Review of Latest Research on Possible Link Between *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and Crohn's Disease

For Advisory Committee on Dangerous Pathogens (ACDP), 26 February 2015

by

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It is my understanding that the topic of a possible link between MAP and Crohn's Disease was last discussed by ACDP in 2005/2006. Therefore, this paper will attempt to summarise: (1) studies to detect MAP in CD patients, (2) current expert opinions on the role of MAP in CD, (3) newly suggested associations between MAP and other human conditions, and (4) the potential for humans to be exposed to MAP via contaminated milk and dairy products, up to the present.

1. Associations between MAP and human disease

MAP and Crohn's disease

The first thing to say is that the possibility that MAP is a causative agent of CD remains controversial. Association between MAP and CD does not prove causality. Causation implies that there is a true mechanism that leads from exposure to disease, whereas association simply implies that exposure might cause disease. Some would argue that Koch's postulates have been fulfilled, others argue that Koch's postulates aren't appropriate and that Hill's Criteria are a more useful framework for assessing causality. Hill's criteria include: (i) strength of association; (ii) consistency of effect; (iii) specificity of effect (iv) temporality; (v) biological gradient or dose response; and (vi) biological plausibility. Uzoigwe et al. (2007) applied Hill's criteria to the available evidence in relation to MAP and CD and claimed they were able to demonstrate that the MAP Crohn's disease phenomenon as fulfilled at least four (i, ii, iv and vi) of the six criteria.

Debate also continues about the role of MAP in CD, whether it is one of the causative agents of CD or if underlying disease has allowed MAP to secondarily infect the bowel and invade the bloodstream. MAP has been isolated from or detected in patients with CD on a fairly regular basis over the years. Feller et al. (2007) carried out a systematic review and meta-analysis on *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's Disease. The following two figures from their paper indicate that for both PCR of tissues or blood and serological (ELISA) detection, in most studies, MAP was more often detected in CD patients than in control subjects.

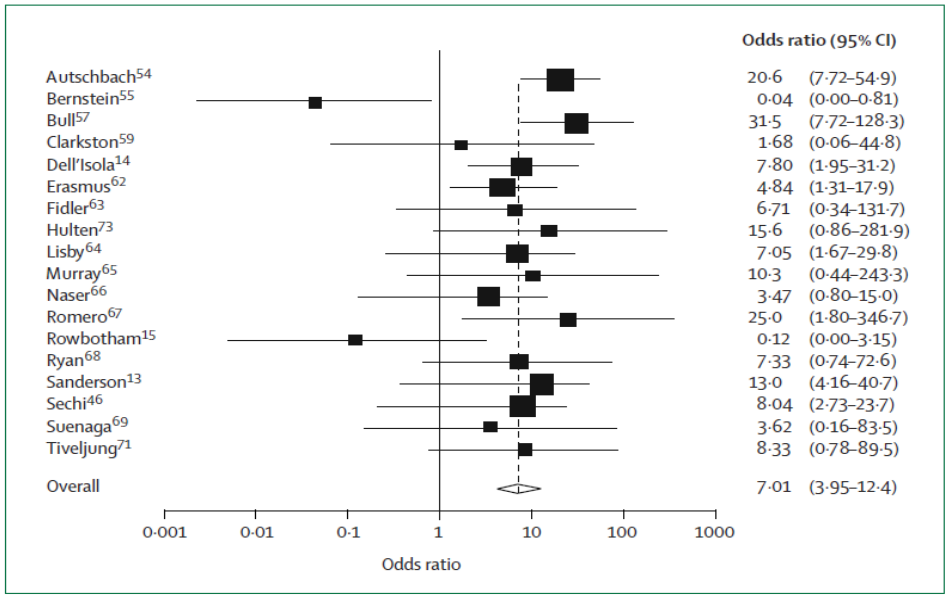


Figure 1: **Meta-analysis of 18 comparisons from case-control studies of patients with Crohn's disease versus controls, with PCR in tissue samples or blood to detect *M avium* subspecies *paratuberculosis* (MAP)**
Odds ratios (ORs) and 95% CI for each study are shown. The size of the square represents the relative weight of each study in the random-effects meta-analysis. The data are displayed on a logarithmic scale. ORs above 1·0 indicate a higher prevalence of MAP in patients with Crohn's disease compared with controls.

