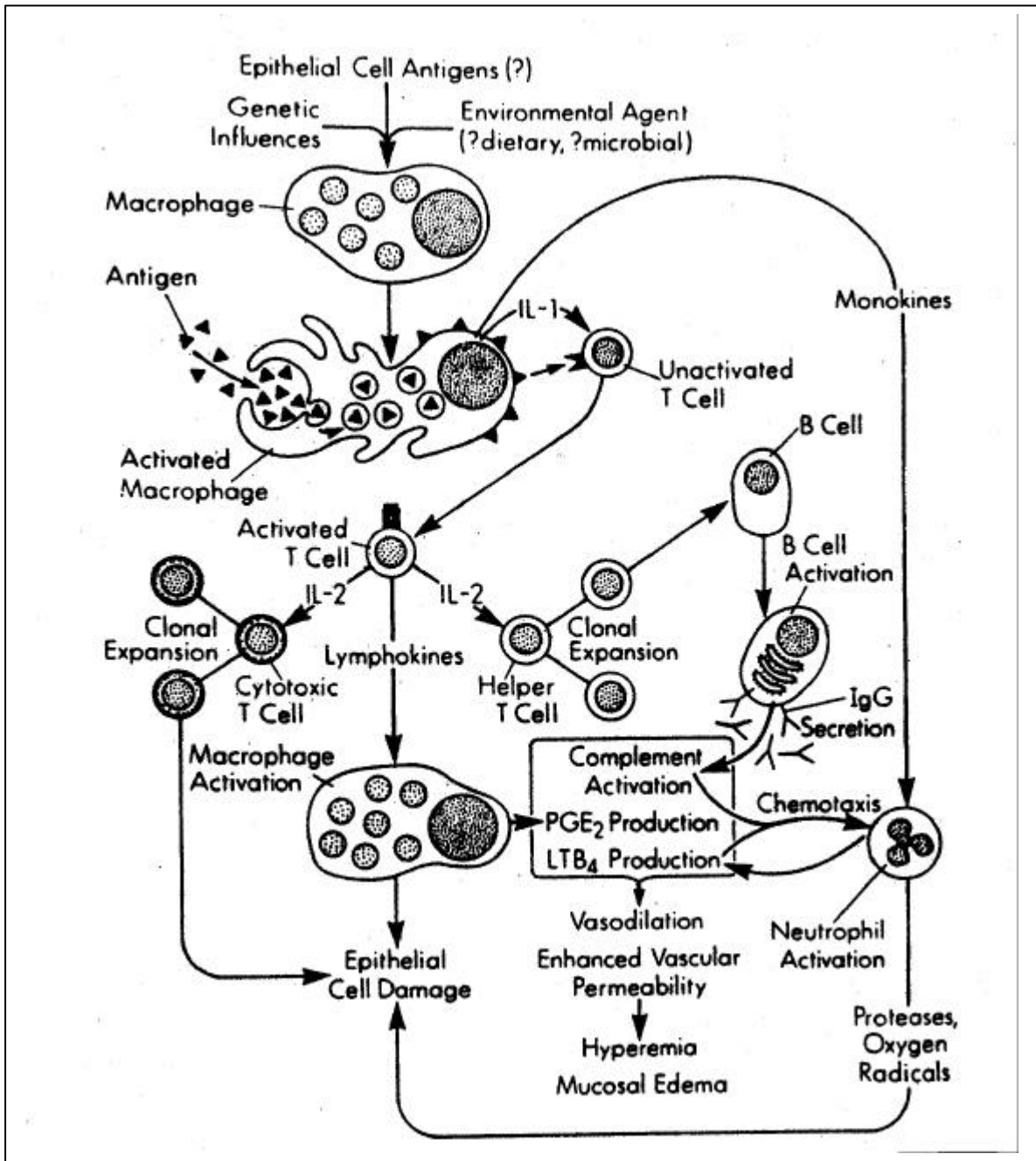
From Stronkhurst, Tytgat & van Deventer (Alan Lancaster web site)

Fig. 6
IL-12 induction



From MacDonald, Monteleone & Pender (2000)

Figure 7
The immune response



From MacDermott & Stenson (1988)

REFERENCES

- ¹ European Commission SANCO/B3/R16 (2000) Possible links between Crohn's Disease and Paratuberculosis *Report of the Scientific Committee on Animal Health and Animal Welfare*.
- ² SAC Report (draft) Assessment of Surveillance and Control of Johne's Disease in farm animals in Great Britain: *Interim Report*: including Part 1: Workshop 1 and Outline of Findings of Part 2.
- ³ Thompson DE (1994) The Role of mycobacteria in Crohn's Disease *Journal of Medical Microbiology* **41** 74-94
- ⁴ Hermon-Taylor J Bull TJ Sheridan JM Cheng J Stellakis ML and Sumar N (2000) *in* Causation of Crohn's Disease by Mycobacterium avium sub-species paratuberculosis. *Canadian Journal of Gastroenterology* **14** 521-523
- ⁵ Dalziel TK (1913) Chronic Intestinal enteritis *British Medical Journal* **ii** 1068-1070
- ⁶ Crohn B Ginzberg L & Oppenheimer G (1932) Regional ileitis, a pathological and clinical entity *Journal of the American Medical Association* **99** 1323-9
- ⁷ Lennard-Jones JE (1996) Clues to the elucidation of Crohn's disease. *Transactions of the College of Medicine of South Africa* Jan –June 34-41.
- ⁸ Jones JH, Lennard-Jones JE, Morson BC *et al* (1973) Numerical taxonomy and discriminant analysis applied to non-specific colitis. *Quart J Med* **42**: 715-732.
- ⁹ Spicer CC, Jones JH, Lennard-Jones JE (1973) Discriminant and Bayesian analysis in the differential diagnosis of Crohn's disease and proctocolitis. *Meth Inform Med* **12**: 118-122.
- ¹⁰ Greenstein RJ and Greenstein AJ (1995) Is there clinical, epidemiological and molecular evidence for two forms of Crohn's Disease? *Molecular Medicine Today* 343-348
- ¹¹ (2000) Crohn's Disease: p6. Booklet published by the National Association for Colitis and Crohn's Disease (NACC) www.nacc.org.uk
- ¹² Rubin GP, Hungin AP, Kelly PJ and Ling J (2000) Inflammatory Bowel Disease: Epidemiology and Management in an English General Practice Population *in Aliment Pharmacol Ther* **27**;14(12):1553-1559
- ¹³ Rangunath AS. and Tsai HH (1999) Gut Abstract 22. *Second International Inflammatory Bowel Disease Meeting* 298.
- ¹⁴ Sunday Telegraph (2001) April 8, p.4. Report of a Study of Infectious Bowel Disease in children by the Institute of Child Health, British Society of Gastroenterology & British Paediatric Surveillance Unit, June 1998-June 1999, and Sawczenko A, Sandhu BK, Logan RFA, Jenkins CJ, Mian S, Lynn R (2001) Prospective survey of childhood inflammatory bowel disease in the British Isles. *Lancet* **357** April 7
- ¹⁵ Hughan, C (2001) Personal communication 10 May.
