

***Mycobacterium paratuberculosis* - A Public Health Issue?**

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1 Abstract

This paper is part of the New Zealand Food Safety Authority's scientific evaluation of foodborne hazards. The focus is the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP), which has been reported as a possible food contaminant, particularly in dairy products. Despite there being no general evidence that MAP causes human foodborne disease, it has been suggested it has a role in Crohn's disease, a chronic inflammatory condition of the lower bowel. Parallels are drawn with Johne's disease in livestock, a condition accepted as being caused by MAP.

Key elements of the ongoing debate on the role of MAP in Crohn's disease are presented. There are summaries of 12 reviews which have been published over the past five years. Background information on MAP and Crohn's and Johne's disease are also included.

There is general agreement that there is insufficient evidence to conclude that MAP is the cause of Crohn's disease.

Data on Johne's and Crohn's diseases are not consistent with a simple cause and effect relationship, as is seen, for example, with brucellosis in cattle and undulant fever in humans.

An association between the isolation of MAP and patients with typical Crohn's disease has been demonstrated. However, this is not a consistent finding and MAP or evidence of MAP has been reported in people not suffering from Crohn's disease. It is possible that MAP is an opportunistic invader, similar to other bowel microflora such as *E.coli*.

Most authorities consider that Crohn's disease is not a single disease entity. There is good evidence that the underlying problem in Crohn's is a dysfunctional immune process in the alimentary tract, and that there is an inherited predisposition for this.

2 Introduction

The aim of the New Zealand Food Safety Authority is to base all activities on sound science. A 'Risk Management Framework' has been established; this entails a systematic evaluation of risks, assessment of management options, implementation of management decisions and follow-up monitoring and reviews [1].

During the past decade there have been reports of contamination of food, especially dairy products, with the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Although there is

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no general evidence of human foodborne disease, for almost a century questions have been asked about the possible role MAP in diseases of the bowel in man. A particular issue has been Crohn's disease, a chronic inflammatory condition generally affecting the lower sections of the small intestine and the colon. The primary impetus for this has been apparent similarities between Crohn's disease in humans and Johne's disease in ruminants, the latter being a condition caused by infection with MAP.

The purpose of this paper is to draw together the key elements of this ongoing debate on the possible importance of MAP as a pathogen in humans and thus allow a considered judgment of this issue. To set the scene, there are short descriptions of MAP, Crohn's disease and Johne's disease. There is then a review of recent papers by food safety regulatory bodies and others on the MAP/Crohn's disease controversy. NZFSA's conclusions follow.

3 The Organism [2, 3]

The mycobacteria are a large, varied group of organisms, which include some significant pathogens (such as *M.tuberculosis*, *M.bovis*, and *M.leprae*) and many free-living non-pathogens. An important sub-group that can survive outside an animal host is the *M.avium* complex; MAP falls within this group.

Like the other mycobacteria, a feature of MAP is a thick complex cell wall, relatively impermeable and rich in lipids. This confers acid-fast properties and may enhance its survival in the environment. Another 'cell wall deficient form', termed a spheroplast, is also postulated. Various research groups have claimed that this is the reason why MAP can be cultured, or MAP DNA demonstrated, in bowel samples from Crohn's disease patients but where no acid fast bacilli, as in Johne's disease, can be seen. It is also claimed that spheroplasts are a key component of the pathogenesis of Crohn's disease, in that they may persist in the body for longer than parent forms, have poor chemotactic activity and resist killing by phagocytosis to a greater extent. However, whether or not this alternative form of MAP exists and, if it does, that it is at the centre of the pathogenesis of Crohn's and Johne's disease, is controversial.

Although MAP is considered an obligatory parasite, it can survive outside the animal for prolonged periods (6 to 12 months in moist, pH neutral conditions). This has led some to argue that MAP can be carried from livestock and wildlife 'reservoirs' into drinking water systems and thus expose human populations, including in cities, at some distance [4].

An important tool for investigating the role of MAP was the discovery of unique IS900 DNA insertion elements in the genome. To date no other useful diagnostic sequences or antigens have been extensively evaluated. However, IS900 amplification as a diagnostic assay is not without problems. The presence in other mycobacteria of insertion elements with similar DNA sequences has complicated diagnosis by PCR. Thus add-on techniques, such as the analysis of PCR products, are sometimes used to check for false-positive results [5].