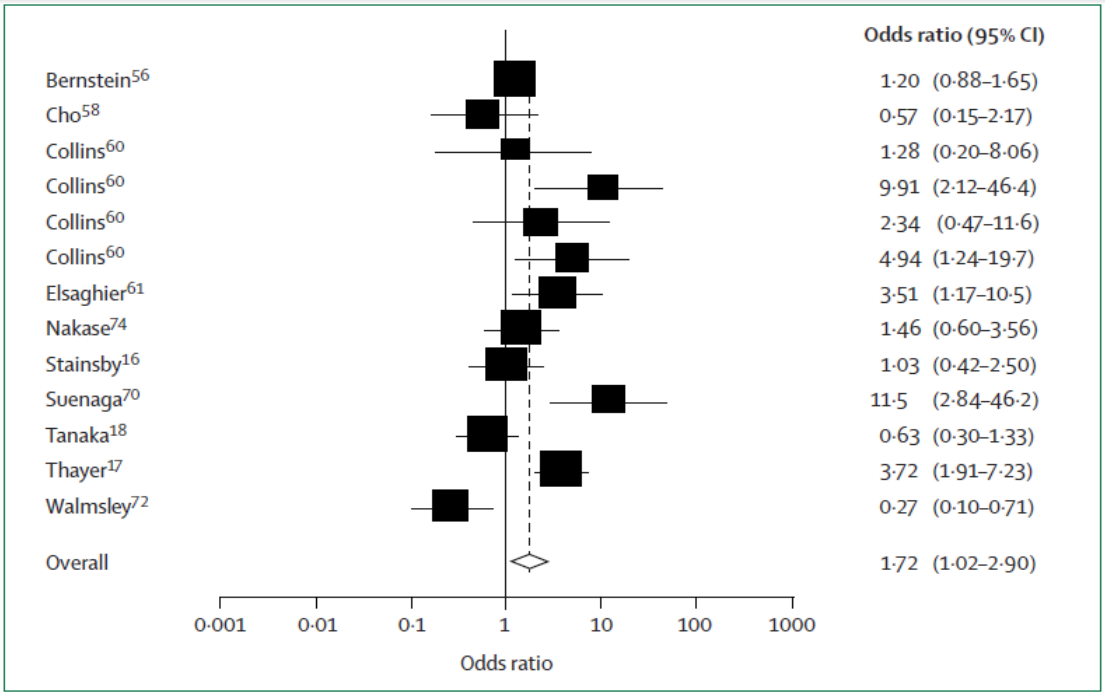


Figure 2: **Meta-analysis of 13 comparisons from case-control studies of patients with Crohn's disease versus controls, with ELISA in serum to detect *M avium* subspecies *paratuberculosis* (MAP)**
Odds ratios (ORs) and 95% CI for each study are shown. The size of the square represents the relative weight of each study in the random-effects meta-analysis. The data are displayed on a logarithmic scale. ORs above 1·0 indicate a higher prevalence of MAP in patients with Crohn's disease compared with controls.

(Source: Feller et al. 2007)

Das and Seril (2012) also suggested that perhaps the higher prevalence of MAP in tissues of patients with CD represents an increased susceptibility of the compromised intestinal mucosa of patients with CD to colonization by a bacterium that is ubiquitous in the environment. Information will be presented later on the prevalence and numbers of MAP in milk and dairy products, which most people consume on a regular basis thereby exposing

