

ASSOCIATION BETWEEN JOHNE'S DISEASE AND CROHN'S DISEASE

A Microbiological Review

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Association between Johne's Disease and Crohn's Disease

A Microbiological Review

There is increasing scientific interest as to the aetiology of Crohn's disease, a chronic, inflammatory bowel disease of humans. Recently, research has focused on the relationship between Crohn's disease in humans and Johne's disease in ruminants. This review will briefly outline the two diseases, with particular focus on the situation in Australia, prior to discussing any potential link.

JOHNE'S DISEASE

Johne's disease is a chronic, contagious, granulomatous, inflammatory disease of ruminants (reviewed by Linnabary et al. 2001). Other animals, such as alpacas, rabbits, foxes, stoats and weasels can also be infected. This review will focus on Johne's disease in cattle.

The main clinical sign of Johne's disease is weight loss, which often progresses to emaciation and death. Diarrhoea is also frequently observed in infected cattle.

The causative agent of Johne's disease is *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (discussed in Section 3). MAP is excreted primarily in the faeces of infected animals and is excreted during both the sub-clinical and clinical stages of disease. MAP can be transmitted both vertically through the placenta to the foetus in advanced infection and also through the calf ingesting colostrum, milk or faeces from an infected animal. MAP is also transmitted horizontally through the faecal-oral route. Young animals are most susceptible to MAP infection (Morgan 1987).

There are two main phenotypes of Johne's disease (reviewed by EC 2000). The first of these is termed the multibacillary form. This is the most common form of disease in cattle and is characterised by numerous acid-fast MAP (MAP with intact cell walls) in the intestinal mucosa. There is a strong antibody response in animals exhibiting this type of disease, but weak or absent cell-mediated response. The second form of Johne's disease, the paucibacillary form, is found more often in small ruminants (e.g. sheep), where it constitutes approximately 30% of cases. Acid-fast MAP are sparse or absent in the mucosal lesions and there is a strong cell-mediated immune response with poor or absent humoral response.

The incubation period for Johne's disease is typically 2-6 years, but many infected cattle do not develop clinical signs during their lifetime. As clinical signs are not observed immediately after infection it is often difficult to control the disease without extensive active surveillance (reviewed by Rubery 2002). Furthermore, MAP can contaminate grazing pastures, water and feed and can survive in favourable environments for a year and possibly longer (reviewed by Rubery 2002). The hardiness of MAP in the environment will complicate disease control measures. Wild animals, such as rabbits, may also act as a reservoir for MAP (Dixon 2002). It is

possible that these animals may introduce or re-introduce MAP into healthy herds by shedding viable¹ MAP through their faeces or urine, thereby contaminating grazing pastures. However, the ability of rabbits and other wild animals to act as a reservoir for MAP and the importance of such a reservoir in the epidemiology of infected farm animals has not been determined. Investigations in Australia have not found evidence of infection in rabbits.

Reliable estimates of the prevalence of Johne's disease are few. Dairy cattle herds have higher incidences of Johne's disease than beef cattle herds due to the intensive husbandry practices used in the dairy industry (Clarke 1997). In dairy herds, prevalence estimates have ranged from 11-17% in the UK (reviewed by EC 2000) to 55% in the Netherlands (Muskens et al. 2000). In Australia, there were 1,328 cattle herds, 659 sheep flocks, 26 goat herds, 10 deer herds and 2 alpaca herds officially classified as infected with Johne's disease as of June 2004 (AHA 2004). The distribution of Johne's disease in Australia is not uniform. It is endemic in the South-Eastern states but Western Australia is considered to be free of the disease.

There is no satisfactory treatment for Johne's disease. Although a variety of antimicrobials have been tried it is not considered a long-term option due to the high costs that will be associated with treating entire herds for prolonged periods (reviewed by EC 2000). A vaccine for Johne's disease is available but the effectiveness of this in eliminating the disease in the field is unclear (reviewed by EC 2000). However, vaccination trials in sheep undertaken recently in Australia indicate that the vaccine does assist in controlling Johne's disease in these animals. Vaccination will also potentially impact on disease surveillance, as vaccinated animals will be seropositive.

The interest in Johne's disease has risen in recent years with the increased debate on whether Johne's disease in cattle is associated with Crohn's disease in humans. Appendix 1 describes the current national approach in Australia to Johne's disease in animals and the current international opinion on the link between Crohn's and Johne's disease.