- ¹⁶ Loftus EV, Silverstein WJ, Tremaine WJ, Harmsen WJ, Zinsmeister AR (1998) Crohn's Disease in Olmsted County, Minnesota, 1940-93: incidence, prevalence, and survival. *Gastroenterology* **114** 1161-68.
- ¹⁷ Lapidus, A, Bernell, O, Hellers, G and Lofberg R (1998) Clinical Course of Colorectal Crohn's Disease: A 35 year follow-up study of 507 patients *in Gastroenterology*, **114** 1151-1160.
- ¹⁸ Michener WM, Caulfield M, Wyllie R, Farmer RG (1990) Management of inflammatory bowel disease: 30 years of observation. *Cleveland Journal of Medicine* Nov-Dec; **57** (8):685-91.
- ¹⁹ Martin A, Leone L, Fries W, Naccarato R (1995) Quality of life in inflammatory bowel disease *Ital J Gastroent* **27** (8): 450-4.
- ²⁰ Hermon-Taylor, J (2001) Personal communication.
- ²¹ Silverstein MD *et al* (1999) Clinical Course and Costs of Care for Crohn's Disease : Markov Model Analysis of a Population-based Cohort. *Gastroenterology* **117**: 49-57.
- ²² Kennedy A (2001) PARA position statement on Mycobacterium avium subspecies paratuberculosis (MAP) in retail supplies of food and its relationship with Crohn's Disease; Paratuberculosis Awareness and Research Association www.crohns.org.
- ²³ Ekblom A and Blomqvist P (1997) Costs to society in Crohn's Disease *Research and Clinical Forums* **20** 33-39 Proceedings of the clinical symposium on IBD in Prague. ASTRA LUND Sweden.
- ²⁴ Crohn B Ginzberg L & Oppenheimer G (1932) Regional ileitis, a pathological and clinical entity *Journal of the American Medical Association* **99** 1323-9
- ²⁵ Wirostko E, Johnson L, Wirostko B (1987) Crohn's disease: Rifampicin treatment of the ocular and gut disease. *Hepato-gastroenterol.* **34** 90-93.
- ²⁶ Chiodini RJ (1989) Crohn's Disease and the mycobacterioses: a review and comparison of two disease entities *Clinical Microbiology Reviews*.
- ²⁷ Dalziel TK (1913) Chronic Intestinal enteritis *British Medical Journal* **ii** 1068-1070
- ²⁸ Abrams JS & Holden WD (1964) Tuberculosis of the gastro-intestinal tract *Arch Surg (Chicago)* **89** 282-293

- ²⁹ Wig KL *et al* (1961) Ileocaecal tuberculosis with particular reference to isolation of *Mycobacterium tuberculosis*. With a note on its relation to regional ileitis (Crohn's Disease) *Am Rev Resp Dis* **84** 169-178
- ³⁰ Sartor RB. Debate with Chiodini: papers for the ACDP – ACDP/55/5.
- ³¹ Bayless TM *et al* (1996) Crohn's Disease: Concordance for site and clinical type in affected family members – potential hereditary influences. *Gastroenterology* **111** 573-579.
- ³² Hugot J-P, Chamallard M, Zoual H *et al* (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **411**, pp.599-603.
- ³³ Ogura T, Bonen D K, Inohara N *et al* (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* **411** 603-606.
- ³⁴ Soderheim JD *et al* (1999) Different intestinal permeability patterns in relatives and spouses of patients with Crohn's Disease: an inherited defect in mucosal defence? *Gut* **44** 96-100
- ³⁵ Roediger WEW (1991) A new hypothesis for the aetiology of Crohn's Disease – evidence for lipid metabolism and intestinal tuberculosis. *Postgraduate Medical Journal* **67** 666-71.
- ³⁶ Grant IR (1997) Zoonotic Potential of *Mycobacterium Paratuberculosis*, in Holland CV (ed) *Modern perspectives in Zoonoses* 75-83 Dublin Royal Irish Academy
- ³⁷ Powell JJ, Harvey RSJ, Thompson RPH (1996) Microparticles in Crohn's disease- has the dust settled? *Gut*: **39** 340-341.
- ³⁸ Hermon-Taylor J (1993) Causation of Crohn's Disease: the impact of Clusters in *Gastroenterology* **104** (2) 643-646.
- ³⁹ McClure HM, Chiodini RJ, Anderson DC, Swenson RB, Thayer WR, Coutu JA. (1987) *Mycobacterium Paratuberculosis* infection in a colony of Stumptail macaques (*Macaca arctoides*) *J Inf Dis* **155** (5) 1011-1019.
- ⁴⁰ Grant IR (1997) Zoonotic potential of *Mycobacterium Paratuberculosis*, in Holland CV (ed) *Modern perspectives on Zoonoses* pp.75-83 Dublin Royal Irish Academy.
- ⁴¹ Sweeney RW, Whitlock RH, Hamir AN, Rosenberger AE, Herr SA (1992) Isolation of *Mycobacterium paratuberculosis* after oral inoculation in uninfected cattle. *American J Vet Res*:**53** 1312-1314.
- ⁴² Sweeney RW, Whitlock RH, Rosenberger AE (1992) *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease. *American J Vet Res*:**53** 477-480.
- ⁴³ Larsen AB, Kopecky KE (1970) *Mycobacterium paratuberculosis* in reproductive organs and semen of bulls *J Am Vet Med Assn Res* **31**: 255-8.
- ⁴⁴ Larsen AB, Stalheim OHV, Hughes DE, Appell LH, Richards WD, Himes EM (1981) *Mycobacterium paratuberculosis* in the semen and genital organs of a semen-donor bull; *JAVMA* **179** (2) 169-171.
- ⁴⁵ Gwozdz JM, Reichel, MP, Murray A, Manktelow W, West DM, Thompson KG. (1997) Detection of *Mycobacterium avium* subsp. *paratuberculosis* in ovine tissues and blood by the polymerase chain reaction; *Vet Microb*: **51** 233-244.
- ⁴⁶ Chang YF, Kim JB, Stehman CA, Rossiter CA, Chang CF, McDonough PL, Jacobson RH, Lein DH (1996) Detection of *M. paratuberculosis* in DNA in blood monocytes of milk macrophages from cattle and sheep affected by Johne's disease by PCR and an oligonucleotide probe: *Proc 5th International Colloquium on paratuberculosis*. Chiodini RJ, Hines ME, Collins MT, eds. Int Assoc for Paratuberculosis. Wisconsin 299-301.
- ⁴⁷ International Dairy Federation (2001) *Mycobacterium paratuberculosis*. Bulletin of the International Dairy Federation 362/2001, ISSN 0250-5118, 61pp.
- ⁴⁸ Grant IR (1997) Zoonotic potential of *Mycobacterium Paratuberculosis* in *Modern perspectives on Zoonoses* Holland CV (ed) pp.75-83 Dublin Royal Irish Academy.