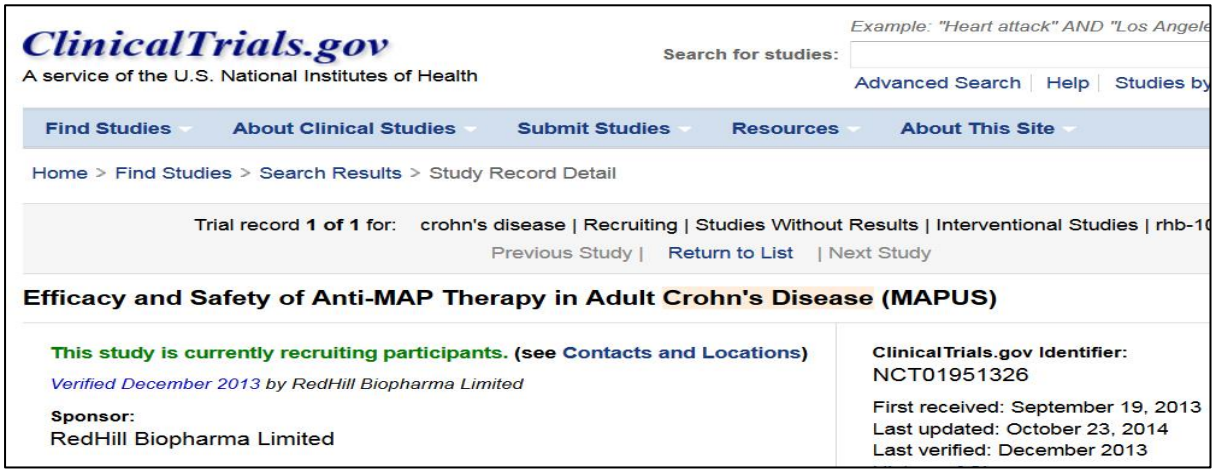
themselves to this bacterium, in countries where JD prevalence in dairy cattle is high at least.

On the basis of an up-to-date review of the literature, Atreya et al. (2014) concluded the following with regard to association of MAP with Crohn's disease: 'We therefore hypothesize intestinal infection with MAP alters the local and potentially later on also the systemic immune responsiveness leading to an impaired mucosal host-microbial homeostasis. In the case of CD, MAP might therefore be considered as an additional risk factor of the disease that acts on a subgroup of CD patients, comparable to already established risk factors such as genetic polymorphisms or specific environmental factors. Further investigations, however, are required to delineate the potential influence of MAP infection on the susceptibility to mucosal inflammation.'

At the 12th International Colloquium on Paratuberculosis, held in Parma in June 2014, Dr Ingrid Olsen, Norwegian Veterinary Institute, and Oslo University Hospital-Rikshospitalet, Norway, was an invited keynote speaker for the MAP and Public Health session of the Colloquium. Dr Olsen made the following assessment: "The importance of immune handling of bacteria differentiates CD pathophysiology from Ulcerative Colitis…..As to which intestinal bacteria are involved in the pathogenesis of CD, much uncertainty remains…..The immune deficiency theory implies that otherwise harmless or opportunistic bacteria may cause a chronic infection in (genetically) susceptible individuals that the host is unable to clear. The result is CD, where the immune system mounted in an attempt to clear the infection, causes more damage than the infection itself." Dr Olsen's conclusion was: "Together with all the genetic susceptibility data emerging over the last decade, it is very hard to reject the hypothesis of mycobacteria being involved in the development of CD in at least a sub-cohort of patients."

Success of anti-MAP treatment for Crohn's disease patients

There have been a few studies reporting the results of treatment of Crohn's patients with antibiotic combinations with presumed effectiveness against intracellular MAP, such as clarithromycin, rifabutin and clofazamine, some of which were associated with a greater rate of remission (including macroscopic healing of the affected gut) in patients with active Crohn's disease as compared with placebo treatment. However, remission was not necessarily sustained. A major criticism of these antibiotic therapy trials is that patients were not confirmed to be MAP infected before treatment commenced, so the specificity of the therapy for MAP is called into question. A large, multi-center, double blind and placebo-controlled Phase III clinical trial, involving 52 US, 2 Canadian and 5 Israeli medical centers, is currently underway. Further details can be found online at ClinicalTrials.gov.