CROHN'S DISEASE

Crohn's disease is a chronic, granulomatous inflammatory disease of humans, which primarily affects the terminal ileum and colon (reviewed by EC 2000; Rubery 2002). Crohn's disease can be differentiated from other inflammatory bowel disorders by the involvement of all layers of the intestinal wall in pathology. The disease is characterised by periods of activity interspersed with periods of remission.

The clinical signs of Crohn's disease include weight loss, abdominal pain, diarrhoea, reduced appetite and fatigue. Crohn's disease has also been associated with arthritis, skin lesions, anaemia and, in the younger age group, reduced growth rate. Patients with Crohn's disease can have their quality of life severely compromised and have to live with the constant threat of significant morbidity.

¹ Viable MAP are able to multiply and therefore be cultured.

Crohn's disease is a heterogeneous disorder with no one feature either present in all cases or absent in similar disorders (Jones et al. 1973; Spicer, Jones, & Jones 1973; Lennard-Jones 1996). However, there does appear to be three major disease syndromes: an inflammatory form, a stricturing form and a perforating form. It has been proposed that the non-perforating and perforating forms of Crohn's disease may have different causes (Greenstein & Greenstein 1995; Greenstein 2003) but more likely represent different host responses to a common stimulus.

The current estimated prevalence of Crohn's disease in Australia is 50 per 100,000 (estimated 1 per 1,000 in western countries world-wide) (Selby 2003). The incidence of Crohn's disease is highest in the 15-35 year age group, followed by the 55-65 year age group. Crohn's disease incidence appears to be increasing worldwide, however this may be due to more sensitive diagnostic measures and an increased awareness of the disease.

There is currently no cure for Crohn's disease (reviewed by Rubery 2002). Treatment methods include anti-inflammatory and/or immunosuppressive medications, medications to treat disease symptoms, antibiotics, diet changes and surgery. Surgery is a significant part of the treatment regime, with 75% of patients having one surgical procedure and 50% of patients having two surgical procedures during the progression of disease (Michener et al. 1990). The most common complication of Crohn's disease that requires surgery is bowel obstruction. Other indications for surgery include perforation of the intestine, significant intestinal bleeding, abscess and fistula formation, severe anal disease and persistence of the disease despite appropriate medical treatment. An Australian double blind antimicrobial trial for Crohn's disease patients will be completed in late 2004 (Selby et al. 2001).

The cost of Crohn's disease in Australia has not been estimated. In Sweden, Crohn's disease is estimated to have cost \$41.3 million in 1991 (Ekbom & Blomqvist 1997). Direct medical costs account for 26% of this with the remaining 74% associated with sick leave and early retirement. These estimates demonstrate the significant morbidity that patients' experience. In 1993 in the USA it was estimated that the average lifetime cost of Crohn's disease per patient was approximately \$US40,000 (Silverstein et al. 1999).

Proposed Causes of Crohn's Disease

Genetic Predisposition

It has been well documented that there is a genetic component associated with developing Crohn's disease (reviewed by Rubery 2002). Studies have indicated a polygenic pattern of disease, with some of the identified loci potentially involved in other inflammatory bowel disorders. Crohn's disease is associated with mutations in the NOD2 gene (chromosome 16), which regulates the activity of macrophages against bacterial pathogens (McGovern et al. 2001). Five additional loci have been implicated in susceptibility to Crohn's disease, but these have not been characterised.

Although a genetic component to Crohn's disease is well accepted, it is not considered the sole cause of disease and other initiating factors are sought.

Geographical and Environmental Factors

Geographical factors also significantly contribute to the incidence of Crohn's disease. For instance, Crohn's disease is more prevalent in populations living in the northern regions of Europe and North America than in southern Europe, Asia and Africa (Shivananda et al. 1996). In addition, people of Asian descent who migrate to western countries become as susceptible to Crohn's disease as the population of their host country (reviewed by Rubery 2002). Further investigation is required to determine the reasons for the discrepancies in the prevalence of Crohn's disease across a particular continent.

