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# *Mycobacterium avium* ssp. *paratuberculosis* vs Crohn's Disease

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## 1. Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease, with the potential to affect any segment of the gastrointestinal tract. Despite the great advances in recent decades, which provided a better understanding of the pathogenesis of the disease, it is yet to be completely elucidated. Presence of genetic factors, luminal factors such as microflora and factors related to the intestinal barrier and immunoregulation are pieces that interact with each other and with environmental factors. The possibility of an infectious etiology has always been widely discussed. In this context, *Mycobacterium avium* subspecies *paratuberculosis* (MAP) has attracted the interest of many researchers because of the similarity between paratuberculosis and CD. In 1913, two decades before the description of CD in 1932, T. K. Dalziel made associations between chronic cases of enteritis in humans and paratuberculosis in cattle (Behr and Kapur, 2008).

Some genetic studies also support the role of MAP in CD and susceptibility genes have been identified, which encode proteins involved in the innate immunity defense against intracellular bacteria. However, no study is conclusive about a causal relationship. It is not possible to conclude that a single agent is solely responsible for the etiology of CD: a multifactorial cause is much more likely (Grant et al., 2001).

Whereas the causal relationship has not been established, therapeutic implications require further studies. Results of these studies could help answer questions about the role of MAP in the etiology of CD.

## 2. *Mycobacterium avium* subspecies *paratuberculosis*

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a small bacillus, Gram-positive, intracellular, acid-resistant bacterium belonging to the Mycobacteriacea family. It grows slowly and when observed under an optic microscope, usually appears to form small clusters (Fig.1). Like other mycobacteria, this microorganism has a thick cell wall, composed mainly of lipids, which determines its acid-resistant, hydrophobicity and high resistance to chemical processes, such as chlorination of water, and physical processes, such as pasteurization (Harris and Barletta, 2001).

Fig. 1. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) stained by Ziehl-Neelsen. Optical microscopy (1000x).

The first observation of the microorganism was made in 1895 by Johne and Frothingham, who isolated an acid-resistant bacterium from the ileum of animals with chronic granulomatous inflammation. The disease was called paratuberculosis, according to its similarity with intestinal tuberculosis. Although the disease had been reported in 1895, identification of the agent was assigned by Twort in 1910 who could grow the microorganism for the first time in laboratory (Cocito et al., 1994).

Morphology of MAP colonies depends on the medium used for growth. In Herrold Egg Yolk Medium (HEYM), colonies are small, measuring about 1 to 2 mm, generally white, convex and smooth, while in Middlebrook Agar they become more wrinkled. Even under optimal conditions, colonies may take 3 to 4 months or longer to become visible (Harris and Barletta, 2001). Other features of MAP, which serve to differentiate it from other bacteria, are its dependence on mycobactin *J* for in vitro growth, a compound extracted from mycobacterial cells that helps in iron uptake, and the presence of insertion element IS*900*, which appears as 14 to 18 copies within the genome (Green et al., 1989).

The complete genome of MAP K-10 was sequenced in 2004 by researchers at the University of Minnesota, USA. Analysis showed that MAP K-10 has a circular sequence of 4,829,781 base pairs with 69.3% G+C. When comparing the genome of MAP and other mycobacteria, researchers have suggested two hypotheses to explain the extremely slow growth of MAP. Firstly, the presence of an insertion sequence, MAP0028c/IS1311, much closer to oriC in MAP compared to *M. tuberculosis*, would be detrimental to chromosomal replication, leading to a wider range of generation. The second theory is the presence of the gene map0638, with a higher replacement rate compared to *M. tuberculosis*. This gene is responsible for regulating synthesis of purine and therefore has a role in rate of protein synthesis and cell growth (Li et al., 2005).

## 3. Paratuberculosis

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the causative agent of paratuberculosis or Johne's disease, a chronic infectious enteritis, characterized by the presence of persistent diarrhea and progressive weight loss, which mainly affects domestic and wild ruminants worldwide and may also affect several other species of mammals, including primates.

In the USA, bovine paratuberculosis is well documented and it is estimated that economic losses for the dairy industry are of the order of millions of dollars annually. These losses are due to decreased production, reduced protein content of milk, premature culling, increased susceptibility to other diseases, reduced fertility and increased health costs (Hendrick et al., 2005).

Under natural conditions, the transmission of the microorganism is usually horizontal, by ingesting food or water contaminated with MAP. Transmission can also occur vertically by intrauterine infection, colostrum from females infected or contaminated semen.

Paratuberculosis usually manifests in young adult cattle. After an incubation period of about 2 to 5 years clinical symptoms begin to appear. However, infected animals eliminate the microorganism in their stools and in minor amounts in milk even before the onset of clinical signs, which contributes to the spread of the agent. Confinement contributes to the infection of animals and can be one of the reasons for higher prevalence of the disease in dairy cattle herds compared to meat cattle. The long incubation period has great importance in the economic impact of disease because infected animals are responsible for the unnoticed spread of the agent and, therefore, of the disease in herds.

In vivo, the primary target of MAP infection is the M cells from Peyer's patches and the primary lesions occur on the walls of the small intestine and mesenteric lymph nodes. The multiplication of the microorganism leads to the extent of injury to the ileum, jejunum, cecum and colon, interfering with the intestinal metabolism. The main gross lesions are characterized by thickening of the intestinal mucosa, which presents an aspect grid with transverse folds, well-exposed and enlarged mesenteric lymph nodes, and the main histopathological findings consist of enteritis and granulomatous lymphangitis and lymphadenitis associated with the presence of acid-fast bacilli resistant into macrophages.