The antibiotic therapy being trialled is RHB-104, "a proprietary and potentially groundbreaking combination drug therapy in oral pill formulation, with potent intracellular, antimycobacterial and anti-inflammatory properties. Use of RHB-104 is based on increasing evidence supporting the hypothesis that CD is caused by MAP infection in susceptible patients rather than being an autoimmune disease." However, once again, it is unclear if recruited patients are being tested for MAP or susceptibility genes before assignment to treatment groups. Estimated study completion date is November 2016.

MAP association with other human diseases

Genome wide analysis and genetic linkage studies have suggested an association between MAP and several other inflammatory diseases. It is no longer just CD in the frame, but quite a list of other chronic human conditions:

- Irritable bowel syndrome
- Type 1 Diabetes mellitus
- Multiple Sclerosis
- Parkinson's disease
- Autism
- Other auto-immune conditions – autoimmune thyroiditis, sarcoidosis, Blau syndrome

MAP antibodies have been detected in blood of patients with type 1 diabetes and multiple sclerosis. MAP has also been isolated by culture from patients with HIV. According to Chamberlin et al. (2007), the full spectrum of MAP infection in humans remains to be determined.

Various hypotheses have been put forward as to MAP's involvement in some of the above auto-immune diseases, for example:

- 'The presence of MAP in Blau syndrome—an autosomal dominant, systemic inflammatory disease—connects genetic and environmental aspects of "autoimmune" disease.' (Dow and Ellingson 2010)

- 'Autoantibodies to brain myelin basic protein (MBP) is a common feature of autism. This article considers the subset of autoimmunity-related autism patients and postulates that MAP, through **molecular mimicry to its heat shock protein HSP65**, triggers autism by stimulating antibodies that cross react with myelin basic protein (MBP).' (Dow, 2011)

- 'Mycobacterial heat shock protein 65 (HSP65) is an immunodominant protein that shares sequential and conformational elements with several human host proteins. This **molecular mimicry** is the proposed etiopathology by which MAP stimulates autoantibodies associated with autoimmune (type 1) diabetes, autoimmune (Hashimoto's) thyroiditis, and multiple sclerosis. This paper proposes that **MAP is a source of mycobacterial HSP65** and acts as a trigger of autoimmune disease' (Dow 2012)
- 'Although the etiology of Type 1 Diabetes mellitus (T1DM) has not been determined, genetic polymorphism in key genes, including *SLC11A1*, and association with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) have been reported. We hypothesize that **molecular mimicry between MAP Heat shock protein 65 K (Hsp65) and human Glutamic Acid Decarboxylase 65 K (GAD65)** may be the trigger leading to autoimmune destruction of beta cells in patients exposed to MAP.' (Dow et al. 2013)

If MAP does have a role in CD or any of the above human conditions, it is likely that it does so in the setting of susceptible individuals, and hence not all persons exposed to MAP will be affected. Genetic susceptibility in individuals appears to play a part in 'MAP-associated' diseases. There is mounting evidence in the literature that susceptibility genes associated with the inability to handle usual gut bacteria effectively, or diminished ability to kill intracellular infections (autophagy), or dysfunctional macrophages and dendritic cells, such as NOD2, CARD15, SLC11A1, IRGM and ATG16L1, are common in affected individuals. The term 'leaky gut' has also been termed in relation to individuals with these susceptibility genes.

2. Potential for human exposure to viable MAP via milk and dairy products

Frequent detection of MAP DNA in healthy controls, in addition to CD patients, might be attributable to the wide environmental distribution of MAP and its presence in the food chain. Infected cattle shed MAP in their faeces and milk. Herd prevalence of Johne's disease, caused by MAP, in dairy cattle worldwide has reached alarming levels - >90% of dairy herds in the USA are infected, 40-50% of dairy herds in the UK are affected. Without effective control programmes MAP infection has spread widely and unquestionably the potential for human exposure to MAP via milk, dairy products and potentially beef has risen too. There is some evidence that the incidence of Crohn's disease in various countries has been rising over recent years.

