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REVIEW ARTICLE

Crohn's disease and the mycobacterioses: A quarter century later. Causation or simple association?

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Abstract

It has been more than 25 years since *Mycobacterium paratuberculosis* was first proposed as an etiologic agent in Crohn's disease based on the isolation of this organism from several patients. Since that time, a great deal of information has been accumulated that clearly establishes an association between *M. paratuberculosis* and Crohn's disease. However, data are conflicting and difficult to interpret and the field has become divided into committed advocates and confirmed skeptics. This review is an attempt to provide a thorough and objective summary of current knowledge from both basic and clinical research from the views and interpretations of both the antagonists and proponents. The reader is left to draw his or her own conclusions related to the validity of the issues and claims made by the opposing views and data interpretations. Whether *M. paratuberculosis* is a causative agent in some cases or simply represents an incidental association remains a controversial topic, but current evidence suggests that the notion should not be so readily dismissed. Remaining questions that need to be addressed in defining the role of *M. paratuberculosis* in Crohn's disease and future implications are discussed.

Keywords: Inflammatory bowel disease, Crohn's disease, etiology, *Mycobacterium paratuberculosis*, infectious agents

Introduction

Crohn's disease remains one of the major challenges in gastroenterology. Crohn's disease is an incurable, chronic, progressive, devastating, inflammatory bowel disease often characterized by severe abdominal pain, diarrhea, bleeding, bowel obstruction, perforation, and fistula, as well as a variety of other intestinal and extra-intestinal symptoms that impair the quality of patient life. The natural history of the disease is variable, but the majority of patients tend to have a relapsing and remitting course (Vatn, 2009). The uncertainty of their condition and the ever-present fear of a flare-up and hospitalization often cause patients to experience anxiety, depression, and isolation. The economic impact of this disease is substantial to the patient, their families, the community, and the health care system (Kappelman et al., 2011). Conservatively, it is estimated that there are 500,000 Crohn's disease patients in the USA and 800,000 in North America (Economou et al., 2009). The incidence of disease is rising, both in developed and developing countries (Molodecky et al., 2011).

There is no cure and treatment is supportive at best. The use of a variety of immunomodulating, immunosuppressive, and anti-inflammatory agents provide temporary relief and symptomatic remission can occur, but flare-ups invariably recur over time (Engel & Neurath, 2010). Many patients endure endless regimens of drugs and hospitalizations to alleviate their symptoms.

The clinical course and manifestations of Crohn's disease and patient's response to various medications are so diverse that the disease is best characterized as a syndrome rather than a single specific disease entity. Within that light, it is generally accepted that Crohn's disease may not have a single etiology, but rather, may represent

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Table 1. Prevailing theories involving bacteria as causative agents in Crohn's disease.

Excessive bacterial translocation (Leaky gut)	Inflammation results from the chronic bacterial translocation into the intestinal tissues as a result of abnormal mucosal permeability.
Altered gut microbiome (Dysbiosis)	Inflammation results from a disruption in luminal commensal bacterial homeostasis (dysbiosis) and loss of the protective barrier provided by normal bacterial populations.
Persistent pathogen	Inflammation results from the persistence of a yet to be identified bacterial or viral pathogen within the intestinal tissues.

a clinical syndrome with the possibility of a variety of etiologies or triggering factors (Arnott & Satsangi, 2003).

Long considered an autoimmune disease, the failure to identify any significant auto-antibodies or other intrinsic factors that could account for the inflammatory response and the failure of the disease to meet standard criteria for classification as such (Marks et al., 2010) has led to a general dismissal of that notion. Current concepts focus on a unique combination of genetic predisposition and environmental triggers that result in immunologic dysregulation releasing a cascade of immunologic inflammatory mediators that result in hyper-responsiveness within the gastrointestinal tract (Lees & Satsangi, 2009, Marks et al., 2010).

Although the precise role of bacteria in Crohn's disease remains unknown, all current etiopathogenic theories revolve around the role of bacterial agents in disease causation. These theories have gained support from genomic studies that have identified deficiencies in innate immunity within a significant number of Crohn's disease patients, particularly those cases with ileal disease (Hume & Radford-Smith, 2002, Barrett et al., 2008, Cooney & Jewell, 2009). The predominant theories (Table 1), which may overlap and are not mutually exclusive, include the following:

1. An abnormally permeable mucosal barrier that results in a continuous translocation of commensal bacteria and/or their antigens into the intestinal wall leading to chronic inflammation (Laffineur et al., 1992, McGuckin et al., 2009).
2. A collapse in the balance between "protective" and "harmful" intestinal bacteria leading to a state of dysbiosis and chronic inflammation (Marteau, 2009, Packey & Sartor, 2009).
3. The existence of an unidentified persistent bacterial pathogen within intestinal tissues which continuously drives the inflammatory response (Behr & Schurr, 2006).

There is evidence to support each of these theories, unilaterally or in combination. Recent advances in defining the intestinal microbiome within normal and diseased patient populations (Manichanh et al., 2006, Frank & Pace, 2008, Qin et al., 2010) may shed some light on the types of bacteria involved in the disease process and provide some insight into new therapeutic approaches.