- ⁴⁹ Mandel, GL, Douglas RG and Bennett JE (1990) in *Principles and Practice of Infectious Diseases*, 3rd edition Churchill Livingstone ISBN 0-443-08688-9, p 1877.
- ⁵⁰ Fine, P (2001) Personal communication.
- ⁵¹ Benenson, AS (ed.) (1990) in *Control of Communicable Diseases in Man* 15th Edition American Public Health Association Washington DC, p244.
- ⁵² Mandel GL, Douglas RG and Bennett JE (1990) in *Principles and Practice of Infectious Diseases*, 3rd edition Churchill Livingstone ISBN 0-443-08688-9 p 1877 *et seq*.
- ⁵³ Fine, P (2001) Personal communication.
- ⁵⁴ Fine, P (2001) Personal communication.
- ⁵⁵ Mandel, GL, Douglas RG and Bennett JE (1990) in *Principles and Practice of Infectious Diseases*, 3rd edition Churchill Livingstone ISBN 0-443-08688-9 p 1915.
- ⁵⁶ Hermon-Taylor J, Bull TJ, Sheridan JM, Cheng J, Stellakis ML and Sumar N (2000) Causation of Crohn's Disease by *Mycobacterium avium* sub-species *paratuberculosis*. *Canadian Journal of Gastroenterology* **14** 521-523

-
- ⁵⁷ SAC Report (draft) Assessment of Surveillance and Control of Johne's Disease in Farm Animals in Great Britain: *Interim Report*: including Part 1: Workshop 1 and outline of findings of Part 2. p 7.
- ⁵⁸ SAC Report (draft) Assessment of Surveillance and Control of Johne's Disease in farm animals in Great Britain : *Interim Report*: including Part 1 : Workshop 1 and Outline of findings of Part 2.
- ⁵⁹ Thorel MF, Krichevsky M, Levy-Frebault VV (1990) Numerical taxonomy of Mycobactin-dependant Mycobacteria, emended description of Mycobacterium avium, and description of Mycobacterium avium subsp. avium subsp nov, Mycobacterium avium subsp paratuberculosis subsp nov and Mycobacterium avium subsp silvaticum subsp nov. *International Journal of Systematic Bacteriology* **40** 254-60.
- ⁶⁰ Pavlik I, Horvathova A, Dvorska L, Bartl J, Svastova P, du Maine R, Rychlik I (1999) Standardisation of restriction length polymorphism analysis for *Mycobacterium avium* subsp *paratuberculosis*. *J Microbiol Methods*: **38** 155-167.
- ⁶¹ Pavlik I, Bartl J, Dvorska L, Svastova P, Machackova M, Yayo Ayele W, Horvathova A (2000) Epidemiology of paratuberculosis in wild ruminants studied by restriction fragment length polymorphism in the Czech republic during the period 1995-1998. *Vet Microbiol* **77** 231-251.
- ⁶² Multharia *et al* (1997) in SAC Report.
- ⁶³ Morgan KL (1987) Johne's and Crohn's: Chronic inflammatory bowel diseases of infectious aetiology? *The Lancet*, **May 2**, 1017-1019.
- ⁶⁴ SAC Report (draft) Assessment of Surveillance and Control of Johne's Disease in farm animals in Great Britain: *Interim Report*: including Part 1: Workshop 1 and Outline of findings of Part 2. p.12.
- ⁶⁵ SAC Report (draft) Assessment of Surveillance and Control of Johne's Disease in farm animals in Great Britain: *Interim Report*: including Part 1: Workshop 1 and Outline of findings of Part 2 p.13.
- ⁶⁶ Hermon-Taylor J, Bull TJ, Sheridan JM, Cheng J, Stellakis ML and Sumar N in Causation of Crohn's Disease by Mycobacterium avium sub-species paratuberculosis. *Canadian Journal of Gastroenterology* **14** 521-523
- ⁶⁷ Hacker J, Blum-Oehler G, Muhldorfer I, Tscape H. (2000) Pathogenicity islands of virulent bacteria: structure function and impact on microbial evolution. 1997: *Molecular Microbiol.* **23(6)**: 1089-1097.
- ⁶⁸ Hermon-Taylor J (2001) Personal communication.
- ⁶⁹ Whittington RJ, Marsh I, McAllister S, Turner MJ, Marshall DJ, Fraser CA (1999) Evaluation of modified BACTEC 12B radiometric medium and solid medium for culture of *Mycobacterium avium* subsp.*paratuberculosis* from sheep. *J Clin Microb* **37** 1077-1083.
- ⁷⁰ Whittington RJ, Marsh I, Turner MJ, McAllister S, Choy E, Eamens GJ, Marshall DJ, Ottaway S (1989) Rapid detection of *Mycobacterium paratuberculosis* in clinical samples from ruminants and in spiked environmental samples by modified BACTEC 12B radiometric culture and direct confirmation by IS900 PCR. *J Clin Microb* **36**: 701-707.
- ⁷¹ SAC Report (draft) Assessment of Surveillance and Control of Johne's Disease in farm animals in Great Britain: *Interim Report*: including Part 1: Workshop 1 and Outline of findings of Part 2, p.13.
- ⁷² Grant IR, Ball HJ, Rowe MT (1999) Effect of higher pasteurisation temperatures and longer holding times at 72 degrees C on the inactivation of *Mycobacterium paratuberculosis* in milk. *Letters in Applied Microbiology* **28** 461-65.
- ⁷³ Whitlock RH and Buergelt C (1996) Preclinical and clinical manifestations of paratuberculosis (including pathology) *Veterinary Clinics of North America: Food animal Practice* **12** 345-356
- ⁷⁴ Tsianos EV, Masalas CN, Mercouropoulos M, Dalekos GN, Logan RFA (1994). Incidence of inflammatory bowel disease in north west Greece: rarity of Crohn's disease in an area where ulcerative colitis is common. *Gut*: **35**: 369-372.
- ⁷⁵ Rees HC and Levison DA (1987) Inflammatory bowel disease in children. *Surgery* **51** 1216-1219.
- ⁷⁶ Calkins BM *et al* Trends in Incidence Rates of ulcerative colitis and Crohn's Disease. *Dig Dis Sci* 1984: **29**: 913-920.
- ⁷⁷ Cosgrove M, Al-Atia RF, Jenkins HR (1996) The epidemiology of paediatric inflammatory bowel disease. *Arch Dis Child*: **74** 460-1.
- Armitage E, Drummond H, Ghosh S, Ferguson, A (1999) Incidence of juvenile -onset Crohn's Disease in Scotland. *Lancet* **353**: 1496-97.
- ⁷⁸ Kyle J (1992) Crohn's Disease in the North eastern and northern Isles of Scotland: an epidemiological review. *Gastroenterology* **103(2)** 392-9.