Detection of MAP in food has always presented problems due to its slow-growing nature, the need for chemical decontamination of samples before culture leading to false negative results or inaccurate counts, and difficulties getting DNA out of MAP cells for PCR detection. Published information on presence and/or levels of viable Map in raw milk arising from use of the traditional culture method has probably been rather misleading; published MAP levels in milk from individual JD affected cattle:

Sweeney et al. (1992) - 2-8 CFU/50 ml
Giese and Ahrens (2000) - <100 CFU/ml
Ayele et al. (2005) - 4-20 CFU/50 ml

The presumption has generally been that by the time milk from multiple animals in a MAP-infected herd is mixed in the bulk tank at farm level, and then again at the processing plant, that reduction of these numbers will occur due to dilution and hence MAP is unlikely to survive milk pasteurisation and subsequent processing. However, with the advent of new and improved detection methods over recent years, particularly real-time PCR, which allows quantitation of MAP based on detection of MAP DNA, and phage amplification-based methods, that can quickly detect and enumerate viable MAP in milk, new information is emerging about the prevalence and possible numbers of MAP in milk and dairy products. Unfortunately, the picture is not very comforting.

Milk from individual cattle:
Slana et al. (2008) - 10-560 cells/ml (= 500-28000 cells/50 ml) (qPCR)
Foddai et al. (2014) – 6-948 PFU/50 ml (PMS-phage assay)

Bulk tank cows' milk:
Slana et al. (2008) – 1-9 cells/ml = 50-450 cells/50 ml (qPCR)
Slana et al. (2009) – 'several tens of cells/ml' (qPCR)
Foddai et al. (2014) – 18-695 PFU/50 ml (PMS-phage assay)

UK pasteurised milk
Rees et al. (2014) – 28% of ~30 retail pasteurised milk samples collected in the Nottingham area were phage assay positive. Only 2 of the samples had MAP counts >10 PFU/50 ml. (Phage assay)

Powdered infant milk formulae
Grant et al. (2014) – 30 of 68 PIF samples sourced from 18 different countries yielded plaques, numbers ranging from 4-678 PFU per 50 ml reconstituted formula (PMS-phage assay)

It is difficult to believe these figures for viable MAP in processed milk products. Further validation of the PMS-phage assay findings is needed to verify that MAP levels indicated are accurate and not due to carryover seed phage leading to false positive results. It is even more difficult to try and explain this apparent survival for a vegetative bacterium, even if it is a *Mycobacterium*. The kind of heat resistance being demonstrated is akin to that of spores not vegetative cells. The possibility of the existence of spore-like forms of MAP has been reported (Lamont et al. 2012), as well as for other *Mycobacterium* spp. (Ghosh et al. 2009). Singh et al. (2010) suggested that sporulation might be an adaptation of lifestyle for mycobacteria under stress; heat treatment and drying would be stressful conditions for MAP. Further research is clearly needed.

3. Concluding remarks

It is difficult to draw any firm conclusions about the role of MAP in CD or in any of the other human disease conditions mentioned above at present. With the application of improved detection methods and alternative research approaches perhaps the situation is clarifying to a degree. The following statements are an attempt to summarise the current state of play:

- CD is described as a syndrome rather than a single disease, and MAP may only be associated with some, not all, cases of CD.
- There is preliminary evidence of MAP or MAP DNA signature in blood or tissues from a range of other, auto-immune, disease conditions, so possible human effects of MAP exposure and infection extend beyond just CD.
- Certain genetic susceptibility genes are frequently demonstrated in individuals that test positive for MAP, which further suggests that not everyone exposed to MAP in their diet or the environment will become infected.
- With the advent of new and improved methods to detect viable MAP in milk and diary products, there is new evidence of higher levels of viable MAP than previously believed in processed dairy products, in particular in infant milk which is consumed by the most susceptible sector of the population. Therefore, the potential for human exposure exists.

Citations for references mentioned in this report can be provided at the Committee's request