Two major strains of MAP are recognised, the so-called sheep and cattle strains. As the name implies, there is a high degree of host preference. Characteristic differences in genome of these strains have been demonstrated [6].

4 Johne's disease

4.1 General Description [7-9]

Johne's disease is a chronic digestive disorder of domestic ruminants and some wildlife. The disease is characterised by a chronic enteritis that provokes a progressive weight loss so severe that the affected animal commonly dies. It was first described in Germany in 1895 and the causative organism, MAP, was isolated in 1910.

Major features of MAP infection or 'paratuberculosis', as it is commonly called, in ruminants are that they are very long standing, often with no clinical signs of disease over the initial stages after infection and there is a marked variation in the number of organisms present in lesions. The typical picture of an advanced clinical case in ruminants is one of a granulomatous enteritis, principally in the lower small intestine and its draining lymph nodes. Clarke and Little [10] have described 'multibacillary' and 'paucibacillary' forms of clinical Johne's disease in sheep, related to a high or a low degree of mycobacterial colonisation. In the multibacillary form of Johne's disease, large numbers of acid-fast staining bacilli are present in macrophages in the granulomas. When appropriate bacterial culture procedures are applied, MAP can invariably be isolated from affected tissues; however, the primary isolation of the sheep strain is very difficult.

Infected animals can be categorized into groups according to the severity of clinical signs, degree of faecal shedding of MAP and the presence and type of immune response. Although large numbers of organisms can be shed in clinically normal animals, very high levels are generally not seen until Johne's disease is apparent.

Transmission is via ingestion of MAP, the main source being faecally contaminated colostrum, milk, water, feeds, and surfaces. In cattle it has also been shown that a proportion of advanced cases may also shed small numbers of organisms in milk [11]. There is a considerable degree of age resistance, so to develop clinical disease the animal must be infected early in life.

For many years Johne's disease was thought to be solely a disease of ruminants. It is now apparent that MAP has a relatively broad host range that includes monogastric animals as well as ruminants. Naturally occurring cases of MAP infections have been reported in rabbits in Scotland and ferrets in New Zealand [12, 13].

Paratuberculosis has been reported in livestock in many countries. The World Organisation for Animal Health (Office International des Épidémiologies, OIE) records show that 44% (i.e. 86/195) of member countries reported paratuberculosis during the past decade. Many indicated that the disease is limited to specific zones; this may be an association with dairy farming. Under-reporting is a widely recognised problem due to the subclinical nature of MAP infection, and to the poor accuracy of many diagnostic tests. The Food Safety Authority of Ireland saw this as a major impediment to future studies of the link between Johne's disease in livestock and Crohn's in humans [14].

4.2 Johne's disease in New Zealand [7]

Johne's disease was first diagnosed in New Zealand in cattle in 1912. It was not reported again until 1928. Thereafter there were steady reports of new infected herds, especially of dairy herds in the Waikato and Taranaki districts. Reports of the national veterinary diagnostic service immediately after World War II confirm this situation. A 'compensation scheme' had been introduced for the owners of affected cattle and 'reported cases' of Johne's disease rose sharply. In 1953 there were 513 cases. In the 1980s, a survey of culled dairy cows was conducted; 5.6% from Taranaki and 0.9% from Wanganui/Manawatu were found to be infected [15].

In a 1986 conference contribution, Milestone reported field data suggesting that 31% of dairy herds in Taranaki were infected [15].

Every Ministry of Agriculture and Fisheries (MAF) office was required to keep a register of Johne's disease infected herds and flocks. In 1990 there were 1,232 cattle herds registered. However, the author of this report commented that this was considered to be just the 'tip of the iceberg' and the true figure was thought to be three to four times higher [16]. Of interest is the high prevalence in some districts, 19% on the West Coast and 14% in Taranaki.

A study of the within-herd prevalence of Johne's disease was conducted in the mid 1980s. Among other activities, faeces from all cattle in six dairy herds were cultured. The mean percent positive was 11.8% (range 6.9% to 18.2%) [15].