- ⁷⁹ Sawczenko A, Sandhu BK, Logan RFA, Jenkins CJ, Mian S, Lynn R (2001) Prospective survey of childhood inflammatory bowel disease in the British Isles. *Lancet* **357**
- ⁸⁰ Lindberg E, Lindquist B, Holmquist L, Hildebrand H (2000) Inflammatory bowel disease in children and adolescents in Sweden, 1984-9995. *J Ped Gastroent and Nutr* **30**: 259-64.
- ⁸¹ Hughan, C (2001) Personal communication, 10 May.

-
- ⁸² Fireman Z, Grossman A, Lilos P *et al* (1989) Intestinal cancer in patients with Crohn's Disease. A population study in central Israel. *Scand J Gastroenterol.* **24**: 346-350.
- ⁸³ Fireman Z, Grossman A, Lilos P, Eschar Y, Theodor E, Gilat T (1989) Epidemiology of Crohn's Disease in the Jewish population of central Israel, 1970-1980. *Am J Gastroenterol* **84**: 255-258.
- ⁸⁴ Jewell DP (1987) Crohn's Disease. In Weatherall DJ, Ledingham JGG, Warrell DA (eds) Oxford Textbook for Medicine. 2nd Edition. Oxford University Press: 12, 121-12, 126.
- ⁸⁵ Weterman IT and Pena AS (1984) Familial incidence of Crohn's Disease in the Netherlands and a review of the literature. *Gastroenterology*: **86** 449-452.
- ⁸⁶ Shivananda S *et al* (1996) Incidence of inflammatory bowel disease across Europe: is there a difference between North and South? Results of the European collaborative study on inflammatory bowel disease (EC-IBD) *Gut* **39** 690-697.
- ⁸⁷ Loftus EV, Silverstein WJ, Tremaine WJ, Harmsen WJ, Zinsmeister AR (1998) Crohn's Disease in Olmsted County, Minnesota, 1940-93: incidence, prevalence, and survival. *Gastroenterology* **114** 1161-68.
- ⁸⁸ Centres for Disease Control 1989 Tuberculosis statistics in the United States. US Department of Health and Human Services, Public Health Service 1991 10-11.
- ⁸⁹ Sathie S and Sarai A (1992) Mycobacterial Infections *Curr Opin Infect Dis* **5** 176-182.
- ⁹⁰ Fine, P (2001) Personal recommendation
- ⁹¹ Bernstein CN, Blanchard JF, Rawsthorne P, Wajda A (1999) Epidemiology of Crohn's disease and ulcerative colitis in a central Canadian province: A population based study. *Am J Epidemiol.* **116** 916-924.
- ⁹² Bernstein CN, Blanchard JF (1999) The epidemiology of Crohn's disease: *Gastroenterol.* **116** 1503-1506.
- ⁹³ Pinchbeck BR, Kirkeikis J, Thomson ABR (1988) Inflammatory bowel disease in Northern Alberta: an epidemiologic study. *J Clin Gastroenterol.* **110** 505-515.
- ⁹⁴ Loftus EV, Silverstein WJ, Tremaine WJ, Harmsen WJ, Zinsmeister AR (1998) Crohn's Disease in Olmsted County, Minnesota, 1940-93: incidence, prevalence, and survival. *Gastroenterology.* 1998 **114** 1161-68.
- ⁹⁵ Wright JP, Froggatt J, O'keefe EA, et al The epidemiology of inflammatory bowel disease in Cape Town 1980-84 *SAfr Med Journ* 1986; **70** : 10-15.
- ⁹⁶ Fireman Z. Grossman A. *et al* Epidemiology of Crohn's Disease in the Jewish Population of Central Israel, 1970-1980 *American Journal of Gastroenterology* 1989 : **84** 255-258.
- ⁹⁷ Odes *et al* (1989) Epidemiological data of Crohn's Disease in Israel: etiological implications. *Public Health Rev* **17** 321-335; and Inflammatory bowel disease in the Bedouin Arabs of Southern Israel: rarity of diagnosis and clinical features. *Gut* 1991 **32** 1024-1026.
- ⁹⁸ European Commission Directorate-General Health and Consumer Protection (2000). SANCO/BR/R16/2000. Possible links between Crohn's disease and Paratuberculosis: Report of the scientific committee on animal health and welfare. Adopted 21 March 2000. p.25, Table 5.
- ⁹⁹ Tamboli CP (1996) A hypothesis for explaining the geographical distribution of Crohn's Disease. *Canadian Journal of Gastroenterology* **10** 173-7.
- ¹⁰⁰ Hermon-Taylor J (1993) Causation of Crohn's Disease: the impact of Clusters in *Gastroenterology* **104** (2) 643-646.
- ¹⁰¹ Van Kruiningan HJ, Colombel JF, Cartun RW, Koopmans M, Kangro HO, Hoogkamp-Korstanje JAA, Lecomte-Houcke M, Devred M, Paris JC, Cortot A (1992) An in-depth study of Crohn's Disease in two French families. *Gastroenterology* **103**:351-360.
- ¹⁰² Allan RN, Pease P, Ibbotson JP (1986) Clustering of Crohn's disease in a Cotswold village. *Quart J Med* **59** 473-478.
- ¹⁰³ Aisenberg J, Janowitz HD (1993). Cluster of inflammatory bowel disease in three close college friends? *J Clin Gastroenterol.* **17** 18-20.
- ¹⁰⁴ Lee JCW and Lennard-Jones, JE (1996) Inflammatory Bowel Disease in 67 families each with three or more affected first-degree relatives. *Gastroenterology* **111** 587-596.
- ¹⁰⁵ Mayberry JF (1989) Recent epidemiology of ulcerative colitis and Crohn's Disease in Cardiff *Int J Colorect Dis.* **4** 59-66.
- ¹⁰⁶ Mayberry JF, Hitchens RAN (1978) Distribution of Crohn's disease in Cardiff. *Soc Sci Med.* **12** 137-138.
- ¹⁰⁷ Peeters M, Nevens H, Baert F *et al* (1996) Familial incidence Crohn's Disease: Increased age –adjusted risk and concordance in Clinical Characteristics *Gastroenterology* **111** 597-603.
- ¹⁰⁸ Weterman IT and Pena AS (1984) Familial Incidence of Crohn's Disease in the Netherlands and a Review of the literature. *Gastroenterology* **86** 449-452.
- ¹⁰⁹ Gent AE *et al* (1994) Inflammatory bowel disease and domestic hygiene in infancy *Lancet.* **343** 766-67.

-
- ¹¹⁰ Hermon-Taylor J, Barnes N, Clarke C and Finlayson C (1998) Grand Round Mycobacterium paratuberculosis cervical lymphadenitis, followed five years later by terminal ileitis similar to Crohn's Disease *British Medical Journal* **316b** 449-453.
- ¹¹¹ Hermon-Taylor J. Personal communication.
- ¹¹² Chamberlin W, Graham D Y *et al* (2001) Mycobacterium avium subsp. paratuberculosis as one cause of Crohn's Disease. *Aliment. Pharmacol.* **15** 337-346.