The geographical incidences of Crohn's disease do not correlate with that of Johne's disease. For instance, the incidence of Crohn's disease is high in parts of Scandinavia where Johne's disease is rare and appears to be similar in Western Australia to other southern Australian States, although WA is officially free of Johne's disease in cattle. There is also no evidence of the incidence of Crohn's disease being higher among demographic groups such as dairy farmers and veterinarians who would be expected to be more heavily exposed to MAP in animal faeces. In fact, Crohn's disease is more common in urban than in rural populations (reviewed by Rubery 2002).

Crohn's disease is more prevalent in people from homes where early hygiene, as measured by having hot water tap and separate bathroom, was good. An argument for the involvement of refrigeration as a risk factor has also recently been proposed (Hugot et al. 2003). Crohn's disease is also more prevalent in people who smoke cigarettes (reviewed by Rubery 2002).

These results indicate that the environment, potentially from early childhood, is an important etiological component for the development of Crohn's disease. The specific etiological components have not been identified but it has been suggested that they are infectious, chemical, physical and/or social factors.

Hypersensitive Inflammatory Response

Crohn's disease is characterized by extensive inflammation of the terminal ileum and colon (reviewed by EC 2000; Rubery 2002). Intestinal lesions are caused by constant stimulation of the mucosal and systemic immune systems that perpetuate the inflammatory cascade. The ileum in Crohn's disease patients is infiltrated with mononuclear inflammatory cells, macrophages and lymphocytes; key cells involved in the inflammatory response. This response may increase the permeability of the intestinal wall.

An increase in the permeability of the mucosal barrier of the intestines may cause a breakdown of tolerance of ubiquitous antigens (Sartor 1997; Soderholm et al. 1999), as it may enable excessive uptake of pro-inflammatory molecules and antigens, including those from normal intestinal flora. The increased intestinal permeability does not appear to have a genetic basis, and has been postulated to be a result of infection of microbial pathogen, or ingestion of a chemical toxin or metallic particulate (reviewed by EC 2000; Rubery 2002; Nazli et al. 2004). In Crohn's disease, production of anti-inflammatory cytokines is deficient or not sufficient to counter the pro-inflammatory response (Elson et al. 1995).

MYCOBACTERIUM PARATUBERCULOSIS

Appendices 2 and 3 list the arguments and experimental evidence for and against MAP as one etiological agent in Crohn's disease. MAP is a member of the *Mycobacteriaceae* family. Fifteen species of this family are known pathogens of man, including *M. tuberculosis*, *M. leprae*, *M. bovis*, *M. ulcerans* and *M. avium*. The disease caused by mycobacteria infection is generally characterised by an indolent course with chronic granulomatous lesions and long incubation periods (reviewed by Rubery 2002). The mycobacteria diseases are either of the pluribacillary or paucibacillary forms.

Twenty eight strains of MAP have been identified, which infect different animals (Pavlik et al. 1999). MAP is slow growing and dependent on an exogenous source of mycobactin, which is an iron-chelating compound, for *in vitro* growth (reviewed by EC 2000; Rubery 2002).

MAP infection is difficult to control as MAP is resistant to treatment with acid and alcoholic compounds. MAP is also not reproducibly eradicated by pasteurisation (Grant et al. 2001; Corti & Stephan 2002; Grant et al. 2002; Stephan, Buhler, & Corti 2002; Djonne et al. 2003) or by chlorination used in most water purification processes (Mishina et al. 1996).

Cell wall-deficient types of MAP exist, called spheroplasts (Ratnam & Chandrasekhar 1976; Hines & Styer 2003). Spheroplasts do not stain using acid-fast staining methods and hence their presence needs to be confirmed by other means, usually PCR of MAP-specific DNA sequences. Many studies employ methods to isolate MAP DNA from tissue samples that select for the vegetative form of MAP, thereby reducing the sensitivity of the diagnostic assay. This may cause significant underestimation of the number of patients with MAP infection. One study which found IS900-specific MAP DNA in tissue sections from patients with Crohn's disease failed to find the vegetative form by staining for MAP with intact cell walls (Sechi et al. 2004).

Once DNA has been successfully isolated, MAP can be confirmed by PCR amplification of MAP-specific sequences. Genes are highly conserved across mycobacteria species with only three sequences identified that are different between MAP and other mycobacteria species (reviewed by Rubery 2002). The most commonly used genetic sequence to differentiate MAP from other mycobacteria species is the insertion sequence IS900. MAP has 14-18 copies of IS900, which encodes a 43kDa DNA binding protein (p43). Recent evidence has shown that the primers used to amplify IS900 also amplify sequences in other mycobacteria (Bull et al. 2003). These mycobacteria species have the amplified sequences in low copy number and are rarely found in the environment. For this reason, it is now recommended that IS900 PCR product be subjected to restriction endonuclease analysis. However, this method has not been routinely used in the studies examining the potential association between MAP and Crohn's disease.