Johne's disease in sheep was first reported in 1952, but there is strong evidence that it had been present on other properties for up to 30 years before this. Over the next 40 years there was a steady flow of notifications of new infected flocks. In 1986 Gumbrell [17] estimated 10% of South Island flocks and 0.8% of North Island flocks were affected, with infection continuing to spread to other flocks. He states that the prevalence with flocks varies, but 'levels up to 1.6% have been recorded'.

Over the past 25 years Johne's disease has been reported in deer in Europe, North America, Australia and New Zealand. As with cattle and sheep, in New Zealand there has been a steady flow of reports of newly uncovered infection. By 2003, 300 infected properties had been identified; a prevalence of approximately 6% and again this was considered an underestimate [18].

Two clinical syndromes have been recognised in farmed red deer in New Zealand [18]. The first is similar to Johne's disease in cattle and sheep: sporadic adult cases of chronic wasting with terminal diarrhoea. The second is in young animals and may involve up to 20% of the group. The course of the clinical phase (i.e. poor growth rates, wasting and diarrhoea) can be relatively short. Of much importance in deer is the similarity between the abdominal and, less commonly, retropharyngeal lesions caused by *M.bovis* and MAP. In MAP lesions, in most cases, there are numerous acid-fast organisms present.

Johne's disease also occurs in goats and has been confirmed in New Zealand in both milking and fibre-producing flocks [19]. Goats are susceptible to both the cattle and sheep MAP strains.

5 MAP in food

5.1 Dairy products

During the past decade the issue of MAP in dairy products, especially milk, has generated much debate [3, 20]. It is generally accepted that this arises from faecal contamination. Shedding by infected cows has been demonstrated but is not considered to contribute significantly.

Early studies were directed at investigating the presence in raw and pasteurized milk of MAP DNA fragments using PCR technology. Following on from this, milk samples were cultured. In the UK Irene Grant has been very active in this field. In 2002, the results of an extensive survey were published: MAP was cultured from 1.6% and 1.8% of raw and pasteurised milks respectively. The authors concluded that viable MAP is occasionally present in commercially pasteurised cows' milk in the UK [21].

There have also been studies and discussion about the efficacy of pasteurisation, including items such as laboratory simulation of commercial processes, standards, adherence to these standards and operational issues. In NZ, a pilot-scale pasteuriser operating under validated turbulent flow was developed. A greater than $7\log_{10}$ kill at 72° was demonstrated and it was concluded that 'in properly pasteurised commercial milk or in dairy products made from such milk, viable MAP micro-organisms are highly unlikely to be present' [22]. O'Reilly et al. reached a similar conclusion from a study of raw and pasteurised milk in Ireland [23]. They draw attention to their visits to processing plants during the study to ensure treatment standards were being met.

5.2 Meat products

The potential for meat to be contaminated with MAP, as a result of faecal contamination during slaughter or systemic foci consequent to a bacteraemia is noted by many authors; for example ground beef [26, 27]. With regard to the latter, some point to the report by Gwozdz et al of evidence of MAP DNA in the blood and/or liver of many sheep with advanced Johne's disease [24]. In a later experimental infection study this was demonstrated in a much smaller proportion of cases [25].

In Ireland, between June and November 2004, 114 beef mince samples were collected from a large mincing plant, which sources cattle less than 30 months of age reared nationwide. After decontamination the samples were cultured using BACTEC® 12B culture vials for a period of up to 14 weeks. No sample was found to contain MAP. The main conclusion of the work was that the presence of MAP in Irish beef typically obtained from young cattle was non-existent or very low. Therefore the exposure of the Irish consumer through this product was found to be negligible. However, further evaluation of the presence of MAP in beef originated from older cattle, imported beef and other meat products that might be carrying the bacterium are recommended (John Egan, *personal communication*).

No studies of carcase contamination with MAP appear to have been conducted.

6 MAP in river systems and water supplies

Surface waters contaminated by run-off from pastures grazed by animals with Johne's disease could theoretically enter water supplies being used to supply drinking water. Herman-Taylor [28] and others have drawn attention to possible widespread exposure of urban populations and, in fact, MAP has been recovered from the water supplying Los Angeles (quoted by Sartor (2005) [29]).