- ¹¹³ SAC Report (draft) Assessment of surveillance and control of Johne's Disease in farm animals in Great Britain: *Interim Report*: including Part 1: Workshop 1, and Outline of Findings of Part 2, p.25.
- ¹¹⁴ Cetinkaya B, Egan K, Harbour DA, Morgan KL (1996) An abattoir –based study of the prevalence of subclinical Johne's disease in adult cattle in southwest England. *Epidemiol Infect.* **116** 373-379.
- ¹¹⁵ Taylor AW () Observations on the incidence of infection with M. Johnei in cattle: *Vet Record* **61** 539-540, and Rankin JD The prevalence of *Mycobacterium johnei* in apparently healthy cattle. *Vet Record* **66** 550-551.
- ¹¹⁶ SAC Report (draft) Assessment of surveillance and control of Johne's Disease in farm animals in Great Britain: *Interim Report*: including Part 1: Workshop 1, and Outline of Findings of Part 2, p.26.
- ¹¹⁷ Benedictus G, Verhoeff J, Schuccken YH, Hesselink JW (2000) Dutch paratuberculosis programme history , principles and development. *Vet Microbiol* **77** (3-4) 399-413.
- ¹¹⁸ Boelaert F, Walravens K, Vermeersch JP, Raskin A, Dufey J, Godfroid J (in publ.) Sample survey for estimating the herd and individual animal seroprevalence for bovine paratuberculosis in dairy herd in Belgium *Vet Microbiol* submitted for publication – quoted I EC report- ref. 1.
- ¹¹⁹ Muskeens J, Barkema HW, Russchen E, van Maanen K, Schukken YH, Bakker D (2000) Prevalence and regional distribution of bovine paratuberculosis in dairy herds in the Netherlands. *Vet Microbiol* – in Press.
- ¹²⁰ Dimarelli Z, Sarris K, Xenos G *et al.* (1992) *The Paratuberculosis Newsletter* **3** 8-9.
- ¹²¹ Juste RA, Badiola JJ, Arnal MC *et al.* *The Paratuberculosis Newsletter* **3** 3-4.
- ¹²² Juste Jordan RA () III Reunion sobre paratuberculosis en Espana. *Informes Tecnicos* **34** Dpto de Agricultura y Pesca. Gobierno Vasco.
- ¹²³ Merkal RS, Whipple DL, Sacks JM, Snyder GR () Prevalence of *Mycobacterium paratuberculosis* in ileocecal lymph nodes of cattle culled in the United States. *J Am Vet Med Assoc* **190** (6) 676-80.
- ¹²⁴ Pavlik I, Bartl J, Dvorska L, Svastova P, Machackova M, Yayo Ayele W, Horvathova A. (2000) Epidemiology of paratuberculosis in wild ruminants studied by restriction fragment length polymorphism in the Czech republic during the period 1995-1998. *Vet Microbiol* **77** 231-251.
- ¹²⁵ Hermon-Taylor. J. Personal communication
- ¹²⁶ FIL/IDF (2001) On-farm Control and Diagnosis of Paratuberculosis (Johne's Disease) 27 January, Brussels, Belgium.
- ¹²⁷ FIL/IDF (2001) On-farm Control and Diagnosis of Paratuberculosis (Johne's Disease) 27 January, Brussels, Belgium.
- ¹²⁸ Pavlik I, Bejcklova L, Pavlas M, Rozsypalova Z, Koskova S. (1995) Characterisation by restriction endonuclease analysis and DNA hybridisation using IS900 of bovine, ovine, caprine, and human-dependent strains of Mycobacterium paratuberculosis isolated in various localities. *Vet Microbiol* **45** (4) 311-18.
- ¹²⁹ Whittington RJ, Hope AF, Marshall DJ, Taragel CA, Marsh I. (2000) Molecular epidemiology of Mycobacterium avium subsp. paratuberculosis: IS900 restriction fragment length polymorphism and IS1311 polymorphism analyses of isolates from animals and a human in Australia. *J Clin Microbiol* **38** (9) 3240-8.
- ¹³⁰ Lennard-Jones JE (1996) Clues to the elucidation of Crohn's disease. *Transactions of The College of Medicine of South Africa* January –June 34-41.
- ¹³¹ Powell JJ, Ainley CC, Harvey RSJ *et al* (1996) Characterisation of inorganic microparticles in pigment cells of human get associated lymphoid tissue. *Gut* **38** pp.390-395.
- ¹³² Powell JJ, Harvey RSJ, Ashwood P *et al* (2000) Immune potentiation of ultrafine dietary particles in normal subjects and patients with inflammatory bowel disease. *Journal of Autoimmunity* **14** 99-105.
- ¹³³ Lomer MCE, Harvey RSJ, Evans SM *et al* (2001) The efficacy and tolerability of a low microparticle diet in a double blind randomised control pilot study in Crohn's disease. *European Journal of Gastroenterology & Hepatology* **13/2** 101-106.
- ¹³⁴ Cosnes J, *et al.* (1996) Effects of smoking on the long-term course of Crohn's Disease. *Gastroenterology*: **110** 424-431.
- ¹³⁵ Fireman Z, *et al* (1989) Intestinal Cancer in Patients with Crohn's Disease: A population study in Central Israel *Scand J Gastroenterol* **24** 346-350.
- ¹³⁶ Daszeh P, *et al* (1997) Detection and comparative analysis of persistent measles virus infection in Crohn's Disease by immunogold electron microscopy *J Clin Pathol* **50** 299-304.

-
- ¹³⁷ Feeney M, *et al* (1997) A case control study of measles vaccination and inflammatory bowel disease *Lancet* **350** 764-766.
- ¹³⁸ From the Chief Medical Officer: Measles, Mumps Rubella (MMR) Vaccine, Crohn's Disease and Autism. PL/CMO/98/227 March 1998. Department of Health
- ¹³⁹ Davis RL, Kramarz P, Bohlke K, Benson P, Thompson RS, Mullooly J, Black S, Shinefield H, Ward J, Marcy SM, Eriksen E, Destefano F, Chen R (2001) Measles-Mumps-Rubella and other Measles-continuing Vaccines do not increase the risk for inflammatory bowel disease: a case-control study from the vaccine safety Datalink project. *Arch Pediatr Adolesc Med* **155** (3) 354-359.
- ¹⁴⁰ European Commission (2000) Possible links between Crohn's Disease and Paratuberculosis: *Report of the Scientific Committee on Animal Health and Animal Welfare*, 21 March.
- ¹⁴¹ Van Wynsberghe D, Noback CR, Carola R (1995) Human Anatomy and Physiology Third Edition. McGraw Hill Inc. p.799.
- ¹⁴² Stronkhorst AS, Tytgat GJH, van Deventer SJH. Immunotherapeutical approaches to Crohn's disease in view of a possible pathogenic role of Mycobacterial antigens [from Alan Lancaster web site].
- ¹⁴³ Macdonald TT, Monteleone G, Pender SLF (2000) Recent developments in the immunology of inflammatory bowel disease. *Scand J Immunol* **51** 2-9.