Some debate currently exists regarding the importance of PCR-positive results, as this detection method does not distinguish between dead and viable bacteria. The results of studies examining MAP in Crohn's disease patients by PCR and/or culture have

varied. Appendices 2 and 3 list the arguments and experimental evidence for and against MAP as one etiological agent in Crohn's disease.

It has been proposed that MAP may be transmitted to humans both through the food supply (milk and milk products, meat and meat products) and through the environment (water supply and direct contact with faeces from infected animals).

TRANSMISSION OF MAP TO HUMANS

Milk and Milk Products

MAP can be excreted into milk and colostrum in both clinically and sub-clinically infected cows. Raw milk may also be contaminated with faeces during the milking process (Clarke 1997). One study has estimated that the average faecal contamination of raw milk is 10 mg of faeces per litre of milk (reviewed by Rubery 2002). Faeces can contain at least 10^6 colony forming units per gram, indicating that faecal contamination of milk may be a critical point at which MAP can enter the food supply.

MAP has been shown to have greater heat tolerance than *M. bovis* and *Coxiella burnetii*, the zoonotic organisms that are the targets of the current milk pasteurisation methods (reviewed by Lund, Gould, & Rampling 2002). The resistance of MAP to heat and the potential transmission of MAP from cattle with Johne's disease to humans through milk and milk products has initiated a series of studies examining pasteurised milk for the presence of MAP. These will be described in the following sections.

Pasteurisation

The effects of pasteurisation on the viability of MAP in milk has been investigated using several pasteurisation methods; standard holder method, laboratory scale pasteurisers, double boilers and capillary tubes in water baths. The heat treatments used were either 63 °C for 30 min or 72 °C for 15 sec. Other experiments have used high temperature short time pasteurisation procedures and found that MAP did not survive but results have been questioned as the MAP inocula were frozen prior to use, which may affect viability. Pasteurisation experiments using different time and temperature combinations found that none of the combinations reliably achieved a complete sterilisation of milk (reviewed by Lund, Gould, & Rampling 2002).

Questions have been raised regarding the applicability of these experiments on industrial commercial pasteurisation methods, as they have been laboratory based and may not reflect conditions in commercial pasteurisation, especially with regard to turbulence. Studies that have tried to mimic commercial pasteurisation have not had consistent findings with viable MAP detected after pasteurisation in some cases but not in others (Hope, Tulk, & Condron 1996; Pearce et al. 2001; Gao et al. 2002; Grant, Ball, & Rowe 2002). However most studies indicated that pasteurisation did reduce viable MAP concentrations in milk by about 5-6 \log_{10} (Stabel et al. 2001).

Further experiments using more sensitive diagnostic methods and different samples and pasteurisation methods are being undertaken.

There have been a number of studies that indicate some MAP may be present in retail pasteurised milk samples. One study undertaken in the UK detected MAP by PCR in 7% of pasteurised retail milk samples (Millar et al. 1996). Of the contaminated samples, only half contained viable MAP. However, viable MAP was also isolated in 16% of the retail milk samples that were negative by PCR. A second study in Ontario detected MAP by PCR in 15% of retail milk samples (Gao et al. 2002). Test results from a proportion of the positive samples could not detect viable MAP. More recently, a study in the USA detected viable MAP in 2.8% of retail milk samples (University of Wisconsin 2004). These and other studies indicate that either some MAP do indeed survive commercial pasteurisation methods (Grant et al. 2001; Corti & Stephan 2002; Grant et al. 2002; Stephan, Buhler, & Corti 2002; Djonne, et al. 2003), that pasteurisation is not effectively done in some facilities, or that retail milk is contaminated after the pasteurisation process.

Unpasteurised Products

Investigation of the presence of MAP in unpasteurised milk has shown between 7.8% (Grant, Ball, & Rowe 2002) and 19.7% (Corti & Stephan 2002) of samples could contain MAP, when examined by PCR. However, when viable MAP was examined only 1.6% of samples tested positive (Grant, Ball, & Rowe 2002). Viable MAP was not examined in the Corti study.