In and around Cardiff, UK a major study of MAP contamination of the River Taff has been undertaken. MAP was detected by IS900 PCR and culture. Parallel studies showed MAP remained culturable in lake water for 2 to 3 years. A hypothesis relating to exposure of parts of Cardiff by contaminated aerosols from the river and this leading to clusters of Crohn's disease in the human population is postulated [30].

7 Crohn's disease

7.1 General description [2, 3, 31, 32]

What is now known as Crohn's disease was first reported in 1913 by Dalziel from Glasgow, UK. He proposed MAP as the agent. In 1932 Crohn and colleagues described a series of regional ileitis cases and thus the disease acquired its name.

Crohn's disease is an 'inflammatory bowel disease' (IBD), most commonly affecting the small and large intestine. It causes the gut wall to become thickened and inflamed and may lead to narrowing of the lumen sufficient to cause obstruction of flow of intestinal contents. The inflammation can progress to ulceration, fistula formation and perforation. Anaemia and arthritis are associated pathologies. Diagnosis presents challenges, especially distinguishing it from other IBDs such as ulcerative colitis. A discriminate analysis has found Crohn's disease to be a heterogeneous condition while ulcerative colitis is more homogeneous. There is no cure presently. The disease follows an unpredictable course, exemplified by periods of activity interspersed with remissions. Therapies have concentrated mainly on the initiation or maintenance of remissions; onsets of relapses seem associated with dietary factors and stress.

A consistent finding in early studies of Crohn's disease was the association between high socio-economic status and increased risk. Recent studies in high incidence areas have failed to demonstrate this. However, socio-economic status is not a biologic exposure as such. It represents differences in factors such as diet, crowding and hygiene, which change over time in a society. Ethnicity also may represent similar factors. Cigarette smoking increases the risk. Many new materials or activities introduced into human existence have been postulated as important in the cause of inflammatory bowel disease. They include toothpaste, chewing gum, fast food, refined breakfast foods, food refrigeration and sedentary, white-collar, indoor employment. The studies examining these hypotheses have used small numbers of cases and have had methodological problems; at best they demonstrate association, not cause. A positive association has been shown between Crohn's disease and oral contraception use but the attributable fraction is small, indicating that a promoter rather than initiator role is more likely.

Genetic studies have provided the foundation for major advances in the understanding of Crohn's disease. They have also provided an insight into the possible role microbes may play in the pathogenesis of this disease. Genetic linkage studies led to the identification of the NOD2 gene (now called CARD15) as a susceptibility gene for Crohn's disease [33]. NOD2 is one of a newly discovered class of intracellularly acting molecules that bind to bacterial cell walls and, after a number of intermediate steps, results in the production of a number of important biologically active chemicals that are known to play an important role in the pathogenesis of Crohn's disease.

There have been a range of proposed bacterial and viral aetiologies of Crohn's disease. In addition to mycobacteria they include *Escherichia coli*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Yersinia* spp, *Streptococcus* species, measles, cytomegalovirus, and Epstein-Barr virus.

Over recent years there have been a number of reports of MAP being cultured or the unique MAP DNA fragment demonstrated in tissues from Crohn's patients; for example Bull et al (2003) [5] Naser et al. (2004) [34], Autschbach et al. [35] and in New Zealand Geary et al (2005) [36]. There is a statistical association but whether this is causal or not is at the heart of the controversy.

Incidence rates for Crohn's disease have been published by a number of reliable overseas agencies. In northern Europe it is reported as 7/100,000/year, in southern Europe 4/100,000/year, with an overall European figure of 5.6/100,000/year (2). In the UK, an annual incidence in the range of 6 to 13 per 100,000 is quoted [20]. This group also estimates the current prevalence to be between 67/100,000 and 133/100,000; i.e. approximately 1 in 1,200 are affected. In Denmark, an analysis of the 'National Registry of Patients' led to an estimated incidence of 4.6 (5.4 for women and 3.7 for men) /100,000/year [37]. In the United States, data collected in 1998/1999 in a telephone survey suggested a prevalence of 150/100,000 [38]. An incidence in the range of 4 to 6/100,000/year is quoted by another reviewer [39]. In this paper the author also quotes rates from intermediate (approximately 2/100,000/year) areas and a low incidence area approximately 1/10th of this (southern Israel / South Africa and Spain respectively).

