Food Safety
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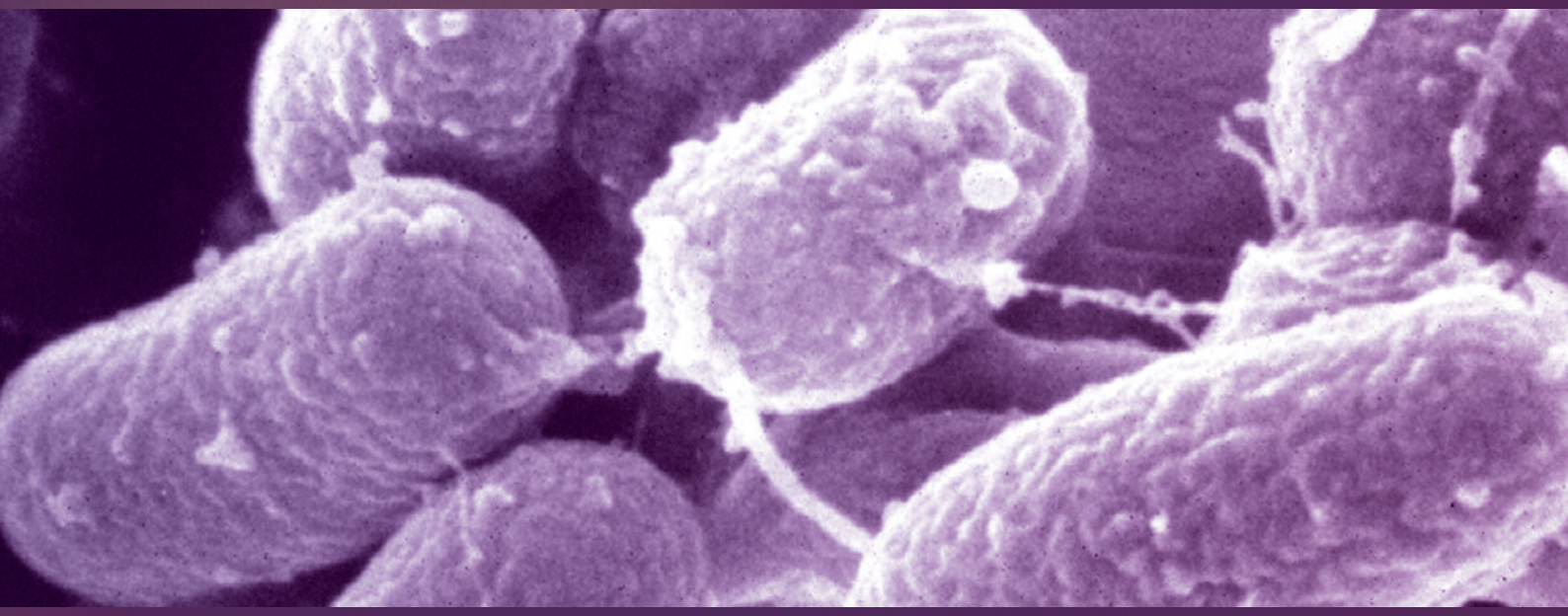
Report of the Scientific Committee
of the Food Safety Authority of Ireland

May 2009

Mycobacterium avium subsp. *paratuberculosis* and the possible links to Crohn's disease



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Mycobacterium avium subsp. *paratuberculosis* and the possible links to Crohn's disease

Published by:
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ISBN 1-904465-62-5

Front cover image courtesy of Johne's Testing Center, University of Wisconsin-Madison

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Executive Summary

In 2000 the Scientific Committee of the Food Safety Authority of Ireland (FSAI) adopted a report entitled *Mycobacterium avium paratuberculosis* (*Map*) does it contribute to Crohn's Disease? (FSAI, 2000). The principal conclusion of that report was that the available data were inconclusive and a direct link between *Map* and Crohn's disease could not be established. However, the report recommended that the Committee keep the issue under review.