Consumption of unpasteurised milk products represents a low proportion of total milk intake (reviewed by Rubery 2002), and so the extent these products could expose a population to MAP is limited. However, it could be hypothesised that unpasteurised products have a greater amount of viable MAP than pasteurised products, due to the absence of any inactivation process during the processing of unpasteurised milk. Following from this, the consumption of unpasteurised milk products may increase the exposure of a person to MAP, which may increase the risk of that person developing Crohn's disease. In spite of this, epidemiological data does not support this hypothesis as Crohn's disease is less common in areas where greater consumption of unpasteurised products is assumed, such as rural areas and developing countries.

Studies have also been undertaken examining MAP in raw milk and cheese products derived from unpasteurised milk (reviewed by EC 2000). These studies, although limited, have shown that the combination of temperature, salt, acids, and aging time are able to reduce viable MAP (Sung & Collins 2000; Spahr & Schafroth 2001).

Meat and Meat Products

Mycobacterium paratuberculosis, although primarily affecting the intestines, can be found systemically in animals with advanced infection (reviewed by EC 2000; Rubery 2002). In addition to the gastrointestinal system MAP has also been detected in milk, mammary glands, lymph nodes, lymph, reproductive organs and foetuses, semen and blood. MAP can be detected in these tissues in animals with advanced sub-clinical infection. Thus it is possible that meat obtained from both clinically infected and apparently 'healthy' animals can be contaminated with MAP. Meat could also be become contaminated with faecal material during slaughtering and processing procedures.

One study has detected MAP in the intestinal lymph nodes or faeces of 34% of 189 healthy thin dairy cows and from 3% of 350 healthy thin beef cows at slaughter (Rossiter & Henning 2001). *Mycobacterium paratuberculosis* was also isolated from 3% and 8% of dairy cows when the peripheral lymph nodes and liver were tested respectively and in one of the beef cows.

No information is available regarding the ability of MAP to survive meat cooking and meat processing methods. It is possible that contaminated meat that is processed at low temperatures or not cooked thoroughly may contain viable MAP. However, the significance of meat as a potential route of food-borne exposure to MAP relative to the potential exposure through milk is not known.

Water

It is possible that water sources may be contaminated with MAP through the excreta of infected animals (ruminant and non-ruminant). Water running off from grazing lands, or lands that have used manure from infected animals as fertiliser, may therefore contain viable MAP (Aronson et al. 1999; Grant et al. 2001; Whan et al. 2001).

MAP has been shown to be able to survive some community water treatment methods currently in place in the UK and USA (Mishina et al. 1996). Other *M. avium* species have been detected in potable water supplies and have been documented as a source of mycobacteria infection of immunocompromised hosts (Aronson et al. 1999). Thus it is possible that MAP is present in potable water supplies.

Wildlife

MAP is able to infect a wide variety of non-ruminant species, such as rabbits and mice (Greig et al. 1999; Daniels et al. 2003). Both natural and experimental infection has been documented. It is possible that wildlife could transmit MAP to humans in a similar way to those described in Sections 4.1, 4.2 and 4.3. However, animals other than ruminants are not regularly used for milk and meat so the exposure to humans would be much lower than that of ruminants. Wildlife may also act as a reservoir for MAP, thereby reducing the effectiveness of MAP control and eradication programs (Greig et al. 1999).

CONCLUSION

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 (or MAP) in ruminants and some cases of Crohn's disease in humans. It is therefore recommended that this paper be regularly reviewed with the emergence of new scientific data.

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APPENDIX 1 - International Opinions on the Link Between Johne's and Crohn's Disease

Johne's disease is classified as a List B disease by the International Animal Health Agency (OIE). List B diseases are transmissible and are considered to be of socio-economic and/or public health importance. These diseases may also significantly impact the international trade of animals and animal products. Johne's disease is notifiable in OIE signatory countries

Internationally, it is generally acknowledged that there is insufficient scientific evidence to prove or disprove a conclusive link between Johne's disease (or MAP) in ruminants and some cases of Crohn's disease in humans. However, the United Kingdom has put in place a strategy for the control of MAP in cows milk (FSA, 2002). This was a precautionary response only due the insufficient evidence for a link between MAP and Crohn's disease. The strategy involves three main areas: controlling MAP in cattle, controlling MAP during milking and reducing MAP in milk for human consumption.