7.2 Crohn's disease in New Zealand

As in many countries, the epidemiology of Crohn's disease in New Zealand is poorly documented. What reports there are must be viewed with caution, recognising that it is a clinical entity and thus open to a degree of interpretation. This was demonstrated in a retrospective study in Auckland, where 19% of the putative Crohn's cases were rejected as failing to meet the investigators' case definition [40]. There is also an issue of how cases are counted, with potential confusion, over incident (new) cases over a given period and prevalent (new and old) cases at any time.

In 1971, Couchman and Wigley published [41] an analysis of hospital admissions data for 1950 to 1966. It would appear that this design was open to individuals with chronic debilitating conditions, such as Crohn's disease, being counted many times. They also relied on the diagnosis at admission. This may account, with reference to later New Zealand data and overseas reports, for the relatively high 'rates' per annum of 119/100,000 in urban populations and 59/100,000 in rural populations. There were 16 cases among Maori, leading to an estimated 'prevalence' of 53/100,000.

A more intensive retrospective study was conducted later in Auckland [40]. A case definition was established and the case notes of all patients with ulcerative colitis/proctitis, Crohn's disease and non-specific inflammatory bowel disease who were outpatients or inpatients of public hospitals from 1969 to 1978 were reviewed. 'New' and 'old' cases were identified. The estimated incidence

of Crohn's disease within the Auckland region was 1.7/100,000/year. Of considerable interest: there were no cases of Polynesian origin (both Maori and Pacific Islanders). Age at onset was similar to that reported overseas, with 50% being between 10 and 29 years old. The authors concluded that 'their data emphasized the extreme infrequency of inflammatory bowel disease among Polynesians and the low overall incidence of Crohn's disease in New Zealand'.

Recently an investigation of inflammatory bowel disease, including Crohn's disease, has begun in the Canterbury district. Preliminary case data suggests an incidence of Crohn's disease of around 5/100,000/year for the period 2001 to 2004. Further, that the incidence has steadily increased, of an order of five or more, over the past 30 years (Gearry, Richard; *personal communication*).

In 2005 this group reported what they believed were spheroplastic phase forms of MAP within macrophages taken from Crohn's patients [36].

8 Reviews of the linkage between MAP and Crohn's disease

This has been a prominent issue during the past decade. Regulatory agencies, research groups and individuals have published on the subject.

8.1 European Commission, 2000 [2]

This comprehensive report of the 'Scientific Committee on Animal Health and Welfare' covers the microbiology, pathology, epidemiology and diagnosis of Johne's and Crohn's diseases. The potential for exposure via milk and drinking water is also reviewed. There are references to 372 scientific papers and articles.

The authors reached two main conclusions. First, the currently evidence is insufficient to confirm or disprove that MAP is a causative agent of at least some cases of Crohn's disease. Second, there are sufficient grounds for concern to warrant increased and urgent research activity to resolve the issue.

A number of supporting conclusions are also included, as follows:

- Crohn's disease is most likely a multifactorial condition
- The incidence is more common in the developed world, in families where there have been other cases and in homes where hygiene in early life has been good
- Although there are similarities between Johne's and Crohn's diseases, there are also some significant differences
- MAP is a relatively common environment contaminant and its association with Crohn's disease may be as a causative agent, pathogenic secondary invader, or non-pathogenic coloniser of changed bowel conditions
- The public could be exposed directly via contact with infected animals or unpasteurised milk. Other possible lower probability sources are pasteurised milk and infected wildlife
- A simple relationship between exposure and development of Crohn's disease does not exist
- If MAP is involved in the causation of Crohn's disease, other factors are required
- Results from drug trials in humans with drugs likely to be active against MAP are encouraging but inconclusive

8.2 Food Safety Authority of Ireland, 2000 [14]

This is in the form of a report of the 'Microbiology Sub-Committee' to the question 'Whether or not MAP contributes to Crohn's disease'. Their simple answer to this was 'inconclusive'.

They note that although the human population may be exposed to MAP via infected animals and milk, 'causation', or even that MAP contributes to the disease processes in Crohn's, has not been established, despite the reported statistical associations.