Since that time, the Committee has periodically revisited the subject as more research has been published. In 2008, the Microbiology Sub-committee conducted a more formal review of the research and opinion published in the years since the original report in 2000. Fifty six key publications were identified between 2000 and 2008. Based on a review of these papers, **the Committee concluded that the balance of available evidence does not support a causal relationship between *Map* and the incidence of Crohn's disease.**

Summary of Key Research Findings since 2000

Crohn's disease (CD) is a form of chronic inflammatory bowel disease. The pathogenic mechanisms are poorly understood, but may involve a dysregulated immune response to commensal intestinal bacteria and possibly defects in mucosal barrier function or bacterial clearance (Sartor, 2006). The possibility of a link between *Map* and CD remains a subject of debate due to the similarities between the pathology of Johne's disease (JD) in cattle and that of human CD. There are many recent reviews available concerning the possible role of *Map* in CD (Charon *et al*, 2004; Chamberlin *et al*, 2001; Hermon-Taylor, 2001; Rodoler, 2004; Shanahan & O'Mahony, 2005; Turenne *et al*, 2007).

In a genome-wide association study, nine genes have been identified as being associated with a predisposition to CD (Welcome Trust Case Control Consortium, 2008), including *Card*15 (formally NOD2 (Inohara *et al*, 2003)), which may affect host interactions with bacterial lipopolysaccharide and newly identified genes which are involved in eliminating intracellular bacteria. The implicated genes in CD patients are involved overall in mucosal barrier integrity and microbial clearance and/or homeostasis. Other microorganisms including *Escherichia coli* and yeast have also been implicated in CD (Pineton deChambrun *et al*, 2008), again related to impaired function in the defence against intracellular bacteria.

Possible links between *Map* and Crohn's disease

1. There have been reports of detection of *Map* in the blood (Naser *et al*, 2004) and tissues (Bull *et al*, 2003; Ryan *et al*, 2002; Scanu *et al*, 2007; Sechi *et al*, 2005) of patients with CD or irritable bowel syndrome more frequently than in control patients.
2. Other researchers have not detected *Map* in patients with CD (Baksh *et al*, 2004; Bernstein *et al*, 2003).
3. A study of farm workers did not find an increased risk of CD among persons in contact with cattle infected with JD (Jones *et al*, 2006). There are also reports of particularly high prevalence of CD in countries with no endogenous JD, such as Sweden (Jones *et al*, 2006).
4. The complete genome sequence of a bovine isolate of *Map* K10, has been published (Li *et al*, 2005). Comparative genomics showed that genome diversity in the *M. avium* subspecies appears to be mediated by large sequence polymorphisms that are commonly associated with mobile genetic elements. Cattle and human isolates have similar genotypes (Paustian *et al*, 2008).

Map in food and water

1. *Map* was detected by culture in the intestinal lymph nodes or faeces of 34% of 189 healthy dairy cows and 3% of 350 healthy beef cows presented for slaughter (Rossiter & Henning, 2001). *Map* was also cultured from the liver of 11.1% of the dairy cows and 0.7% of the beef cows examined. While *Map* primarily affects the intestines in the later stages of infection, it is spread throughout the animal (Collins, 1997; Report 35, 2004).

2. In a survey of 133 minced beef samples obtained from a meat processing plant in the Republic of Ireland, no viable *Map* was detected (Maher, 2006). A survey of 200 retail ground beef samples were analysed by Jaravata *et al.* (2007) in the USA and *Map* was not detected using PCR (IS900) and conventional culture methods.

3. There is conflicting evidence regarding the ability of *Map* to survive in milk during pasteurisation i.e. high temperature short time (HTST) pasteurisation at 72°C for 15 seconds[1]:

   a) Pilot scale studies undertaken in Ireland using milk samples spiked with *Map* demonstrated that *Map* did not survive HTST pasteurisation conditions of 72.5°C x 27 seconds (Lynch *et al*, 2007). Similar data were obtained in an earlier study from New Zealand (Pearse *et al*, 2001).

   b) Surveys of commercially pasteurised cow's milk have demonstrated that low levels of viable *Map* are sometimes detected. During a large-scale UK survey, viable *Map* was cultured from 1.8% (10/567) of samples (Grant *et al*, 2002). All ten of the *Map*-positive samples had been pasteurised using conditions that met or exceeded the EU minimum requirements; seven samples had been pasteurised at 72-74°C for 15 seconds and three had been pasteurised at 72-75°C for 25 seconds. A similar level of viable *Map* was found in retail pasteurised milk in the USA, with the organism cultured from 2.8% (20/702) of samples (Ellingson *et al*, 2005). This US study did not state what pasteurisation conditions the retail milk samples had been subjected to. In the Czech Republic, viable *Map* was detected in 1.6% (4/244) of commercially pasteurised milk samples and 2% (2/100) of samples of milk pasteurised in small-scale local establishments (Ayele *et al*, 2005). The indication from Ireland however, is that current pasteurisation procedures are considered to be effective since no viable *Map* were detected during a survey of 357 samples of commercially pasteurised milk from approved Irish liquid-milk pasteurisation plants (O'Reilly *et al*, 2004). In this study the researchers reported that, based on the pasteurisation records of the milk sampled, 90% were treated at temperatures in excess of 75°C and that 62% of samples were treated at holding times in excess of 25 seconds. Overall 56% of samples were treated commercially at or above a time temperature combination of 75°C for 25 seconds (O'Reilly *et al*, 2004)[2]. Similarly, in a survey of retail milk samples in Ontario, Canada, no viable *Map* was detected (Gao *et al*, 2002).