Approach to Controlling Johne's Disease in Australia

While there is still uncertainty about a possible link between Crohn's disease and Johne's disease, Animal Health Australia, the cattle industries and governments are implementing the National Bovine Johne's Disease Strategic Plan, the first goal of which is to reduce contamination of farms and farm products by MAP. The strategic plan is implemented through the National Johne's Disease Control Program.

A copy of the National Bovine Johne's Disease Strategic Plan can be obtained from <http://www.aahc.com.au/bjd/index.htm#program>.

More information regarding the National Johne's Disease Control Program can be obtained from <http://www.aahc.com.au/jd/njdcp.htm>.

A new National Approach to Ovine Johne's Disease has also been implemented to help control Johne's disease in sheep. Information regarding this program can be obtained from <http://www.aahc.com.au/ojd/index.htm#wha>.

APPENDIX 2 - MAP is the causative agent of Crohn's disease

Argument	Details	Counter arguments
Crohn's disease is similar to Johne's disease.	<ul style="list-style-type: none"> • Crohn's disease and MAP were initially associated because the disease MAP causes in cattle resembles Crohn's disease. • MAP isolated from a Crohn's disease patient induced Johne's disease in goats and mice. • MAP has tropism for the gastrointestinal tract regardless of site of inoculation. • Johne's disease is a systemic infection. Viable MAP has been isolated from the peripheral blood of Crohn's disease patients. 	<ul style="list-style-type: none"> • Other microbial species have been detected in Crohn's disease patients; <ul style="list-style-type: none"> ○ <i>M. avium</i> complex ○ <i>Helicobacter</i> sp, ○ <i>Listeria monocytogenes</i> and ○ <i>E coli</i> ○ <i>Bacteriodes vulgaris</i> ○ measles virus. • Pathology in Crohn's disease commonly includes fissuring ulceration, fistula formation and fibrosis, whereas Johne's disease in cattle does not. • There is no caseation in the granulomata in Crohn's disease as in Johne's disease. • Causative agent is easy to recover in Johne's disease but not in Crohn's disease. • Crohn's disease does not have any evidence of organism on histochemical examination.
Mycobacteria all produce similar characteristics to what has been proposed for MAP in humans.	<ul style="list-style-type: none"> • Mycobacterial infection causes chronic inflammation of the intestine in many species of animals including humans. 	
MAP has been isolated from patients with Crohn's disease.	<ul style="list-style-type: none"> • The first isolation of MAP from Crohn's disease patients required mycobactin J and had cultural characteristics similar to MAP. Strains had initially been isolated as non-acid fast coccobacillary forms that had the ultrastructural appearance of spheroplasts, which after several months in culture had transformed into the characteristic mycobacterial forms. • MAP is harboured deep in mucosal tissue and thus is not a contaminant passing through to gut. • Viable MAP has been cultured from the peripheral blood of patients with Crohn's disease. Only MAP DNA was detected in healthy controls. 	<ul style="list-style-type: none"> • MAP has been detected in some healthy controls and in patients with other inflammatory bowel disorders. • Histological examination rarely shows acid-fast staining of MAP. • MAP has been detected by faecal culture in approximately 5% of cases. • MAP has been detected by blood culture in approximately 50% of cases. • MAP has been detected by PCR in approximately 19-46% of cases. • MAP has been detected by in situ hybridisation in 40-92% of cases.

APPENDIX 2 - MAP is the causative agent of Crohn's disease

Argument	Details	Counter arguments
Johne's disease and Crohn's disease are increasing world-wide.	<ul style="list-style-type: none"> The prevalence of Johne's disease and Crohn's disease are increasing world-wide. 	<ul style="list-style-type: none"> There is a lack of reliable data on incidence and prevalence of both diseases. The increases may be due to better diagnosis and detection methods and increased awareness of Crohn's disease.
MAP is present in dairy herds and retail pasteurised milk	<ul style="list-style-type: none"> 22% and 55% of dairy herds are infected with MAP. MAP has been detected in retail pasteurised milk. 	
Treatment of Crohn's disease patients with drugs specifically targeting MAP have produced promising results.	<ul style="list-style-type: none"> Mycobacterial infections are difficult to eliminate. Multiple drug regimes may be more effective than single drug regimes. Specific drugs against MAP have shown promise in anecdotal reports. 	<ul style="list-style-type: none"> There are no published controlled trials of anti-MAP therapy in Crohn's disease. The results of the Australian placebo-controlled trial of anti-MAP antibiotics are awaited and should be available in late 2004. The antimycobacterial antibiotics used to treat Crohn's are not restricted in activity to MAP or mycobacteria species. They have a much broader spectrum of activity and may be removing other microbial flora.