Typical clinical signs of the disease are rapid and progressive weight loss (Fig. 2) and intermittent diarrhea, which becomes progressively more severe. Animals continue with a normal appetite but cannot effectively absorb nutrients. Lower body condition scores are generally found. In the final stage, chronic diarrhea of animals becomes untreatable and then, they will die in cachectic state. During the early stages of paratuberculosis, the immunity is characterized by a strong cell-mediated immune response and in the later stages there is a humoral immune response. Antibody concentrations become higher with the progression of the disease when the lesions become more extensive, reflecting the amount of antigen present.

Diagnosis can be based on the detection of the etiologic agent or detection of immune response to this agent. Several methods have been used to diagnose the disease such as fecal culture, immunological tests, histopathological tests and molecular tests.

MAP isolation by fecal culture is considered the "gold standard" for diagnosis of paratuberculosis, despite having low sensitivity and requiring up to 16 weeks until the first colonies can be seen. To detect infected animals in the early stages of the disease by this method is very difficult due to the slow growth of MAP. The agent is grown on specific

medium and confirmation of the identity of the colonies is done by its dependence of micobactin J or targeting the insertion sequence IS*900* on molecular tests, an element pattern that enables the genetic identification of MAP.

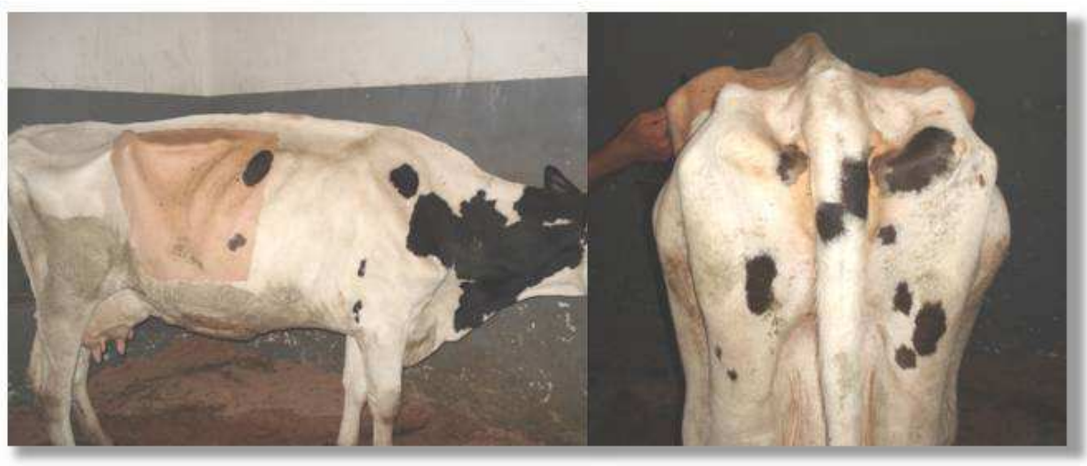

Fig. 2. Animal with paratuberculosis showing low body score

Several serological tests have been used for rapid detection of infected animals including complement fixation, agar gel immunodiffusion and ELISA. However, because disease immunity is mediated by cells in the early stages and humoral in the later stages, these tests generally have high sensitivity in infected animals showing clinical signs, and low sensitivity in animals that do not. Therefore they are most useful in the clinical stages of disease. Among the serological tests, ELISA is the most commonly used for its high sensitivity and acceptable specificity. Despite the low sensitivity and specificity, intradermal tests have also been used. Infected animals show a cellular response when in contact with purified MAP proteins. These tests are not recommended because it may cause cross-reactivity of these proteins with tuberculin during the tuberculin test, leading to false positive results. This is a test performed by control programs for diagnosis of tuberculosis, a disease caused by *M. bovis* in countries where this disease is present.

Rapid detection of microorganisms of slow growth has become possible through use of molecular biology techniques. Discovery of insertion sequence IS*900* in the MAP genome and the development of the polymerase chain reaction (PCR) revolutionized this field. PCR is used successfully to detect MAP DNA in samples of pasteurized milk and fresh milk and is more sensitive than fecal culture. Using PCR it is possible to detect concentrations as low as 10UFC/mL milk. It is a highly sensitive and specific technique for the detection of MAP, as well as fast, and can reduce diagnosis time from months to just two days. PCR is also very versatile, considering that it can be used in stool, tissues, milk, blood and semen. There is currently no satisfactory treatment for animals affected by MAP. Vaccines currently available against MAP do not protect animals completely and there is also the aforementioned problem of possible cross-reactions. Thus, vaccination is not recommended.

General management measures such as general hygiene facilities, separating animals by age, and identification and disposal of infected animals can be cited as preventative measures.

### 3.1 Paratuberculosis in Brazil

In Brazil, the first notification of paratuberculosis occurred in Rio de Janeiro, in an imported animal (Dupont, 1915). Afterwards, the disease was reported in the Southeastern (Santos and Silva, 1956; Dacorso Filho et al., 1960; Silva, 1961; Nakajima et al., 1991; Ristow et al., 2007; Costa et al., 2010), Southern (Portugal et al., 1979; Ramos et al., 1986; Driemeier et al., 1999), Mid-western (Brautingam et al., 1996; Acypreste et al., 2005), and Northeastern (Mota et al., 2010; Oliveira et al., 2010) regions of the country in animals born and raised in Brazil. The first report in raw milk samples is recent (Carvalho et al., 2009).

The first report of the presence of MAP in milk samples in the country was fairly recent. Although researches on paratuberculosis in Brazil have increased considerably, the studies published in the area are still few and the economic impact of the disease has not been measured in the country. In Brazil, the estimated prevalence of paratuberculosis is higher than in other countries. Further studies are needed to subsidize control measures in the national herd, since, in Brazil, there is no health program for this disease.