   c) Using DNA detection methods rather than conventional culture techniques, in particular, the IS900 insertion sequence unique to *Map* (Collins *et al*, 1989; Green *et al*, 1989), *Map* DNA was detected in 9.8% (35/357) of Irish pasteurised milk samples (O'Reilly *et al*, 2004) and 15% (110/710) of the Canadian samples (Goa *et al*, 2002). *Map* was detected in 11.6% (67/573) of the pasteurised milk samples in a UK study (Grant *et al*, 2002). While most studies have focused on cows' milk, *Map* DNA has been detected in raw goat's and sheep's milk (Grant, 2006). Furthermore, a survey of 51 powdered infant formula products from ten producers in the European Union detected *Map* DNA (IS900) in 49% (25/51) of samples (Hruska *et al*, 2005)[3].

> "In a survey of 133 minced beef samples obtained from a meat processing plant in the Republic of Ireland, no viable *Map* was detected"

[1] The legal temperature requirement for the heat treatment of milk laid down in Regulation (EC) No 853/2004 as amended by Commission Regulation (EC) No 1662/2006.

[2] Following publication of the FSAI Scientific Committee report in 2000 the dairy industry in Ireland increased its milk pasteurisation times and temperatures to 75°C for 25 seconds where possible.

[3] Research related to detection of *Map* may use culture methods to detect viable organisms or molecular methods to detect *Map* DNA. Culture based methods are limited because *Map* is difficult to grow in the laboratory and required prolonged incubation. Molecular methods (such as PCR) are more readily applied. However, it is important to emphasise that in general a positive result with PCR-based methods does not differentiate between viable and non-viable organisms. A positive PCR result in a sample of pasteurised milk may be expected even if the heat treatment has effectively inactivated the organisms.

4. Raw and pasteurised milk is used in the manufacture of cheese. As *Map* may be present in milk, and because *Map* is relatively resistant to salt and acid conditions, the extent of survival of *Map* during cheese maturation has been studied (Collins *et al*, 2001). Sung and Collins (2000) detected *Map* after 30 days of ripening in soft Hispanic-style cheese made from milk to which *Map* had been added. Viable *Map* was also found after 120 days maturation in semi-hard and hard cheese made from raw milk to which *Map* had been added (Spahr & Schafroth, 2001). Viable *Map* was also detected in cheddar cheese prepared from pasteurised milk to which *Map* had been added at the end of a 27-week maturation process (Donaghy *et al*, 2004). This study suggested that because mild cheddar is usually ripened for up to 16 weeks, a higher margin of safety (with respect to *Map*) may be provided by medium or mature cheddar as they are ripened over a longer time period. Viable *Map* was also found in 4.7% (2/42) of samples of five brands of feta cheese (made from a mixture of sheep and goats' milk) available on the Greek market although using PCR, *Map* DNA was found in 50% (21/42) of the same samples (Ikonomopoulos *et al*, 2005). In the same survey, cows' milk cheese available for sale in the Czech Republic was studied and viable *Map* was detected in 4.3% (1/23) of samples of a hard cheese but not detected in five samples of a semi-hard cheese and 14 samples of a soft cheese (Ikonomopoulos *et al*, 2005). In contrast, *Map* DNA was detected by PCR in 17.4% (4/23) of the same samples of hard cheese, 20% (1/5) of the same samples of semi-hard cheese but was not found in any of 14 samples of soft cheese examined (Ikonomopoulos *et al*, 2005). A significant association (P=0.0018) between *Map* infection in humans and the consumption of handmade cheese directly from farms in Sardinia, has been published (Scanu *et al*, 2007).

