The following articles have been compiled to aid doctors, researchers, medical professions and patients in their research of how *Mycobacterium avium* subspecies *paratuberculosis* (MAP) relates to Crohn's disease. These are the core published peer-reviewed journal articles and studies that have been put forth to make the case that a human version of MAP (hMAP) plays a critical role in some Crohn's disease patients. There are, in fact, hundreds of published scientific and clinical studies that demonstrate a relationship between MAP infection and Crohn’s disease.

**Human Exposure to MAP from Food, Veterinary and Environmental Exposure:**

1. **Isolation of *Mycobacterium avium* subsp *paratuberculosis* from breast milk of Crohn’s disease patients.**
   Of 7 samples analyzed, MAP was found in the breast milk of the 2 Crohn's disease patients but not in the 5 controls. This highlights transmission potential of MAP from mother to infant, similar to transmission in Johne's disease.
   

2. **Detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in retail pasteurized whole milk by two culture methods and PCR.**
   Although MAP DNA has been found in retail pasteurized milk, this study detected live MAP via culture and PCR analysis in 2.8% of milk samples studied in the United States. 22 brands were tested, and 12 were found to contain live MAP. MAP culture was highest in the samples tested from July through September. 64% of the samples showed evidence of MAP exposure.
   

3. **Contamination of food products with *Mycobacterium avium paratuberculosis*: a systematic review.**
   A review of journal articles discussing the possibility of MAP contamination in the human food supply. It concludes that MAP contamination from beef and dairy sources have a higher rate of contamination than first thought. MAP can survive pasteurization, and contamination of products that enter the human food supply chain should not be ignored.
   

4. ***Mycobacterium avium* subspecies *paratuberculosis* in Animal Derived Foods and the Environment.**
   Dr. Grant discusses MAP contamination in beef and dairy products and the rates of MAP surviving processes associated with dairy products, like pasteurization. Environmental contamination of MAP by infected animals also may have increase human exposure to the bacteria. Further studies and improved detection techniques will hopefully lead to a better idea of human risk from veterinary MAP exposure.
   

5. **Assessment of food as a source of exposure to *Mycobacterium avium* subspecies *paratuberculosis* (MAP).**
   The committee was not charged to determine if MAP is a human pathogen, but rather to investigate, based on available literature, the prevalence and exposure routes of MAP to humans. They concluded that the highest risk of MAP exposure to the human population is from raw milk, but other exposure routes may include pasteurized milk, beef, cheese, drinking water, soil and fruits and vegetables contaminated with farm runoff. Current detection techniques are lacking, and if
MAP was eliminated in cattle, other exposure routes may be insignificant. Research into these areas would become critical if MAP is determined to be a human pathogen.


6. Detection of viable Mycobacterium avium subspecies paratuberculosis in powdered infant formula by phage-PCR and confirmed by culture.

Researchers from Cyprus, the Czech Republic and the United Kingdom used multiple MAP detection methods to test powdered infant formula. The premise of the experiment was that since viable MAP is found in milk, and infant formula is derived from milk, infant formula may also contain viable MAP. 32 samples were tested. Using phage-PCR assay, viable MAP was found in 13% of the infant formula samples. 9% of samples grew MAP in culture. The positive culture samples were also phage-PCR positive. The conclusion was that, "the presence of viable MAP in [powdered infant formula] is a potential public health concern."


MAP Detection in Crohn's Disease Patients:

1. Culture of Mycobacterium avium subspecies paratuberculosis from the blood of patients with Crohn's disease.

By utilizing PCR detection, MAP was found in the blood mononuclear cells of 46% of Crohn's disease patients, 45% of Ulcerative Colitis patients, and 20% in controls. The researchers also found nine different MAP strains from 11 isolates.


This meta-analysis shows a strong correlation between Crohn's disease and the presence of MAP DNA. MAP is detected more frequently in Crohn's disease patients than in controls. Differences in laboratory techniques and sample site could account for variations in results.