## 4. Crohn's disease

Crohn's disease (CD) is an inflammatory bowel disease of unknown etiology, which is characterized by chronic, focal, asymmetric, transmural and granulomatous inflammation, and can affect any segment of the digestive tract, from mouth to anus, although with preference for the distal small intestine and proximal large intestine. The disease has three phenotypes which are: stenosing, penetrating, and not stenotic and non-penetrating.

Incidence and prevalence of CD varies greatly with geographic location. USA, Britain, Scandinavia (especially Norway and Sweden), Italy and countries of northern Europe are considered areas with greater impact. Intermediate incidence areas are represented by the countries of southern Europe, South Africa, Australia and New Zealand. Low incidence is reported in Asia and South America.

The disease can affect individuals of any age, but occurs with greater frequency in patients between 20 and 40 years old. It affects people in their most productive period, with an enormous impact on quality of life of patients. A second peak in incidence, less obvious, is described in patients between 60 and 80 years old, setting a bimodal presentation. The disease has no predilection for sex.

Pathogenesis of CD, although not fully understood, fundamentally involves four aspects that interact with each other and with environmental factors: a) genetic factors; b) luminal factors related to the intestinal microbiota, its antigens, metabolic products and food antigens; c) factors related to the intestinal barrier, including aspects related to innate immunity and intestinal permeability; and d) factors related to immunoregulation, based on the adaptive or acquired immunity.

CD is characterized by periods when the disease is active and others where it is in remission. Symptoms depend on the severity and location of intestinal involvement. In approximately one third of patients, CD involves the small intestine, especially ileum, and in some cases the jejunum. About 20% to 25% of cases present only colonic lesions. The isolated involvement of the jejunum is rare, as well as cases involving the esophagus, stomach and duodenum. The most common clinical manifestations are abdominal pain, diarrhea and weight loss. CD progresses with periods of remission and exacerbation, even after surgical resection of the affected areas. It has a high percentage of complications with

formation of abscesses, fistulas, stenosis, cavity free perforation and anoperineal involvement (Fig. 3). About 70% of individuals affected by the CD undergo surgery and 30% suffer from repeated bowel resections. This disease may be associated with extraintestinal manifestations, and rheumatological, dermatological, ophthalmological, and hepatobiliary nephrology manifestations are the most frequent.

Diagnosis of the disease represents a major challenge, especially when it comes to early diagnosis. It is based on a combination of clinical, laboratory, radiological, endoscopic and histopathological findings. There is no pathognomonic test for the diagnosis of CD.

To date there is no medical or surgical treatment that provides healing of CD. As inflammation is the maximum expression of the disease pathophysiology, clinical therapy, which involves different groups of medications such as aminosalicylates, corticosteroids, immunomodulators and biologic therapy are aimed at blocking the inflammatory cascade and ending the inflammation and scarring of the intestinal mucosa.

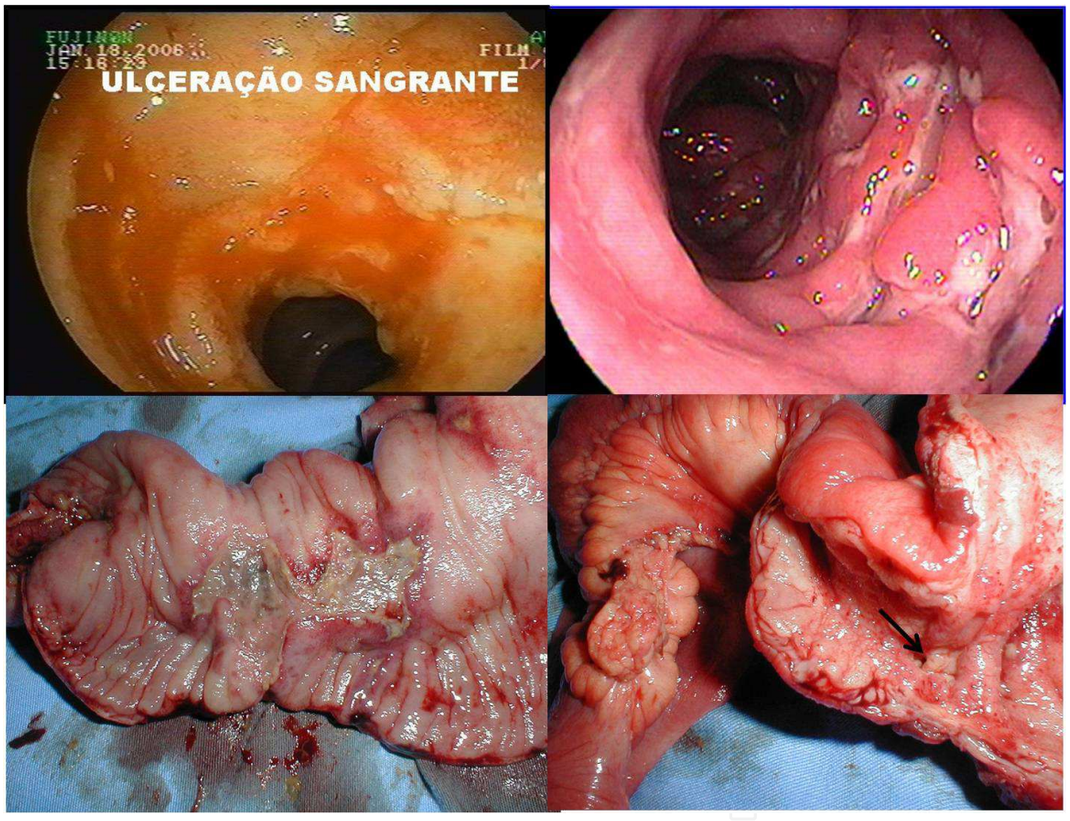


Fig. 3. Endoscopic appearance of Crohn's disease (CD). A: bleeding ulcerated lesion; B: serpiginous ulcerations in the mucosa showing cobblestones appearence. Surgical specimens. C: ileum with extensive ulcerated lesion, D: intestinal wall thickening and opening of the fistulous orifice (arrow).