They consider that in future studies of Johne's and Crohn's disease linkages, an important limiting factor will be the poor accuracy of diagnostic tests for Johne's disease in cattle and other animals.

Despite this, the sub-committee concluded that it was reasonable to prohibit the use of milk from Johne's disease clinical cases. Further, that raw milk from farms where Johne's disease is present should not be used for human consumption or for making cheese; it should first be pasteurised.

8.3 UK Food Standards Agency, 2002 [3]

This report by Dr E Rubery is of a similar scope as that commissioned by the EU. It covers, in some detail, the microbiology, pathology and epidemiology of Crohn's disease, MAP, and Johne's disease. There are 242 referenced articles and during the study 20 experts were consulted and/or reviewed the report.

Dr Rubery concludes that the link between MAP and Crohn's disease has been extensively investigated, but there continues to be insufficient evidence for a link. She adds "it is clear that if MAP is causally linked to Crohn's disease it forms only one strand in the picture: genetic and immunological factors also playing a significant part".

As in the other reports, the possibility of exposure via milk is noted. In line with a 'precautionary approach to food safety', she says that the UK Food Safety Agency is working with industry to improve the efficacy of the pasteurisation process with respect to MAP.

8.4 Food Standards Australia New Zealand (2004) [26]

This review concludes that Crohn's disease is a multifactorial disease or syndrome, with no one etiological factor appearing to dominate. At present there is insufficient scientific evidence to prove or disprove a conclusive link between Johne's disease in ruminants and some cases of Crohn's disease in humans.

8.5 Other reviews

8.5.1 Hermon-Taylor (2001) [28]

This paper develops a case for MAP being the root cause of Crohn's disease.

It sets a scene of widespread sub-clinical Johne's disease of domestic animals (possibly induced by animal culling practices) and a MAP wildlife reservoir. This, in his view, has led to human populations being exposed via milk and other dairy products, and possibly by raw and processed meats. An additional pathway via drinking water supplies is also argued.

A prerequisite for individuals developing Crohn's disease is 'a particular inherited or acquired susceptibility'. Drawing on the established pathogenesis of Johne's disease, Hermon-Taylor proposes that MAP initiates and then sustains an inflammatory response in man.

Reasons for the failure to demonstrate consistently MAP association with Crohn's disease are discussed.

The review concludes with a statement that 'proof' of concept may be difficult to achieve, but if the scenario portrayed is correct, then a 'public health issue of substantial proportions for which a range of remedial measures is needed'.

8.5.2 Harris & Lammerding (2001) [42]

This is a comprehensive review of the literature by two members of the 'Microbial Food Safety Risk Assessment Unit' of 'Health Canada'.

Their overall conclusion is there is insufficient evidence to implicate any one factor, including MAP, as the definitive cause of Crohn's disease.

They list a number of key issues that should be considered in this debate, including:

- Strong evidence of a genetic predisposition to developing Crohn's disease
- An active immune system is necessary for Crohn's disease to develop
- In most cases of Crohn's disease, it is impossible to determine which came first, the microbial infection or the disease manifestations
- Crohn's disease diagnosis is based on the observed enteric pathology
- There are higher incidence rates of Crohn's disease in urban than (exposed) rural populations

8.5.3 Chamberlin et al (2001) [43]

The premise put forward in this review is that a large proportion of Crohn's disease cases (perhaps of the order of 40%) arise from oral infection with MAP followed, in genetically predisposed individuals, by a dysfunctional immune response. In their view 'the mycobacterial theory and the autoimmune theory are complementary: the first deals with the aetiology of the disorder, the second deals with its pathogenesis'.

The key issues they raise include:

- The role of the cell wall deficit MAP forms in the pathogenesis of MAP
- Culture of MAP from the milk of some lactating mothers with Crohn's disease, underscoring the similarities between Crohn's disease and Johne's disease
- There may be different forms of Crohn's disease not triggered by MAP, and this would explain the inconsistent outcomes from anti-mycobacterial therapy

- Possible exposure via dairy products

8.5.4 Davies (2002) [44]

This review from the EpiCentre, Massey University, New Zealand, concludes that the ‘basis for concluding that MAP has a causal role in the aetiology of Crohn’s disease is unconvincing’.