A letter to the editor describing Anti-MAP antibiotic therapy success in Crohn's disease patients. Nine of the Crohn's disease patients (47%) tested positive for MAP, while all controls were negative.


MAP detection by IS900 analysis was investigated in treatment naive pediatric Crohn's disease patients. MAP was found more often in the mucosal biopsies of Crohn's disease patients than in controls (39% - 22/56 v 15% - 6/39), which suggests that MAP may play a role in the early stages of the disease.


5. Association of Mycobacterium avium subspecies paratuberculosis with Crohn disease in pediatric patients.

Biopsies were taken from pediatric Crohn's disease and control subjects. Using IS900 sequence detection, MAP was found in ileal biopsies in 35% of Crohn's disease patients and 5.2% of controls.

The Role of hMAP in Crohn's disease:

1. **Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease.**
   Mounting evidence supports the theory that Crohn's disease may have an infectious cause, with the prime suspect being MAP. MAP causes a Crohn's-like disease in cattle (Johne's disease) and is frequently found in the environment, suggesting human exposure may be common. Trials using macrolide antibiotics which have effectiveness against MAP in Crohn's patients illustrate that a cure may be possible.

2. **MAP-associated Crohn's disease: MAP, Koch's postulates, causality and Crohn's disease.**
   The case for MAP causing human disease is discussed utilizing Koch's postulates and Relman's guidelines, which are applicable with newer technologies available today.

3. **Crohn's disease and the mycobacterioses: a quarter century later. Causation or simple association?**
   The debate over whether MAP is the etiological agent of Crohn's disease is more than 25 years old. A review of both sides of the research is presented, and questions that need to be answered with further research are asked. Even the skeptics should use caution before dismissing this theory.

4. **Mycobacterium avium subspecies paratuberculosis causes Crohn's disease in some inflammatory bowel disease patients.**
   A meta-review of 60 studies that support the role of MAP in at least 30-50% of Crohn's disease patients. PCR and culture were used to detect MAP in humans, though the same techniques used to detect MAP in cattle do not always translate to detecting MAP in the human samples Naser S, Sagramsingh S, Naser A, Thanigachalam S. World J Gastroenterol. 2014 June 21; 20(23): 7403-7415.

5. **The association of Mycobacterium avium subspecies paratuberculosis with Inflammatory Bowel Disease.**
   Colonoscopy biopsies of 105 subjects (42 controls) were tested for the MAP via three different PCR assays. While only 29% of Crohn's patients were positive for all three PCR assays, this was a significant association that was unrelated to age, gender, place of birth, smoking and alcohol intake. No evidence of MAP was detected in the patients with aphthous (mouth) ulcers, which have been suggested as a precursor to Crohn's disease. One of the cultures, from a UC patient, grew at 5ºC and was resistant to ethambutol, streptomycin, clofazamine and rifampin. The authors made note of this culture and raised the Selby Study, wherein this isolate would have been resistant to the antibiotic regime and deemed ineffective. This highlights the need for improved detection and monitoring MAP in humans.
6. **Dysbiosis of the Fecal Microbiota in Cattle Infected with Mycobacterium avium subsp. paratuberculosis.**

The bacterial communities from the fecal samples of 20 MAP infected cows, 25 of their herdmates and 25 non-herd MAP negative cows were analyzed. In the MAP positive group, the Actinobacteria class was highly abundant compared to the other two groups. The Bacteroidetes and Firmicutes groups decreased in the cows with MAP vs. the other two groups, where they made up 95% of the bacterial population. Additionally, there were bacterial strains that were found in the MAP infected group that were absent in the other two groups. The presence of the Anthrobacter genus in the MAP infected group was of interest since it may have a synergistic interaction with MAP to produce disease, but more research is necessary. This shift from bacterial with anti-inflammatory properties found in healthy controls to bacteria with pro-inflammatory properties in MAP infected cattle is similar to what is seen in humans with Crohn's disease.