Although it is a benign disease with low mortality rate, CD is accompanied by high morbidity, with an unpredictable course and the phases of activity, exteriorized through uncomfortable manifestations and/or complications, even when the best care and treatments are implemented. Patients have to live for a long time with limitations that affect directly their daily lives, impacting greatly on the quality of life (Sands, 2006; Brand, 2009).

### 4.1 Crohn's disease in Brazil

Brazil is located in South America, a region considered to have a low incidence of CD. Despite the paucity of epidemiological studies that allow us to know the real incidence and prevalence of the disease, reports of cases, predominantly from universities, have shown that this disease is not rare, and that its incidence had increased in recent decades. This epidemiological profile, now recognized in Brazil, has been previously observed in different countries with a high incidence. Nowadays, these countries have been evolving for stability in the frequency of the disease. For the different areas of Brazil, CD is most frequently observed in states located in the south and southeast, areas with higher socioeconomic development. Currently, there is a joint effort of hospitals for inflammatory bowel diseases to collect reliable statistical data that would serve as an international database.

In one follow up study, researchers evaluated 100 patients with CD in university referral center for inflammatory bowel disease. The mean follow-up was 47.3 months, with variations from one month to 9.5 years. As for gender distribution, 59% were female and 41% male. Age of patients ranged from 16 to 69 years old, with an average of 29.9 and a median of 27 years old. 5% of patients had a family history of ulcerative colitis or CD. Among the clinical manifestations, abdominal pain was the most common symptom observed in 98% of cases, followed by chronic diarrhea in 83% and weight loss in 82% of patients. Regarding the behavior of the disease, stenosing form was observed in 35% of cases, followed by non-penetrating and not stenosing form in 34% of cases and fistulizing form was described in 31% of cases. Of the extraintestinal manifestations, rheumatological manifestation was the most frequently observed, followed by skin and eyes manifestations. 50% of patients underwent surgical procedures and 63% were hospitalized at least once. The authors conclude that the profile of CD in evaluated patients was similar to that described in the literature, and these data were corroborated by other Brazilian authors (Faria et al., 2004; Santana et al., 2008; Torres et al., 2010).

## 5. *Mycobacetrium avium* subspecies *paratuberculosis* vs Crohn's disease

Paratuberculosis and Crohn's disease (CD) are two diseases that share many clinical and histopathological similarities. Both diseases are characterized by chronic inflammation, weight loss and there is no cure.

While there have been, in recent years, various hypotheses about the etiology of CD and the mechanisms that trigger it may be cited as diet, environmental factors, genetic predisposition and autoimmune responses, the two main causal theories are infection and the autoimmune response. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) has been cited as the leading candidate from the point of view of infection.

Considering the high prevalence of MAP in dairy herds and the resistance of microorganisms to disinfectants and pasteurization, several countries have created programs to control the disease considering its possible zoonotic potential.

### 5.1 Facts that support MAP in Crohn's disease

Despite extensive research and large and important advances in the past few decades, the etiology of Crohn's disease (CD) remains unknown, hindering the development of a specific therapy. Due to the similarity of clinical signs and histopathological findings between the two diseases, associations between paratuberculosis in cattle and chronic ileocolitis in humans have been made. Interest in participation in the infectious etiology of CD has

increased with the isolation and detection of DNA of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in samples from patients with the disease. MAP is not classified as a zoonotic agent, but it represents a major concern in the field of public health because of the associations that have been made.

The controversial zoonotic potential of CD grows as more research has been seeking an association between MAP and CD. In recent studies, MAP strains were isolated from human intestinal tissues and blood from patients with CD. It is known that patients with CD are substantially more positive for MAP, regardless of method used, when compared with individuals with ulcerative colitis or individuals without inflammatory bowel disease. The isolation of MAP and detection of MAP DNA from breast milk of nursing mothers with a diagnosis of CD has also been reported and a possible maternal-fetal transmission of MAP may be suggested. These results, however, were not replicated by other researchers and there is no evidence of increased frequency of CD in children of mothers with CD (Naser et al., 2000; Schwartz et al., 2000; Bull et al., 2003; Naser et al., 2004).

In several published reports, presence of antibodies against MAP has also been demonstrated in serum samples from patients with CD by serological tests. Using these tests, there are also significant differences when compared with sera from patients with CD, ulcerative colitis and control patients. This is another fact that supports the association of MAP with CD (Olsen et al., 2009; Rosenfeld and Bressler, 2010).

Molecular techniques have been used for providing faster results than conventional diagnostic techniques. Using PCR-based sequence IS*900*, MAP DNA has also been found in a significantly higher proportion of patients with CD than in patients with ulcerative colitis or patients without inflammatory bowel disease (Abubakar et al., 2008).

Besides all this evidence, CD's patients have been treated successfully using antimycobacterial drugs. If MAP is involved in the etiology of CD, it is expected that antimycobacterial drugs should improve the clinical status of affected patients (Hermon-Taylor, 2002).

Contaminated milk would be the main, but not the only, vehicle of transmission of MAP from animals to human beings. Infected animals can eliminate the organism in this way and furthermore can occur a milk contamination with via fecal material, a route through which the organism is eliminated at higher concentrations. It is known that occurrence of MAP in milk is well documented and several studies have shown that the microorganism can remain viable after being subjected to standard conditions of pasteurization and processes used to produce cheese if it is present in large concentrations. In addition, although not recommended, it is known that raw milk is consumed fresh in many parts of the world, besides being used for the manufacture of several dairy products. A causal association of MAP with CD would have important implications for the processing of milk and other dairy products (Grant et al., 2001).