APPENDIX 3 - MAP is not the causative agent of Crohn's disease

Argument	Details	Counter arguments
MAP is not reliably detected in 100% of Crohn's disease patients.	<ul style="list-style-type: none"> • Histological examination rarely shows acid-fast staining of MAP. • MAP has been detected by faecal culture in approximately only 5% of cases. • MAP has been detected by blood culture in approximately only 50% of cases. • MAP has been detected by PCR in approximately only 19-46% of cases. • MAP has been detected by in situ hybridisation in only 40-92% of cases. 	<ul style="list-style-type: none"> • MAP may be in low abundance. Similar problems with detection of low abundance bacterial pathogens have been reported in disease tissues of people with tuberculosis, lymphocytic leprosy, lyme disease and brucellosis. • MAP may be present as spheroplasts, which do not stain using acid-fast methods. Some studies have shown IS900-specific MAP DNA, but no MAP with cell walls, in tissue sections from Crohn's disease patients, suggesting the presence of the cell-wall deficient form. • MAP DNA is more difficult to purify than other bacterial species- isolation requires a mechanical disruption step that, when incorporated, enhances MAP detection (this method has not been routinely used). • Where MAP has been isolated from Crohn's disease patients it has usually been isolated as spheroplasts which can be easily destroyed during the treatment steps required for culture. • The majority of IS900 positive samples from Crohn's disease were obtained from resected tissues and the positive controls were from biopsy specimens. This might indicate a bacterial infection that persists deep in the tissue and not a contaminant passing through the gut when the biopsy was obtained. • Detection of MAP by PCR may not correlate to viable MAP.
MAP found in 'healthy' controls.	<ul style="list-style-type: none"> • MAP has been detected in some healthy controls and in patients with other inflammatory bowel disorders. 	<ul style="list-style-type: none"> • The detection of MAP in patients other than Crohn's disease is not unexpected as even with <i>M. tuberculosis</i>, only ~10% of infected individuals experience clinical disease. • Differential diagnosis of inflammatory bowel disorders may not be accurate.
IS900 is not specific for MAP.	<ul style="list-style-type: none"> • Product has been obtained from other mycobacterial species after PCR amplification using IS900 primers. 	<ul style="list-style-type: none"> • The primers used to amplify IS900 also amplify low copy regions of some rare mycobacteria.
The pathology of Crohn's disease is not identical to Johne's disease.	<ul style="list-style-type: none"> • Pathology in Crohn's disease commonly includes fissuring ulceration, fistula formation and fibrosis, whereas Johne's disease in cattle does not. • There is no caseation in the granulomata in Crohn's disease as in Johne's disease. • Causative agent is easy to recover in Johne's disease but not in Crohn's disease. • Crohn's disease does not have any evidence of 	<ul style="list-style-type: none"> • The paucibacillary form of Johne's disease, which is more common in small ruminants than in cattle, most resembles that in Crohn's disease. • A spheroplast form of MAP can exist in the intestinal tissue, which does not stain using acid-fast methods. • MAP isolated from a Crohn's disease patient induced Johne's disease in goats and mice. • Crohn's disease is a heterogenous disorder, so some disease characteristics may not be the same in all cases.