It approaches the issue along six lines: the diagnosis of Crohn’s disease, the aetiology of Crohn’s disease, the comparative pathology of Crohn’s disease and other MAP conditions, the presence of MAP in Crohn’s disease lesions, the immune responses to MAP in Crohn’s disease and, finally, responses to therapy.

Some key points raised include:

- The uncertainty in the case definition of Crohn’s disease and that considerable heterogeneity among Crohn’s disease cases is recognised
- It is commonly accepted that Crohn’s disease arises from chronic inflammation resulting from an interaction of the persistent stimulus of microbial antigens with genetically determined host susceptibility factors that determine the individual’s immune response or mucosal barrier function, and there is little evidence to claim that MAP have more significance than ubiquitous intestinal microflora
- There are marked differences between the pathology of Crohn’s disease and Johne’s disease
- Concerning evidence of MAP in Crohn’s disease lesions, the possibility remains that this is opportunistic colonisation of diseased tissues rather than a causal role of MAP
- The immune response in Crohn’s disease does not support the MAP causal hypothesis.
- Reported response to therapy neither supports nor refutes involvement of MAP in Crohn’s disease

8.5.5 Shanahan (2002) [45]

The main focus of this review is the possible immune basis of Crohn’s disease. However, a case is briefly put against Crohn’s disease being a specific infection with MAP. He considers it difficult to reconcile the therapeutic efficacy of anti-tumour necrosis factor with such an intracellular infectious cause. Although blockage of this factor is a substantial risk for active and disseminate *M. tuberculosis* infection, disseminate MAP has not been reported in patients so treated, even in those with metastatic or extra-intestinal Crohn’s disease.

8.5.6 Greenstein (2003) [46]

Greenstein argues for the case that MAP, like *M. tuberculosis*, is an effective, insidious and important pathogen of humans.

The case is put as follows:

- There is ample opportunity for exposure (i.e. dairy products, wildlife, water supplies)
- MAP has been shown to be a human pathogen (i.e. two cases, one an AIDS patient and the other a boy of five years)

- The difficulties demonstrating MAP in Crohn's disease cases is related to cell wall deficient forms and problems of culturing MAP
- MAP specific DNA and RNA have been associated with Crohn's disease
- There are analogies between the pathology of Crohn's disease and other conditions accepted as being caused by infection with *Mycobacteria* species (i.e. leprosy and Johne's disease)
- The good results of antibiotic therapy using drugs that have activity against *M. avium*.

He also states that the presence of a gene that is associated with an increased susceptibility to develop a disease does not preclude the possibility that the disease may be infectious in aetiology.

He does note the paradox, assuming an infectious aetiology, that immune modulation therapy may lead to clinical improvement.

8.5.7 UK National Association for Colitis and Crohn's (2003) [20]

This is a report of the 'Expert Review Group' to the National Association for Colitis and Crohn's (NACC). It was commissioned to review published evidence for a link between MAP and Crohn's disease, to consider the likelihood of MAP being a cause of Crohn's disease and to consider what research should be undertaken to answer the question of the relationship of Crohn's disease and MAP definitively.

The group concluded that a causative link between MAP and Crohn's disease is unproven.

They also commented as follows:

- If MAP is involved in the aetiology, it is not acting as a conventional infective agent
- MAP is present in the bowels of some Crohn's disease cases and a lower proportion of non-Crohn's disease controls, but whether this is cause or consequence has yet to be established
- The hypothesis that Crohn's disease is a collection of different conditions with different aetiologies, either genetic or environmental, is likely to be correct
- Despite these conclusions, the group welcomed efforts to reduce exposure of MAP via milk.
- The way forward should be well designed epidemiological studies
- Further key evidence concerning MAP involvement would come from the results of targeted therapy

8.5.8 Bannantine et al (2004) [47]

While this review primarily addresses the potential for new diagnostic and investigative tools to be developed from the knowledge of the MAP genome, the authors draw attention to reports of mycobacteria being commonly found in the human intestine. Further, they reference (10) clinical studies that have demonstrated the presence of several different species of mycobacteria in intestinal biopsy tissues from Crohn's disease patients.