There is no doubt therefore that there is a potential source of zoonotic infection, considering (1) the widespread dissemination of MAP in dairy herds in Europe, America and Australia, (2) the elimination of MAP in milk of infected animals, (3) the relative strength of MAP to pasteurization process used currently and (4) the recovery of viable MAP in milk samples, water and beef, other potential sources of transmission of MAP. So, considering the association between CD and MAP infection to be correct, the fact that MAP has been detected in foods could be a public health problem.

It is known that the vast majority of studies using many different techniques have detected MAP DNA or cultured the microorganism most frequently in tissue from patients with CD, rather than in those with ulcerative colitis and other disorders. These results are consistent

with two possibilities: either MAP infection could cause CD in a subgroup of patients that are selectively exposed to this microorganism or are genetically susceptible to infection, or alternatively, this microorganism, relatively common in the diet, can colonize selectively to ulcerated mucosa of patients with CD, but not initiate or perpetuate intestinal inflammation (Behr and Kapur, 2008; Hermon-Taylor, 2009; Rosenfeld and Bressler, 2010).

The most plausible theory that would explain a role for MAP in the etiology of CD is related to the recipient NOD2/CARD15. NOD2/CARD15 is an intracellular receptor for muramyl dipeptide (MDP), the smallest immunologically active component of bacterial peptidoglycan. The binding of MDP to the receiver NOD2/CARD15 activates nuclear factor kB. This may contribute to the elimination of intracellular bacterial infection and secretion of α defensins by Paneth cells, which constitutively express NOD2/CARD15. The three most common polymorphisms of this gene lead to a defective activation of nuclear factor kB by the MDP and they are found in 35% of Caucasian patients with CD. NOD2/CARD15 mutations in CD are associated with a decreased expression of α defensins in the mucosa. Thus, a plausible explanation linking NOD2/CARD15 to CD is that a defect in this gene could not result in elimination of intracellular infection by MAP and decreased secretion of luminal α defensins in the mucosa, allowing a greater adhesion and epithelial invasion by the microorganisms ingested (Sartor, 2005).

Despite all the evidence implying an association of MAP with CD, it is not possible to conclude that a single agent is solely responsible for the cause of CD - a multifactorial cause is more likely. The role of MAP in the etiology of CD cannot yet be confirmed or refuted with certainty. The organism can act as a causative agent, it may have a role in the context of infection, it can exacerbate the disease, or it may be non-pathogenic. Clearly, more studies are needed to determine whether MAP infection causes the disease or whether this environmental contaminant innocently lodges in ulcerated mucosa. Well-designed studies are needed to definitively resolve this debate.

### 5.2 Facts that do not support MAP in Crohn's disease

It has been suggested that a relationship between MAP infection and Crohn's exists due to the clinical and pathological similarities between Crohn's disease (CD) in human patients and paratuberculosis in animals. However, there are also many arguments against MAP being the causative agent of CD. Despite the similarities between clinical signs and histopathological features of both diseases, there may be differences in clinical and pathological responses between both diseases, which are not expected if both diseases are caused by the same microorganism.

Another important factor to be considered is the lack of epidemiological support considering the infection's transmission. If animals eliminate MAP in large quantities in feces and milk, it would be expected that the prevalence of CD in people in direct contact with animals infected with MAP was great if the association between the CD and paratuberculosis is true. These facts have not yet been reported (Jones et al., 2006).

Not all patients with CD respond well to treatment with anti-MAP. The failure of treatment in such cases can be attributed to the fact that CD can exist in two forms: one form caused or triggered by infection with MAP and otherwise, induced by some other unknown cause. If this is true, the treatment may be ineffective due to inability to identify patients infected with MAP before the start of treatment. There is not enough evidence to assert the effects of antimycobacterial therapy in patients with CD, but it is suggested that this therapy can help maintain remission of clinical signs of disease (Selby et al., 2007).

Genetic profiles of different strains already isolated have been outlined and possible epidemiological associations between the species from which they were isolated has been studied. Studies have shown that human isolates have profiles more similar to those isolated from sheep and goats than to those isolated from cattle. This fact also contrasts with the idea that cow's milk would be the main source of transmission of MAP from cattle to human beings. In contrast to this fact, there are studies reporting that both animals and humans are susceptible to infection by MAP isolates with similar genotypes (Jones et al., 2006).
Just as there are several research groups associating MAP to CD using detection of MAP DNA in samples of intestinal tissues and blood of patients with CD, other groups have shown the opposite: the same levels of detection of MAP in patients with CD compared with patients with ulcerative colitis or patients without inflammatory bowel diseases. Moreover, the absence of MAP DNA in patients with CD was also reported. Data are very variable for all groups. There is a variation from 0 to 100% of MAP by PCR detection (Abubakar et al., 2008).
Another argument against a possible etiology of MAP in CD is related to the observation of a good response from patients with CD undergoing immunosuppressive therapy.