APPENDIX 3 - MAP is not the causative agent of Crohn's disease

Argument	Details	Counter arguments
	organism on histochemical examination.	<ul style="list-style-type: none"> MAP causes different forms of disease in different host species. Johne's disease is a systemic infection. Viable MAP has been isolated from the peripheral blood of Crohn's disease patients.
Geographical incidences of Johne's disease do not correlate with that of Crohn's disease.	<ul style="list-style-type: none"> Crohn's disease shows strong geographical variation. Johne's disease is found globally, but distribution does not always correlate with occurrence of Crohn's disease, eg in Scandinavia and Western Australia. 	<ul style="list-style-type: none"> There is a lack of reliable data on incidence and prevalence of both diseases. Crohn's disease, MAP infection and Johne's disease are increasing globally. Animals may have subclinical infection, which reduces the reliability of incidence and prevalence rates of Johne's disease. Due to the long incubation period the likely time of exposure, infection and expression of disease needs to be examined. Retrospective analysis of Johne's disease 10-15 yrs ago needs to be compared to Crohn's disease today. Other factors, such as environment, may play an important role in the development of Crohn's disease. There may be a large proportion of sub-clinical infection in places with low Crohn's disease rates. Other environmental or geographical factors present in high incidence areas may initiate clinical disease.
There is no apparent humoral response to MAP in Crohn's disease patients.	<ul style="list-style-type: none"> All bar one study have failed to find differences in MAP reactive antibodies in Crohn's disease patients compared to healthy people. Cross reactivity was observed in patients with tuberculosis or leprosy and in vaccinated people and some 'healthy' controls. The results indicate a relatively weak recognition of MAP in Crohn's disease, which is only visible if certain immunological targets are selected. Crohn's disease patients have 'leaky' intestine and thus elicit a response to normal intestinal flora and bystander mycobacteria. 	<ul style="list-style-type: none"> Antibodies tend to be a late stage manifestation of disease and so may not be detectable in all samples. Considerable proportion of the population has been exposed to other mycobacteria species, which may cross react in tests. The lack of a humoral response could be indicative of MAP evading immune response. Antibodies with greater specificity to MAP (p35 and p36) were statistically more frequent and of higher titre in Crohn's disease patients compared to controls. Seroreactivity to these antigens was found in 77% of Crohn's disease, 8% of ulcerative colitis and 0% of healthy controls.
Treatment of Crohn's disease with antimycobacterial drugs does not completely eliminate the disease from patients.	<ul style="list-style-type: none"> There are no published controlled trials of anti-MAP therapy in Crohn's disease. The results of the Australian placebo-controlled trial of anti-MAP antibiotics are awaited and should be available in late 2004. 	<ul style="list-style-type: none"> Overall only a subset of Crohn's disease patients responded to treatment with antimycobacterial drugs. There is no guarantee that if the disease were caused by MAP that there would be a response to drugs. The antimycobacterial antibiotics used to treat Crohn's are not restricted in activity to MAP or mycobacteria. They have a much broader spectrum of activity and may be removing other microbial flora.

APPENDIX 3 - MAP is not the causative agent of Crohn's disease

Argument	Details	Counter arguments
		<ul style="list-style-type: none"> • Multiple drug regimes may be more effective than single drug regimes. • MAP in Crohn's disease would be present in low abundance, which may change the effectiveness of the drugs. • Spheroplasts may not be affected by these drugs. • Mycobacteria infections in immunocompromised hosts are difficult to cure.
<p>Other bacteria species have been detected in Crohn's disease.</p>	<ul style="list-style-type: none"> • Other microbial species have been detected in Crohn's disease patients; <ul style="list-style-type: none"> ○ <i>M. avium</i> complex ○ <i>Heliobacter</i> sp, ○ <i>Listeria monocytogenes</i> and ○ <i>E coli</i> ○ <i>Bacteriodes vulgaris</i> ○ measles virus. 	<ul style="list-style-type: none"> • The first isolation of MAP from Crohn's disease patients required mycobactin J and had cultural characteristics similar to MAP. Strains had initially been isolated as non-acid fast coccobacillary forms that had the ultrastructural appearance of spheroplasts, which after several months in culture had transformed into the characteristic mycobacterial forms. • The principal property that distinguishes MAP from all other candidate organisms in the causation of Crohn's disease is the specific ability of MAP to cause chronic inflammation of the intestine of a large range of histopathological types in many species of animals including primates. Its presence in individuals with chronic inflammation of the intestine of the Crohn's disease type has lead some people to suggest a causal relationship. • Crohn's disease and MAP were initially associated because the disease MAP causes in cattle resembles Crohn's disease. • Results are not consistent between studies.
<p>Some risk factors do not correlate to the development of Crohn's disease.</p>	<ul style="list-style-type: none"> • There have been reports that Crohn's disease patients are less likely to ingest unpasteurised milk and have non-tap water as their primary water source. 	<ul style="list-style-type: none"> • Confounding factors (i.e. genetic susceptibility, stress etc) may be need for an individual to develop Crohn's disease.