**Treatment Applications of hMAP in Crohn’s Disease Patients:**

1. **Two-year outcomes analysis of Crohn’s disease treated with rifabutin and macrolide antibiotics.**

A reduction in the Crohn’s disease activity index was seen in 89% of patients after treatment with rifabutin and a second macrolide antibiotic. Steroid dependence decreased and a reduction in ESR and CRP was noted after 18 months. Patients with ileocolitis responded better than those with ileitis. This treatment may result in a significant improvement for Crohn’s disease patients.


2. **Antimycobacterial treatment for Crohn’s disease: does it prevent surgery and how fast does it act?**

Using rifabutin, clofazimine and clarithromycin, this study sought to assess the impact of this therapy on patients who had difficulty managing their Crohn's disease. The result was a rapid improvement, with half achieving more long term remission. Some patients were able to avoid surgery with this therapy.


3. **Treatment of severe Crohn's disease using antimycobacterial triple therapy—approaching a cure?**

With a goal of studying long term antibiotic use (rifabutin, clofazimine and clarithromycin) in severe Crohn's disease patients, the researchers observed 12 patients for up to 54 months. Half achieved full remission, and some were able to discontinue antibiotic use while maintaining long term remission up to 10 years. Results indicate that a cure may be available.


4. **Open clinical trial of rifabutin and clarithromycin therapy in Crohn's disease.**

58.3% of acute Crohn's disease patients treated with rifabutin and clarithromycin achieved sustained improvement without the need for additional Crohn's disease medications. Only 13.8% did not respond to the therapy. Prior to treatment, the patients tested positive for MAP antigens.


5. **Australian Gastroenterology Week Meeting, 19-22 October 2005, Brisbane, Queensland, Australia.**

Increased doses of rifabutin, clofazimine and clarithromycin resulted in a 61.5% remission rate among Crohn's patients. The addition of clofazimine to the therapy seemed to prove helpful.

*Borody TJ et al. (2005).*

52 patients with severe Crohn's disease received a triple cocktail of clarithromycin, rifabutin, and clofazimine and were monitored by colonoscopies and for histological progress. 56.4% healed but showed an unusual scarring pattern. 38.5% had histological reduction of inflammation. In 2 of 6 patients who continued therapy for more than 3 years, scarring eventually changed to normal mucosal tissue. This longitudinal scarring pattern of the mucosa shows a benefit of the Anti-MAP therapy.


7. On the action of 5-amino-salicylic acid and sulfapyridine on M. avium including subspecies paratuberculosis.

Three often prescribed treatments for Inflammatory Bowel Disease, 5-ASA, methotrexate and 6-MP, may be effective in Crohn's disease by inhibiting MAP growth. Of the three, 5-ASA had the most profound effect on inhibiting MAP growth. Patients who have been treated with these therapies should be excluded from Anti-MAP studies.


8. On the action of methotrexate and 8-mercaptopurine on M avium subspecies paratuberculosis.

The effectiveness of methotrexate and 6-MP in Crohn's disease patients may be primarily due to a MAP killing effect rather than the decrease in pro-inflammatory cytokines.


A case study describing the treatment of a MAP positive, severe Crohn's disease patient who experienced significant improvement after a 6 month course of triple antibiotic treatment consisting of clarithromycin, rifabutin and levofloxacin. Following treatment, MAP was no longer present in the blood due to the antibiotic choice, and not due to an anti-inflammatory effect of the antibiotics since the patient did not respond to prednisone.


10. Two year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Crohn's disease.

In a large randomized trial of 213 Crohn's disease patients taking either a triple antibiotic therapy (clarithromycin 750 mg/day, rifabutin 450 mg/day, clofazimine 50 mg/day) or a placebo, a significant number showed improvement at week 16 in the antibiotic group compared to the placebo. During the first year of the study, fewer patients from the antibiotic group relapsed vs. the placebo group.