### 5.3 Conditions in Brazil that could facilitate the transmission from MAP to humans

MAP has been detected in several states in the Southeast, South, Midwest and Northeast regions of Brazil. Despite reports of paratuberculosis in several states, few research groups working in this field are still doing surveys on the disease. In addition to bovine paratuberculosis, there are some groups in the country researching the presence of MAP in goats and sheep.
Although there is a strong and effective control program for tuberculosis in the country, there is not a control program for paratuberculosis. Research into this disease is still preliminary and there has been no survey even made, even superficially, about the economic losses caused by the disease.
Currently, Brazil is the sixth largest milk producing country, with a volume that corresponds to approximately 4.5% of world production (IBGE, 2006). The Southeast region is the largest producing region, accounting for 38.4% of all domestic production. Despite the high production, productivity of dairy herds in Brazil is low. The milk industrial chain is important from the standpoint of economic and social development, generating significant income and jobs in all sectors. Despite all these factors, no studies in the country are aimed at detection of MAP in dairy products and the first detection of MAP in milk was quite recent. MAP was detected by PCR using primers based on the IS*900* sequence in an initial survey about the disease in the Southeast region (Carvalho et al., 2009).
Brazil has also not been reported the presence of MAP in pasteurized milk. A survey in this sense, in the same region where it was detected MAP DNA in raw milk samples, is already in the early stages. In parallel, the resistance of MAP to pasteurization temperatures in the laboratory is being tested.
Brazil has a volume estimated at around 112 billion cubic meters of fresh water of the planet. Moreover, in Brazilian subsoil there is the Brazilian Guarani aquifer, the largest subsoil reservoir of freshwater on the planet. This enormous underground wealth extends over an area of 1.6 million square kilometers, of which two thirds are in Brazilian territory. Even with all that water, there have been no studies in the country to verify the presence of MAP in water.
There are no studies about the possible association between MAP infection and Crohn's disease (CD). A first attempt of an association between MAP infection and CD is being performed. Intestinal biopsies of patients with CD, ulcerative colitis patients and patients

without inflammatory bowel disease are being collected with the aim of isolating MAP and/or detection of DNA of the microorganism for molecular tests.

## 6. Future prospects

Historically, one of the ways to make the connection between a potential agent of an infectious disease and the disease itself was considering whether Koch's postulates were true. For the relationship between MAP infection and Crohn's disease (CD), postulates "The organism can be isolated from a sick patient" and "The organism can be cultured in the laboratory" can still be controversial, but are true. "If the causative microorganism is introduced into another susceptible host, the same disease should be generated" is a postulate a little harder to prove. Disease results from interaction between infection and immune response by the microorganism that causes it. Clinical manifestations of infection depend on several variables such as genetics of the host and the state of the immune system, among others (Rosenfeld and Bressler, 2010).

CD certainly involves host genetic influences and environmental influences that interact to cause clinically evident disease. It is known that MAP is widely present in the human food chain and that MAP DNA can be recovered from intestinal samples of patients with CD. Although existing data do not necessarily involve MAP as a causative agent of CD, this possibility cannot be definitively excluded.

Besides this, there is the difficulty in obtaining experimental models for studies involving human pathogens or potential human pathogens. Such studies are complicated to performed, since although the relationship between MAP and CD is not yet established, it is reckless inoculate MAP in human patients for testing.

There is still much to be learned about MAP and diseases that it can cause in humans. CD may be a syndrome with multiple etiologies that result in clinical, endoscopic, radiological and pathological findings that define the disease. MAP may be one of the etiologies of this syndrome.

## 7. Conclusion

Some studies have shown that the etiology of Crohn's disease (CD) may involve a variety of viral and bacterial agents, including MAP, or an immunological origin. Evidence supports an interaction between a persistent environmental stimulus, such as a microbial antigen, and genetic factors that regulate an immune response or a function of the intestinal mucosa. Recent discoveries in many research fields have generated favorable results suggesting an association between CD and MAP, but that does not necessarily indicate that the microorganism is involved in the etiology of the disease. It is not possible to conclude that a single agent is solely responsible for the cause of CD as a multifactorial cause is more likely. For patients and their physicians, a clear answer on this association is an important step toward establishing control measures and treatment for this debilitating disease. Theories of mycobacterial and autoimmune etiologies of CD should be seen as complementary rather than mutually exclusive. The causal association between CD and MAP infection remains unanswered.

If MAP is responsible for a subset of CD, public health measures should be implemented to eliminate the source of infection in the human food chain and food processing practices must be modified. If there is no evidence of a causal association of MAP and CD, we must direct resources to other research fields. This controversy has persisted for too long and needs to be resolved.

When appropriate methods are used, most patients with CD are detected and there is no data showing that MAP is harmless to human patients. More epidemiological studies seeking to rigorously analyze both diseases are needed. While CD is likely to be a multifactorial condition, MAP can be a primary etiologic agent or a significant secondary invading agent. It is well documented that MAP is present in humans and that there are many routes of transmission and consumption of milk and dairy products are the main, and perhaps the biggest, problem link on the subject, since it has been shown that the microorganism can resist milk pasteurization processes and also the chlorination processes of drinking water. Therefore, until MAP is declared as a non-pathogenic agent for humans, it should be treated as such.

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## 9. References

Abubakar, I., Myhill, D., Aliyu, S.H.& Hunter, P.R. (2008). Detection of *Mycobacterium avium* subspecies *paratuberculosis* from patients with Crohn's disease using nucleic acid-based techniques: A systematic review and meta-analysis. *Inflammatory Bowel Diseases*, Vol.14, No.3, pp.401-410

Acypreste, C.S., Juliano, R.S., Riveira, F.E.B., Silva, L.A.F., Fioravanti, M.C.S.& Filho, F.C.D. (2005). Uso da técnica do ELISA indireto na detecção de anticorpos anti-*Mycobacterium paratuberculosis* em vacas em lactação. *Ciência Animal Brasileira*, Vol.6, pp. 41-45

Behr, M.A.& Kapur, V. (2008). The evidence for Mycobacterium paratuberculosis in Crohn's disease. *Current Opinion in Gastroenterology*, Vol.24, No.1, pp.17-21

Brand, S. (2009). Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease. *Gut*, Vol.58, No.8, pp.1152-1167 1468-3288 (Electronic) 0017-5749 (Linking)

Brautingam, F.E., Glass, R.& Mendy, W., 1996. Levantamento sorológico utilizando a técnica de ELISA para paratuberculose em cinco rebanhos de corte do Mato Grosso do Sul e quatro rebanhos de leite do Estado de São Paulo. In: XV Congresso Panamericano de Ciências Veterinárias, Campo Grande, MT, p. 271.