- ¹⁴⁴ Elson CO, Sartor RB, Tennyson GS, Riddell RH (1995) Experimental models of inflammatory bowel disease. *Gastroenterology*: **109** 1344-67.
- ¹⁴⁵ Stronkhorst A, Radema S, Yong SL, *et al.* (1997) CD4 antibody treatment in patients with active Crohn's disease: a phase one dose-finding study. *Gut* **40** 320-7.
- ¹⁴⁶ Stronkhorst A, Tytgat GNJ, Vas Deventer SJH (1992) CD4 antibody treatment in Crohn's disease. *Scand J Gastroenterol: (Supplement)* **27** 61-65.
- ¹⁴⁷ Boirivant M, Marini M, Di Felice G *et al.* (1999) Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. *Gastroenterology*: **116** 225-232.
- ¹⁴⁸ Trianchieri G (1994) Interleukin 12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood* **84** 4006-27.
- ¹⁴⁹ Trianchieri G (1994) Interleukin 12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood* **84** 4006-27.
- ¹⁵⁰ Parronchi P, Romagnani P, Annunziato F *et al.* Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. 1997; *Am J Pathol I* **150** 823-32.
- ¹⁵¹ Macdonald TT, Monteleone G, Pender SLF (2000) Recent developments in the immunology of inflammatory bowel disease: *Scand J Immunol* **51** 2-9.
- ¹⁵² Sartor RB (1997) Pathogenesis and immune mechanisms of chronic inflammatory bowel disease. *Am J Gastroenterol.* **92** 5S-11S.
- ¹⁵³ Soderheim JD, *et al.* (1999) Epithelial permeability to proteins in the non-inflamed ileum of Crohn's Disease? *Gastroenterology* **117** 65-72.
- ¹⁵⁴ Soderheim JD *et al* (1999) Different intestinal patterns in relatives and spouses of patients with Crohn's Disease : an inherited defect in mucosal defence? *Gut*: **44** 96-100.
- ¹⁵⁵ Peeters M *et al* (1997) Clustering of increased small intestinal permeability in families with Crohn's Disease. *Gastroenterology* **113** 802-807.
- ¹⁵⁶ Baldassano RN. *et al* (1993) Crohn's Disease Monocytes are primed for accentuated release of toxic oxygen metabolites *Gastroenterology* **105** 60-66.
- ¹⁵⁷ Rogler G, Andua T () Cytokines in Inflammatory bowel disease. *World J Surg* **22** 382-389.
- ¹⁵⁸ van Devanter S, Camoglio L, te Velde. A Immune modulation of Crohn's disease. Laboratory of Experimental Internal Medicine, Amsterdam website e-mail SJ vanDeventer@amc.uva.nl
- ¹⁵⁹ Plevy, SE, Landers CJ, Prehn J, Carramanzana NM, *et al.* (1997) A role for TNF-alpha and mucosal T helper -1 cytokines in the pathogenesis of Crohn's disease. *J Immunol.* **159** 6276-6282.
- ¹⁶⁰ MacDonald, T (2001) Personal communication, 12 June.
- ¹⁶¹ Chiodini RJ, *et al.* (1984) Characteristics of an unclassified *Mycobacterium* species isolated from patients with Crohn's Disease. *Journal of Clinical Microbiology* **20** 966-71 and McFadden JJ *et al* (1987) Crohn's Disease-isolated mycobacteria are identical to *Mycobacterium* Paratuberculosis as determined by DNA probes that distinguish between mycobacterial species. *J Clin Microbiol* **25** 796-801.
- ¹⁶² Gitnik *et al.* (1989) Preliminary Report on Isolation of *Mycobacteria* from patients with Crohn's Disease *Digestive Diseases and Sciences* **34** 925-932.
- ¹⁶³ Chiodini RJ (1989) Crohn's Disease and the mycobacterioses: a review and comparison of two disease entities *Clinical Microbiol Reviews* **2** 90-117.
- ¹⁶⁴ Chiodini *et al.* (1986) Spheroplastic phase of *Mycobacteria* isolated from patients with Crohn's Disease *J Clin Microbiology* **24** 357-363.

- ¹⁶⁵ Ratnam S and Chandrasekhar S (1976) The pathogenicity of spheroplasts of *Mycobacterium tuberculosis*. *Am Rev Resp Dis* **114** 549-554.
- ¹⁶⁶ Kobayashi K, Blaser MJ, and Brown WR (1989) Immunohistochemical examination for mycobacteria in intestinal tissues from patients with Crohn's Disease. *Gastroenterology* **96** 1009-1015.
- ¹⁶⁷ Graham DY *et al* (1989) Mycobacteria as the cause of Crohn's Disease *Gastroenterology* **97** 1354-1356.
- ¹⁶⁸ Graham DY *et al* (1987) Mycobacteria and Inflammatory Bowel Disease: Results of Culture. *Gastroenterology* **92** 436-442.
- ¹⁶⁹ Naser SA, Schwartz D, and Shafran I (2000) Isolation of *Mycobacterium avium* subsp paratuberculosis from breast milk of Crohn's Disease Patients *American Journal of Gastroenterology* **95** (4) 1094-1095.
- ¹⁷⁰ Mishina *et al* (1996) On the etiology of Crohn's Disease *PNAS USA*: **93** 9816-9820.
- ¹⁷¹ Mc Fadden JJ and Fidler HM (1996) Mycobacteria as possible causes of sarcoidosis and Crohn's Disease *Journal of Applied Bacteriology* **81** 47S-52S.
- ¹⁷² Fidler HM (1994) Specific detection of *Mycobacterium Paratuberculosis* DNA associated with granulomatous tissues in Crohn's Disease. *Gut* **35** 506-510.
- ¹⁷³ Sanderson JD *et al* (1992) *Mycobacterium paratuberculosis* DNA in Crohn's Disease tissue. *Gut* **33** 890-896.
- ¹⁷⁴ Thompson DE (1994) The Role of mycobacteria in Crohn's Disease. *Journal of Medical Microbiology* **41** 74-94.
- ¹⁷⁵ European Commission SANCO/B3/R16 (2000) Possible links between Crohn's Disease and Paratuberculosis. *Report of the Scientific Committee on animal Health and Animal Welfare*.
- ¹⁷⁶ Pavlik I, Bejkova L *et al* () Characterisation by restriction endonuclease analysis and DNA hybridisation using IS900 of bovine, ovine, caprine and human dependent strains of *Mycobacterium paratuberculosis* isolated in various localities *Vet Microbiol* **45** (4).
- ¹⁷⁷ Whittington RJ *et al* (2000) Molecular epidemiology of *Mycobacterium avium* subsp paratuberculosis : IS900 restriction fragment length polymorphism and IS 1311 polymorphism analysis of isolates from animals and a human in Australia *J Clin Microbiol* **38** (9) 3240-8
- ¹⁷⁸ Cartun *et al* (1993) An immunocytochemical search for Infectious agents in Crohn's Disease *Modern Pathology* **6** 212-219.