8.5.9 Sarton (2005) [29]

After reviewing data showing an association between MAP and Crohn's lesions, Sarton comments that the results are consistent with two possibilities: either MAP infection could cause Crohn's disease in a subset of patients that are either selectively exposed to this organism or who are

genetically susceptible to infection or, alternatively, this relatively common dietary organism may selectively colonise (or a dead organism selectively lodge in) the ulcerated mucosa of Crohn's disease patients but not initiate or perpetuate intestinal inflammation.

He notes that Crohn's disease certainly has environmental and host genetic influences that interact to cause clinically evident disease.

His conclusions are that well designed clinical, microbiological and mechanistic experiments are urgently needed to definitely settle this still unresolved debate. We need to determine if clearance of MAP selectively changes the natural history of disease in an infected subset of patients.

8.5.10 Grant (2005) [27]

This is a comprehensive 11 page review which cross references and updates many of the major reviews listed above.

Key points are as follows:

- There is prolonged environmental survival in soil and water, but MAP is unable to multiply outside the host.
- In humans genetic mutations are associated with susceptibility to Crohn's disease.
- There are reports of successful treatment of some Crohn's patients with antibiotics which are active against MAP.
- That the majority of studies of pasteurization show that MAP is more heat-resistant than other mycobacteria and that low numbers of viable MAP may survive occasionally.

A case is described of persistently active Crohn's which is the first documented report of the coexistence of MAP disease and a permissive *NOD2/Card15* mutant.

She concludes that MAP has not been unequivocally established as a cause of significant human disease, but some kind of association, not necessarily causal, exists.

9 Discussion

A decade after coming to prominence in public health, there is still general agreement, and this includes many of those arguing for a role of MAP in Crohn's disease, that an accepted level of proof of causation has not been met. We concur with this, for the following reasons.

Of much importance is the situation that exposure to MAP appears not to be correlated with the incidence of Crohn's disease. For example, in New Zealand the data on Johne's disease in the 30s, 40s and 50s indicates that a high proportion of the then considerable rural population was exposed to MAP, but there is good evidence, despite difficulties of diagnosis, that Crohn's is a disease of the developed, hygiene-conscious world. Likewise, groups most exposed to MAP, such as dairy farmers and large animal veterinarians, have not been shown to have higher rates. Internationally, it appears that the incidence of Crohn's disease in the developed world is around 3 to 6 per 100,000 per year, and is not related to the Johne's disease status of livestock.

The issue of exposure to MAP preceding the development of Crohn's has not been satisfactorily addressed. The proponents might argue that a long incubation period and frequent exposure to MAP would make a temporal relation difficult to establish. However, this has not been a barrier to the understanding of many other diseases.

The lack of consistency in reports of MAP in Crohn's cases also points away from a simple cause-effect relationship. However, if MAP is as ubiquitous as suggested it is unlikely that the question of causation will be satisfactorily resolved by the methods used to date. The report by Ryan et al. (2004) [48] of *E.coli* in Crohn's lesions illustrates this problem; i.e it is possible that MAP is an opportunistic invader, similar to other bowel microflora. An understanding of the cellular and molecular processes involved would seem to be a more productive way forward.

There is also the unique pathology of Crohn's disease. A commonly stated opinion is that Crohn's disease is not a single disease entity. It is a clinical syndrome that potentially could arise from many different disease processes. The simple premise that Crohn's disease is the human equivalent of Johne's disease is not supported widely. There is general acceptance that the underlying problem in Crohn's is a dysfunctional immune process in the alimentary tract, and that there is an inherited predisposition for this. The key question in this debate is whether or not this is MAP dependent or not.

Considering the epidemiology of Johne's disease, the issue of the establishment of MAP infection in the neonate, a significant epidemiological factor, is not discussed by the advocates of the MAP/Crohn's hypothesis.

Behind a lot of this literature is the premise that MAP is an obligate pathogen of some animal species, and, from a public health perspective, they are 'potentially' a classical reservoir host. A parallel is brucellosis in cattle and goats resulting, directly or indirectly, in undulant fever in humans. Although there are claims that MAP is a common environmental contaminant, whether or not this depends on the presence of infected livestock is not clear. There are many uncertainties with respect to our understanding of MAP in the environment.

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