> "Viable *Map* was also detected in cheddar cheese prepared from pasteurised milk to which *Map* had been added at the end of a 27-week maturation process"

5. A survey of treated and untreated water in the UK did not find *Map* in any samples, but other *Mycobacterium* spp. were detected in 11% (19/170) of samples (Hunter *et al*, 2001).

6. In Northern Ireland, *Map* was detected by culture and/or PCR in 7.8% (15/192) of one litre samples of water entering a water treatment plant (Whan *et al*, 2005). It is not possible to determine the effect water treatment had, as treated water was not tested. Previously, investigations in the same laboratory showed that *Map* was not killed by chlorine at levels as high as 2.0 µg/ml with a contact time of 30 minutes (Whan *et al*, 2001). However, it was noted that *Map* had been added to the water used at initial concentrations higher than would be expected in the natural environment (106 cfu/ml) in order to ensure that the number of survivors after chlorination was above the sensitivity of the detection method used.

7. *Map*-contaminated water and contact with infected animals or people have been suggested as vehicles of transmission to humans (Shanahan, 2002).

8. A case-control study in the UK carried out during 1999–2004 by Abubakar *et al*, (2007), assessed the possible role of drinking water and dairy products potentially contaminated with *Map* in the aetiology of CD. No such role was identified for contaminated water or dairy products. In a retrospective questionnaire survey, the authors speculated on a possible statistical association with meat consumption and a negative association with pasteurised milk consumption and recommended further study on both these observations.

> "*Map*-contaminated water and contact with infected animals or people have been suggested as vehicles of transmission to humans"

Risk Associated with the Ingestion of *Map* in the Food Chain

A number of reports have been published since 2000 including three from the EU (EC 2000; Gould *et al*, 2004 and NCCA, 2003), a report from Food Standards Australia New Zealand (FSANZ, 2004) and an American Academy of Microbiology (AAM) report (AAM, 2008) There has also been a compilation of information on *Map* by the International Dairy Federation (IDF, 2001).

1. European Commission DG - Health and Consumer Protection in 2000 commissioned a report from the Scientific Committee on Animal Health and Animal Welfare on the possible links between Crohn's disease and *Map* (EC, 2000). After a substantial review of the research literature available in 2000, it was concluded that the available evidence was "insufficient to confirm or disprove that [*Map*] is a causative agent of a least some cases of Crohn's disease in man" and that there were "sufficient grounds for concern to warrant increased and urgent research activity to resolve the issue".

2. The International Dairy Federation established a task force to brainstorm current practices and research on *Map* (IDF, 2001). The outcome included a bulletin reporting on methodologies for isolation, diagnostic techniques, destruction of *Map* and on-farm management of JD. The conclusions drawn were that there was 'no gold standard' method for the identification of *Map*. There was poor reporting of the disease in cattle even though paratuberculosis is a notifiable disease in some countries. The limited and conflicting nature of data on heat inactivating *Map* in milk and milk products made definitive conclusions impossible.

3. The National Association for Colitis and Crohn's disease (NACC) in the UK commissioned a report from an expert review group into the evidence linking *Map* and Crohn's disease in 2003 (NACC 2003). The conclusion from this report stated: "*Map* both alive and dead is present in human foods" and "the evidence for this is strongest in milk" and "DNA from *Map* can be found in the bowel tissue of a proportion of patients with Crohn's' disease but also in lesser quantities in the bowel tissue of some people who do not have Crohn's disease". The report stated that *Map* involvement in Crohn's disease is unknown.

4. The International Life Science Institute (ILSI) Europe in 2004 published a report from its Emerging Pathogen Task Force on *Map* and the food chain (Gould *et al*, 2004). In its conclusion, it stated that "the public health importance of such survival of *Map* depends on their possible involvement in human disease, in particular Crohn's disease. At the present time, despite substantial research……the possible involvement of *Map* in human disease remains under discussion. Further studies are needed to clarify the issue". This opinion followed a review of *Map* survival characteristics in food which noted in particular, studies demonstrating survival of viable *Map* in pasteurised milk.