The remission rate remained higher in the antibiotic group until 156 weeks, when there was no statistical difference. Critics of this study note the suboptimal dosage of antibiotics, and flaws in the delivery system for clofazimine. Despite the study flaws, Anti-MAP therapy shows higher remission rates than anti-TNF medication. (ITT remission rate of 41% for Anti-MAP at 52 weeks; 24% for Humira at 52 weeks and 26% for Remicade at 26 weeks using the highest dosages for Humira and Remicade.)

11. **Antimycobacterial therapy for Crohn’s disease: a reanalysis.**
In response to the Selby study, the researcher here points out flaws in the methodology including deviation from an intention-to-treat analysis. When reanalyzed, the significance of the antibiotic group is clear with significance at each time point compared to placebo; p=0.02 at 16 weeks, p=0.003 at 52 weeks and p=0.005 at 104 weeks.

12. **Effects of interactions of antibacterial drugs with each other and with 6-mercaptopurine on in vitro growth of Mycobacterium avium subspecies paratuberculosis.**
The synergistic effect of 6-MP with macrolides and the rifamycin derivatives against MAP was demonstrated.

13. **Long-term antibiotic treatment for Crohn’s disease: Systematic review and meta-analysis of placebo-controlled trials.**
A meta-analysis indicated that long term antibiotic treatment of Crohn’s disease with clofazimine or nitroimidazoles was superior over a placebo.

14. **Primary Treatment of Crohn’s Disease: Combined Antibiotics Taking Center Stage**
This article discussed the role of antibiotics in Crohn's disease by looking at the published studies from 1976 to 2011. The table contained within summarizes the antibiotic trials with success rates ranging from 44% to 98%. The 2007 Selby study is analyzed, and the authors conclude that properly chosen antibiotics are beneficial for treating Crohn's disease.

15. **Treatment of Crohn’s disease patients with infliximab is detrimental for the survival of Mycobacterium avium subsp. paratuberculosis within macrophages and shows a remarkable decrease in the immunogenicity of mycobacterial proteins.**
In 20 Crohn's disease patients showed significant decrease in antibody reactivity to MAP proteins during treatment with infliximab. Antibody levels were in the range of negative controls. MAP survival decreased when infected macrophages were exposed to infliximab. Anti-TNF therapy may inadvertently decrease intracellular MAP.

16. **Resolution of Crohn's disease and complex regional pain syndrome following treatment of paratuberculosis.**
In this series of case reports, five family members who tested positive for MAP by culture or antibody screening. Two were treated successfully with a combination of Anti-MAP antibiotics and Ultraviolet blood irradiation, at which point MAP testing was negative. The patients were diagnosed with a variety of ailments, including Crohn's disease, asthma, complex region pain syndrome, Raynaud's phenomenon, type 1 diabetes, hypothyroidism, lymphangiomatosis and rosacea. This study showed evidence of the pathogenic role of MAP humans.
17. **RHB-104 triple antibiotics combination in culture is bactericidal and should be effective for treatment of Crohn's disease associated with *Mycobacterium paratuberculosis*.**

The raw active antibiotics at the percentages contained in RHB-104 (clarithromycin, rifabutin, and clofazimine) were tested alone and in combination in culture against 16 strains of MAP isolated from human Crohn’s disease patients, 9 strains of *Mycobacterium avium* and 10 other mycobacterial species. (NOTE: The researchers did not test RHB-104 itself, but the active antibiotics of RHB-104 in their relative percentages.) They measured the Minimum Inhibitory Concentration of each antibiotic individually, in dual combinations and as a triple therapy against these species to determine how much of each antibiotic/combination it would take to inhibit bacterial growth. The results showed that the triple combination of clarithromycin, rifabutin, and clofazimine contained at the relative percentages in RHB-104 was the most effective at inhibiting mycobacterial growth. 

*Alcedo, K et al, Gut Pathogens 2016 8:32.*