- ¹⁷⁹ Cho S-N, Brennan PJ, Yoshimura HH, Korelitz BL, Graham DY (1986) Mycobacterial etiology of Crohn's disease : Serologic study using common mycobacterial antigens and a species-specific glycolipid antigen from *Mycobacterium paratuberculosis*. *Gut*: **27** 1353-6.
- ¹⁸⁰ Chamberlin W, Graham DY, Hulten K, *et al* (2001). Review Article: *Mycobacterium avium* subsp. paratuberculosis as one cause of Crohn's disease. *Aliment Pharmacol Ther.* **15** 337-346.
- ¹⁸¹ Merhal *et al* (1970) and Milna *et al* (1990) in SAC report.
- ¹⁸² Chiodini RJ, *et al.* (1984) Characteristics of an unclassified *Mycobacterium* species isolated from patients with Crohn's Disease. *Journal of Clinical Microbiology* **20** 966-71 and McFadden JJ *et al* (1987) Crohn's Disease-isolated mycobacteria are identical to *Mycobacterium Paratuberculosis* as determined by DNA probes that distinguish between mycobacterial species. *J Clin Microbiol* **25** 796-801.
- ¹⁸³ Tizard M, Bull T, Millar D, *et al.* (1998) A low G+C content in *Mycobacterium avium* subsp. paratuberculosis and *M avium* subsp silvaticum with homologous genes inn *Mycobacterium tuberculosis*. *Microbiology* **144** 3413-3123 and Hermon-Taylor, J. Personal communication.
- ¹⁸⁴ El-Zaatari, FAK, Naser SA, Graham DY (1997) Characterisation of a specific *Mycobacterium paratuberculosis* recombinant clone expressing 35 000 molecular weight antigen and reactivity with sera from animals with clinical and subclinical John's disease. *J Clin Microbiol* **35** 1794-99.
- ¹⁸⁵ El-Zaatari FAK, Naser S, Engstrand L, Hachem CY, Graham DY (1994) Identification and characterisation of *Mycobacterium paratuberculosis* recombinant clones expressed in *E coli*. *Curr Microbiol.* **29** 177-84.
- ¹⁸⁶ El-Zaatari FAK, Naser SA, Engstrand L, *et al* (1996) Identification of *Mycobacterium avium* complex in sarcoidosis. *J Clin Microbiol.* **34** 2240-5.
- ¹⁸⁷ Naser S, Shafran I, El-Zaatari FAK (1999) *Mycobacterium avium* subsp paratuberculosis in Crohn's disease is serologically positive. *Clin Diag Lab Immunol.* **6** 282.
- ¹⁸⁸ Naser SA, Hulten K, Shafran I, Graham DY, El-Zaatari FA (2000) Specific seroreactivity of Crohn's disease patients against p35 and p36 antigens of *M.avium* subsp. paratuberculosis. *Vet Microbiol.* **77** 497-504.
- ¹⁸⁹ Olsen I, Wiker HG, Johnson E, Langeggen H, Reitan LJ (2001) Elevated antibody responses in patients with Crohn's disease against a 14-kDa secreted protein purified from *Mycobacterium avium* subsp paratuberculosis. *Scand J Immunol.* **53** 198-203.
- ¹⁹⁰ Chamberlin W, Graham DY, Hulten K, *et al* (2001) Review Article: *Mycobacterium avium* subsp. paratuberculosis as one cause of Crohn's disease. *Aliment Pharmacol Ther.* **15** 337-346.

-
- ¹⁹¹ Baldassano RN, Schreiber S, Johnston RB, Fu RD, Muraki T Mac Dermott RP (1993) Crohn's disease monocytes are primed for accentuated release of toxic oxygen metabolites. *Gastroenterology* **105** 60-65.
- ¹⁹² Sartor RB (1997) Pathogenesis and immune mechanisms of chronic inflammatory bowel disease. *Am J Gastroenterology* **92** 5S-11S.
- ¹⁹³ Van Kruiningen HJ *et al* (1986) Experimental disease in infant goats induced by a *Mycobacterium* isolated from a patient with Crohn's Disease. a preliminary report. *Digestive Diseases and Science* **31** 1351-1360.
- ¹⁹⁴ Chiodini RJ *et al* (1984) Possible role of Mycobacteria in Inflammatory Bowel Disease: 1. An unclassified *Mycobacterium* Species isolated from Patients with Crohn's Disease. *Digestive Disorders and Sciences* **29** 1073-1079.
- ¹⁹⁵ Thompson DE (1994) The Role of mycobacteria in Crohn's Disease *Journal of Medical Microbiology* **41** 74-94.
- ¹⁹⁶ Tamboli CP (1996) A hypothesis for explaining the geographical distribution of Crohn's Disease. *Canadian Journal of Gastroenterology* **10** 173-7.
- ¹⁹⁷ Streeter RN *et al* (1995) Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of sub-clinically infected cows. *American journal of Veterinary Research* **56** 1322-1324.
- ¹⁹⁸ Merkal RS; Crawford JA Whipple DL (1979) *Applied Environmental Microbiol.* **38** (5) 831-835.
- ¹⁹⁹ Chiodini RJ and Hermon-Taylor J (1993) The thermal resistance of *Mycobacterium paratuberculosis* in raw milk under conditions simulating pasteurisation. *Journal of Veterinary Diagnostic Investigation* **5** 629-631.
- Grant IR *et al* (1996) Inactivation of *Mycobacterium paratuberculosis* in cows milk at pasteurisation temperatures *Applied and Environmental Microbiology* **62** 631-6.
- ²⁰⁰ Grant IR, Ball HJ, Neill SD, Rowe MT (1996) Inactivation of *Mycobacterium paratuberculosis* in cows' milk at pasteurisation temperatures. *Applied and Environment Microbiology* **62** (2).
- ²⁰¹ Grant IR, Rowe MT, Dundee L, Hitchings E (2001) *Mycobacterium avium ssp. paratuberculosis*: its incidence , heat resistance and detection in milk and dairy products. *International Journal of Dairy Technology* **54** 2-14.
- ²⁰² Grant IR, Ball HJ Rowe MT. (1999) Effect of higher pasteurisation temperatures and longer holding times at 72 degrees C on the inactivation of *Mycobacterium paratuberculosis* in milk. *Letters in Applied Microbiology* **28** 461-65.
- ²⁰³ Amor D (1998) Why milk needs reassessing. *Food Industry News* **10**.
- ²⁰⁴ ACM/485. Preliminary results from the national study on the microbiological quality and heat processing of cow's milk: *Mycobacterium Avium subsp Paratuberculosis*: UK Advisory Committee on the Microbiological Safety of food (ACMSF) www.foodstandards.gov.uk/pdf_files/papers/acm485.pdf.
- ²⁰⁵ Millar D *et al* (1996) IS900 PCR to detect *Mycobacterium paratuberculosis* in retail supplies of whole pasteurised cows' milk in England and Wales. *Applied and Environmental Microbiology* **62** 3446-3452.