Bull, T.J., McMinn, E.J., Sidi-Boumedine, K., Skull, A., Durkin, D., Neild, P., Rhodes, G., Pickup, R.& Hermon-Taylor, J. (2003). Detection and verification of Mycobacterium avium subsp. paratuberculosis in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. *Journal of Clinical Microbiology*, Vol.41, No.7, pp.2915-2923

Carvalho, I.A., Silva, A., Jr., Campos, V.E.& Moreira, M.A. (2009). Short communication: detection of *Mycobacterium avium* subspecies *paratuberculosis* by polymerase chain reaction in bovine milk in Brazil. *Journal of Dairy Science*, Vol.92, No.11, pp.5408-5410, ISSN 1525-3198 (Electronic) 0022-0302 (Linking)

Cocito, C., Gilot, P., Coene, M., de Kesel, M., Poupart, P.& Vannuffel, P. (1994). Paratuberculosis. *Clinical Microbiology Reviews*, Vol.7, No.3, pp.328-345

Costa, J.C.M., Pieri, F.A., Souza, C.F., Espeschit, I.F., Felippe, A.G., Santos, G.M., Tobia, F.L., Silva Jr., A.& Moreira, M.A.S. (2010). Levantamento sorológico de *Mycobacterium*

*avium* subesp. *paratuberculosis* em bovinos leiteiros no estado do Espírito Santo. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Vol.62, No.6, pp.1491-1494

Dacorso Filho, P., Campos, I.O.N., Faria, J.F.& Langenegger, J. (1960). Doença de Johne (Paratuberculose) em bovinos nacionais. *Arquivos do Instituto Biológico*, Vol.3, pp.129-139

Driemeier, D., Cruz, C.E.F., Gomes, M.J.P., Corbellini, L.G., Lorett, A.P.& Colodel, E.M. (1999). Aspectos clínicos e patológicos da paratuberculose em bovinos no Rio Grande do Sul. *Pesquisa Veterinária Brasileira*, Vol.19, No.3/4, pp.109-115

Dupont, O. (1915). *Jornal do Commercio*, pp.8

Faria, L.C., Ferrari, M.L.A.& Cunha, A.S. (2004). Aspectos clínicos da doença de Crohn em um centro de referência para doenças intestinais *GED Gastroenterologia e Endoscopia Digestiva*, Vol.23, No.4, pp.151-163

Grant, I.R., Rowe, M., Dundee, L.& Hitchings, E. (2001). *Mycobacterium avium* ssp. *paratuberculosis*: its incidence, heat resistance and detection in milk and dairy products. *International Journal of Dairy Technology*, Vol.54, No.1,

Green, E.P., Tizard, M.L., Moss, M.T., Thompson, J., Winterbourne, D.J., McFadden, J.J.& Hermon-Taylor, J. (1989). Sequence and characteristics of IS*900*, an insertion element identified in a human Crohn's disease isolate of *Mycobacterium paratuberculosis*. *Nucleic Acids Research*, Vol.17, No.22, pp.9063-9073

Harris, N.B.& Barletta, R.G. (2001). *Mycobacterium avium* subsp. *paratuberculosis* in Veterinary Medicine. *Clinical Microbiology Reviews*, Vol.14, No.3, pp.489-512

Hendrick, S.H., Kelton, D.F., Leslie, K.E., Lissemore, K.D., Archambault, M.& Duffield, T.F. (2005). Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds. *Journal of the American Veterinary Medical Association*, Vol.227, No.8, pp.1302-1308 0003-1488 (Print) 0003-1488 (Linking)

Hermon-Taylor, J. (2002). Treatment with drugs active against *Mycobacterium avium* subspecies *paratuberculosis* can heal Crohn's disease: more evidence for a neglected public health tragedy. *Digestive and Liver Disease*, Vol.34, No.1, pp.9-12

Hermon-Taylor, J. (2009). *Mycobacterium avium* subspecies *paratuberculosis*, Crohn's disease and the Doomsday scenario. *Gut Pathogens*, Vol.1, No.1, pp.15 1757-4749 (Electronic) IBGE, 2006. Produção da Pecuária Municipal. Online available: http://www.cnpgl.embrapa.br/nova/informacoes/estatisticas/producao_leite/index.php. Acessed: 5 Nov 2006

Jones, P.H., Farver, T.B., Beaman, B., Cetinkaya, B.& Morgan, K.L. (2006). Crohn's disease in people exposed to clinical cases of bovine paratuberculosis. *Epidemiology and Infection*, Vol.134, No.1, pp.49-56 0950-2688 (Print) 0950-2688 (Linking)

Li, L., Bannantine, J.P., Zhang, Q., Amonsin, A., May, B.J., Alt, D., Banerji, N., Kanjilal, S.& Kapur, V. (2005). The complete genome sequence of *Mycobacterium avium* subspecies *paratuberculosis*. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.102, No.35, pp.12344-12349

Mota, R.A., Peixoto, P.V., Yamasaki, E.M., Medeiros, E.S., Costa, M.M., Peixoto, R.M.& Brito, M.F. (2010). Ocorrência de paratuberculose em búfalos (*Bubalus bubalis*) em Pernambuco. *Pesquisa Veterinária Brasileira*, Vol.30, No.3,

Nakajima, M., Maia, F.C.L.& Mota, P.M.P.C., 1991. Diagnóstico da Paratuberculose em Minas Gerais. In: IV Simpósio Brasileiro em Micobactérias, Bauru, São Paulo.