5. Food Standards Australia New Zealand conducted a microbiological review of the association between JD and CD (FSANZ, 2004). It concluded that CD was a "multifactorial disease or syndrome, with no 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"

6. The American Academy of Microbiology issued a review of the evidence of pathogenicity in *Map* (ASM, 2008). It noted that "there is a suspicion, supported by reports of genetic inability to interact appropriately with certain bacteria or bacterial products in some patients, that CD may have a currently unrecognised infectious origin, perhaps environmentally derived". Suspected bacterial agents were cited as *Map* and a variant of *E.coli*. It also noted that "the possibility of more than one infectious cause that leads to a similar set of symptoms confounds the research agenda to find both a cause and a cure for CD". The report lists five reasons why *Map* has a suspected role in CD including *Map*'s ability to survive milk pasteurisation and the success in some CD patients of antibiotic therapy against *Mycobacteria*. The report lays out the pros and cons of a possible association between *Map* and CD. Finally, they concluded that "more research support and substantial additional research effort by both scientists and clinicians is necessary before we will know whether CD has an infectious aetiology, and whether *Map* is the culprit".

Conclusion

Based on this review by it's Microbiology Sub-committee, the Scientific Committee of the Food Safety Authority of Ireland has concluded that on balance, the available evidence does not support a causal relationship between *Mycobacterium avium* subsp. *paratuberculosis* and the incidence of Crohn's disease.

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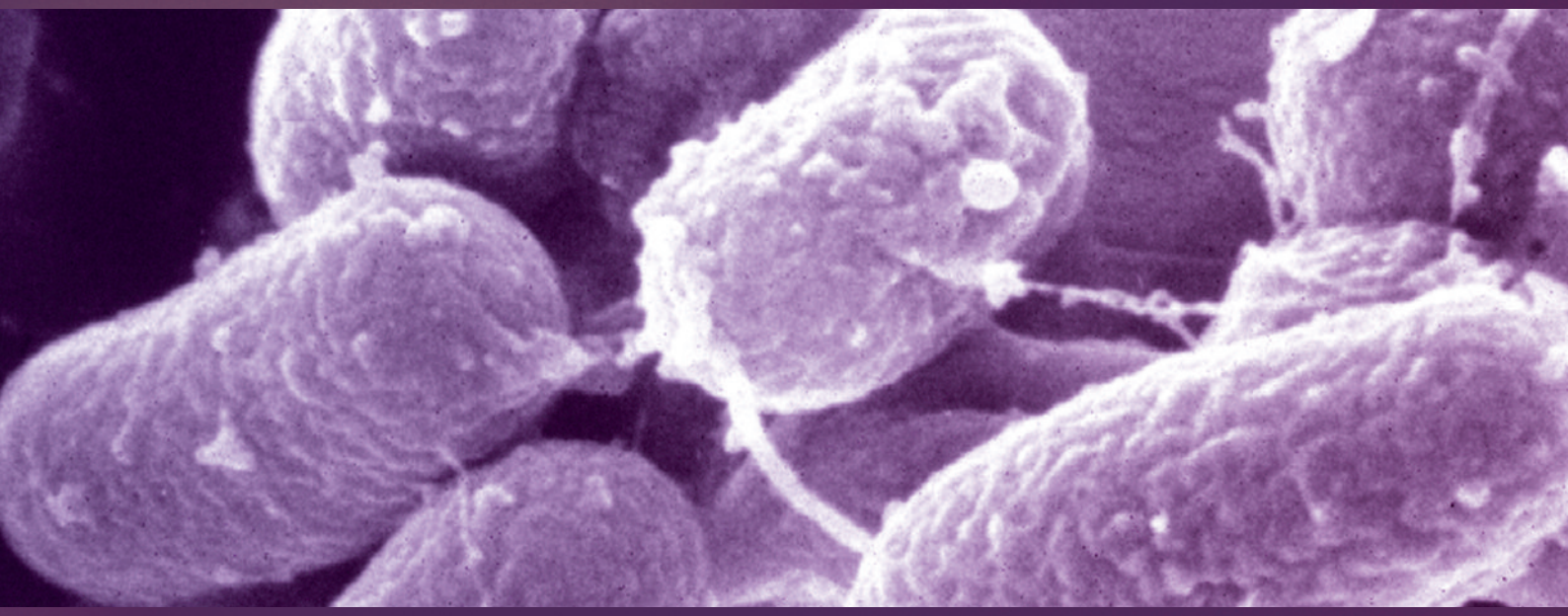
* A review of the literature of *Map* and Crohn's disease was prepared in the first instance by Dr Catherine Adley for discussion by the Microbiology Sub-committee assisted by Dr Judith O'Connor (FSAI).



Food Safety
AUTHORITY OF IRELAND

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