- ²⁰⁶ Miller D S *et al* (1994) IS900 PCR testing for *Mycobacterium paratuberculosis* in unit of whole pasteurised milk widely obtained from retail outlets in England and Wales. In Chiodini RJ *et al*. *Proceedings of the 4th International Colloquium on Paratuberculosis*. Providence, International Association for Paratuberculosis, p.320.
- ²⁰⁷ Advisory Committee on the Microbiological Safety of Food (2000) Preliminary results from the national study on the microbiological quality and heat processing of cows' milk: *Mycobacterium avium subsp. paratuberculosis*. Paper ACM/485 presented to the Committee on the 19 September. Available on www.foodstandards.gov.uk/pdf_files/papers/acm485.pdf.
- ²⁰⁸ Grant IR, Ball HJ, Rowe MT (1997) A novel staining technique for assessing clumping and viability of *Mycobacterium avium subsp paratuberculosis* during pasteurisation. *Proceedings of the Fifth International Colloquium on Paratuberculosis*, pp 374-376. Chiodini RJ, Hines ME, Collins MT. (eds). Rehoboth , MA: International Association for Paratuberculosis.
- ²⁰⁹ Grant IR, Ball HJ, Rowe MT (1996) Thermal inactivation of several *Mycobacterium spp.* in milk by pasteurisation: **22** 253-256.
- ²¹⁰ Sung N, Collins MT (1998) Thermal; tolerance of *Mycobacterium paratuberculosis*. *Applied and Environmental Microbiology* **64** 999-1005.
- ²¹¹ Keswani J and Frank JF (1998) *Journal of Food Protection* **61** 974-978.
- ²¹² Hammer P (2000) *Mycobacterium paratuberculosis* in cattle and milk hygiene. *Bulletin of the International Dairy Federation* **345** 19-22.
- ²¹³ Grant IR, Ball HJ, Rowe MT (1999) Effect of higher pasteurisation temperatures , and longer holding times on the inactivation of *Mycobacterium paratuberculosis* in milk. *Letters in Applied Microbiol.* **28** 461-485.
- ²¹⁴ Grant IR, Rowe MT, Dundee L, Hitchings E (2001) *Mycobacterium avium ssp. paratuberculosis*: its incidence , heat resistance and detection in milk and dairy products. *International Journal of Dairy Technology* **54** 2-14.

- 215 Grant IR, Ball HJ, Rowe, M (1998) Isolation of Mycobacterium paratuberculosis from milk by immunomagnetic separation. *Applied Environmental Microbiology* **64** (9) (Sept) 3153-8.
- 216 Grant IR, Pope CM, O’Riordan LM, Ball HJ, Rowe MT (2000) Improved detection of Mycobacterium avium subsp paratuberculosis in milk by immunogenetic PCR. *Vet Microbiol.* **77** 369-378.
- 217 Grant IR, Ball HJ, Rowe MJ (1998) High Temperature , short time pasteurisation of milk containing low levels of *Mycobacterium paratuberculosis*. *Letters in Applied Microbiology* **26** 166-70.
- 218 Grant IR, O’Riordan LM, Ball HJ Rowe MT () Incidence of *Mycobacterium paratuberculosis* in raw sheep and goats milk in England , Wales and Northern Ireland. *Veterinary Microbiology* **79** 123-131.
- 219 Rowe MT, Grant IR, Dundee L, Ball HJ (2000) Heat resistance of *Mycobacterium avium* subsp. *paratuberculosis* in milk. **39** 203-208.
- 220 MacDonald, T (2001) Personal communication (12 June).
- 221 Grant IR (1997) Zoonotic potential of Mycobacterium Paratuberculosis, in *Modern perspectives on Zoonoses* Holland CV (ed) pp.75-83, Dublin Royal Irish Academy.
- 222 Chiodini RJ, Van Kruiningen HJ and Merkal RS (1984) Ruminant paratuberculosis (Johne’s Disease): the current status and future prospects *Cornell Vet.* 218-262.
- 223 Grant IR (1997) Zoonotic potential of Mycobacterium Paratuberculosis in *Modern perspectives on Zoonoses* Holland CV (ed) pp.75-83, Dublin Royal Irish Academy.
- 224 Mishina D, Kats P, Brown ST and Greenstein RJ (1996) On the etiology of Crohn’s Disease *Proc Natl Acad Sci.* **93** (18) 9816-20
- 225 Hermon-Taylor J and Pickup R. MRC/NERC Research Grant.
- 226 Beard PM, Daniels MJ, Henderson, D, *et al* (2001). Paratuberculosis infection of non-ruminant wildlife in Scotland: *J Clin Microbiol.* **39** (4) 1517-21.
- 227 Heights LB (1996) Clarithromycin against Mycobacterium avium complex infections *Tuber Lung Dis* **77** (1) 19-26.
- 228 Heifats LB (1996) Clarithromycin against Mycobacterium avium complex infections *Tuber Lung Dis* **77** (1) 19-26.
- 229 Thomas GAO, Swift GL, Green JT, *et al* (1998) Controlled trial of anti-tuberculous chemotherapy in Crohn’s disease: a five year follow up study. *Gut* **42** 497-500.
- 230 Quirke P, Taylor GR and Dixon MF; and Mapstone NP and Quirke P (1990-1992) Reports to CICRA.
- 231 Rastogi N and Labrousse V (1991) Extracellular and intracellular activities of clarithromycin used alone and in association with ethambutol and rifampicin against *Mycobacterium avium* complex. *Antimicrob agents and Chemotherapy* **35** 462-470.
- 232 Hermon-Taylor, J. Personal communication.
- 233 Hermon-Taylor, J. Personal communication.
- 234 Kennedy, A. Position paper, Paratuberculosis Awareness and Research Association, www.crohns.org.uk
- 235 El-Zaatari, FAK, Naser SA, Graham DY (1997) Characterisation of a specific *Mycobacterium paratuberculosis* recombinant clone expressing 35 000 molecular weight antigen and reactivity with sera from animals with clinical and subclinical Johne’s disease. *J Clin. Microbiol.* **35** 1794-99.
- 236 The BSE Inquiry Report (2000). Lord Justice Phillips, October.
- 237 The Interim Response to the Report of the BSE Inquiry (2001) HM Government in Consultation with the devolved administrations: CM 5049, February.
- 238 (2001) *The Interim Response to the Report of the BSE Inquiry*. HM Government in consultation with the devolved administrations, February, CM 5049.
- 239 (1996) Microbial Risk Assessment: An interim Report. June. HMSO.
- 240 Kennedy, A. Position paper. Paratuberculosis Awareness and Research Association, www.crohns.org.uk
- 241 Stronkhorst AS, Tytgat GJH, van Deventer SJH. Immunotherapeutical approaches to Crohn’s disease in view of a possible pathogenic role of Mycobacterial antigens [from Alan Lancaster web site].
- 242 Chiodini RJ and Rossiter CA (1996) Paratuberculosis : A potential Zoonosis? *Veterinary Clinics of North America: Food animal practice* **12** 457-467.