Naser, S.A., Ghobrial, G., Romero, C.& Valentine, J.F. (2004). Culture of *Mycobacterium avium* subspecies *paratuberculosis* from the blood of patients with Crohn's disease. *The Lancet Infectious Diseases*, Vol.364, No.9439, pp.1039-1044

Naser, S.A., Schwartz, D.& Shafran, I. (2000). Isolation of *Mycobacterium avium* subsp *paratuberculosis* from breast milk of Crohn's disease patients. *American Journal of Gastroenterology*, Vol.95, No.4, pp.1094-1095

Oliveira, D.M., Riet-Correa, F., Galiza, G.J.N., Assis, A.C.O., Dantas, A.F.M., Bandarra, P.M.& Garino Jr, F. (2010). Paratuberculose em caprinos e ovinos no Brasil. *Pesquisa Veterinária Brasileira*, Vol.30, No.1,

Olsen, I., Tollefsen, S., Aagaard, C., Reitan, L.J., Bannantine, J.P., Andersen, P., Sollid, L.M.& Lundin, K.E. (2009). Isolation of *Mycobacterium avium* subspecies *paratuberculosis* reactive CD4 T cells from intestinal biopsies of Crohn's disease patients. *PLoS ONE*, Vol.4, No.5, pp.e5641 1932-6203 (Electronic)

Portugal, M.A.S.C., Pimentel, J.N., Saliba, A.M., Baldassi, L.& Sandoval, E.F.D. (1979). Ocorrência de paratuberculose no estado de Santa Catarina. *O Biológico*, Vol.4, No.1/2, pp.19-24

Ramos, E.T., Poester, F.P., Correa, B.L., Oliveira, S.J., Rodrigues, N.C.& Canabarro, C.E. (1986). Paratuberculose em bovinos no Estado do Rio Grande do Sul. *A Hora Veterinária*, Vol.34, No.6, pp.28-32

Ristow, P., Marassi, C.D., Rodrigues, A.B., Oelemann, W.M., Rocha, F., Santos, A.S., Carvalho, E.C., Carvalho, C.B., Ferreira, R., Fonseca, L.S.& Lilenbaum, W. (2007). Diagnosis of paratuberculosis in a dairy herd native to Brazil. *The Veterinary Journal*, Vol.174, No.2, pp.432-434

Rosenfeld, G.& Bressler, B. (2010). *Mycobacterium avium paratuberculosis* and the etiology of Crohn's disease: a review of the controversy from the clinician's perspective. *The Canadian Journal of Gastroenterology*, Vol.24, No.10, pp.619-624 0835-7900 (Print) 0835-7900 (Linking)

Sands, B.E., 2006, Crohn's Disease, In: Feldman, M.e.a. (Ed.), Sleisenger and Fordtran's Gastrointestinal and liver Disease, Saunders Elsevier, Philadelphia, pp. 2459-2498.

Santana, G.O., Souza, L.R., Azevedo, M., Sá, A.C., Bastos, C.M.& Lyra, A.C. (2008). Application of the Vienna classification for Crohn's disease to a single center from Brazil. *Arquivos de Gastroenterologia*, Vol.45, pp.64-68

Santos, J.A.& Silva, N.L. (1956). Sobre a 1ª observação da paratuberculose no Brasil. *Boletim da Sociedade Brasielira de Medicina Veterinária*, Vol.24, pp.5-14

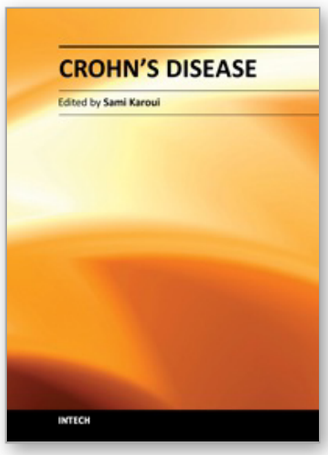
Sartor, R.B. (2005). Does *Mycobacterium avium* subspecies *paratuberculosis* cause Crohn's disease? *Gut*, Vol.54, No.7, pp.896-898, ISSN 0017-5749 (Print) 0017-5749 (Linking)

Schwartz, D., Shafran, I., Romero, C., Piromalli, C., Biggerstaff, J., Naser, N., Chamberlin, W.& Naser, S.A. (2000). Use of short-term culture for identification of *Mycobacterium avium* subsp. *paratuberculosis* in tissue from Crohn's disease patients. *Clinical Microbiology and Infection*, Vol.6, No.6, pp.303-307 1198-743X (Print) 1198-743X (Linking)

Selby, W., Pavli, P., Crotty, B., Florin, T., Radford-Smith, G., Gibson, P., Mitchell, B., Connell, W., Read, R., Merrett, M., Ee, H.& Hetzel, D. (2007). Two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Crohn's disease. *Gastroenterology*, Vol.132, No.7, pp.2313-2319

Silva, N.M. (1961). Estudos sôbre a paratuberculose. *Arquivos do Instituto Biológico*, Vol.4, pp.169-173

Torres, U.S., Rodrigues, J.O., Junqueira, M.S., Uezato, S.& Netinho, J.G. (2010). The Montreal classification for Crohn's disease: Clinical application to a Brazilian single-center cohort of 90 consecutive patients. *Arquivos de Gastroenterologia*, Vol.47, No.3, pp.279-284

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In this book, several important points regarding Crohn's disease are discussed. In the first section, we focus on etiopathogeny of Crohn's disease and the recent advances in our overall understanding of the disease - specifically, the role of the gut epithelium, alterations of the epithelial crypts, and the roles of the different cytokines in the pathophysiology of Crohn's disease. In the second section, a diagnosis of Crohn's disease is discussed. Another particular area of focus is in the diagnosis of intestinal tuberculosis, and the role of mycobacterium avium in Crohn's disease. In the third and final section, the management of Crohn's disease is discussed, with a focus on recent evidence-based medicine recommendations.

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