This review covers only the unidentified persistent bacterial pathogen theory, specifically as related to *Mycobacterium avium* subspecies *paratuberculosis* (hereafter referred to as *M. paratuberculosis*[1]).

Throughout the years, a variety of infectious agents have been proposed as causal factors, but most of these suggestions have been short lived and rejected or dismissed, usually because of limited evidence (Hansen et al., 2010, Marks et al., 2010) (Table 2). The inability to correlate any recognized infectious agent with Crohn's disease and the number of "false starts" have caused a possible infectious etiology of some cases of Crohn's disease to be disregarded. However, recent findings of a genetically driven deficiency in innate immunity related to the ability of patients to deal with intracellular organisms in a significant proportion of Crohn's disease patients (Shaoul et al., 2004, Barrett et al., 2008, Cooney & Jewell, 2009, Lees & Satsangi, 2009) have lead many investigators to rethink their position related to the involvement of an underlying infectious agent.

In 1984, the isolation of *M. paratuberculosis* from the diseased intestinal tissues of some patients with Crohn's disease led to the suggestion that this organism may be a causative agent in select cases (Chiodini et al., 1984a). Since that time, *M. paratuberculosis* has been demonstrated at a disproportionately higher frequency in Crohn's disease patients as compared with ulcerative colitis and other control groups, both by cultural methods and the demonstration of specific genetic components of *M. paratuberculosis* in diseased tissues. In addition, sources of human exposure to this agent have been identified within the human food chain and anti-mycobacterial therapy has shown efficacy in some patients (Table 3).

However, the literature is conflicting and difficult to interpret. The result has been the ever increasing division of committed advocates and confirmed skeptics. For more than a quarter century, the suggestion that *M. paratuberculosis* might be an etiologic agent of Crohn's disease has continued to linger; proponents of the suggestion have failed to provide convincing evidence of a causal role and opponents have failed to provide convincing evidence of an incidental role. As a result, the role of *M. paratuberculosis* as an etiology of Crohn's disease remains a controversial subject.

A major contributing factor to the controversy relates to the difficulties in dealing with mycobacteria in general, and in particular, difficult to grow organisms such as

[1]The official name of this organism is *Mycobacterium avium* subspecies *paratuberculosis,* commonly abbreviated as MAP. The authors prefer the original approved name which more accurately reflects its' clinical significance and phenotypic properties.

Table 2. Bacterial agents implicated in the etiology of Crohn's disease and their current disposition.

Infectious agent	Status	Reference
Bacterial		
Listeria monocytogenes	Dismissed	(Liu et al., 1995, Chen et al., 2000)
Pseudomonas maltophilia	Dismissed	(Parent & Mitchell, 1976)
Mycobacterium kansasii	Dismissed	(Burnham et al., 1978)
Bacteroides fragilis	Dismissed	(Persson & Danielsson, 1979)
Chlamydia trachomatis	Dismissed	(Schuller et al., 1979, Müller et al., 2006)
L-form bacteria	Active[a]	(Belsheim et al., 1983, Chiodini et al., 1986)
Adherent-invasive *Escherichia coli*	Active	(Barnich & Darfeuille-Michaud, 2007, Friswell et al., 2010, Hansen et al., 2010)
Mycobacterium paratuberculosis	Active	(Chiodini, 1989, Grant, 2005)
Viral		
Measles virus	Dismissed	(Mikula, 2000)
Cytomegalovirus	Dismissed	(Levesque et al., 2009, Lawlor & Moss, 2010)
Other	Dismissed	(Lidar et al., 2009)

[a]As part of *M. paratuberculosis.*

Table 3. Currently recognized subspecies of the *M. avium* complex.

Subspecies	Disease	Identifying genomic features
paratuberculosis	Johne's disease (paratuberculosis)	IS*1311*+, IS*1245*–, IS*900*+, IS*901*–
avium	Avian tuberculosis	IS*1311*+, IS*1245*+, IS*900*–, IS*901*+
hominissuis	Opportunistic environmental organism	IS*1311*+, IS*1245*+, IS*900*–, IS*901*–

M. paratuberculosis. The unique culture peculiarities of mycobacteria led to the failure of many groups to reproduce the results reported by other investigators due to the lack of experienced technical personnel.

The controversy is best exemplified by the fact that almost all publications primarily related to *M. paratuberculosis* bring to light the association with Crohn's disease and the possible causal role of this agent. In contrast, *M. paratuberculosis* is rarely mentioned in publications focused on Crohn's disease. Within the specialty of gastroenterology, the notion that *M. paratuberculosis* is etiologically related to Crohn's disease, in either a primary or secondary causal role, has largely been discounted but available data fail to support this conclusion. The premature discrediting of this theory by gastroenterologists has hampered funding and hindered progress, scientific inquiry, and resolution of the controversy. The controversial and polarizing issues of *Helicobacter pylori* and peptic ulcers (Barry Marshall; Nobel Laureate, 2005) and transposable elements (Barbara McClintock; Nobel Laureate, 1983) remind us that a mistake is not a mistake unless we fail to learn from it.

The purpose of this article is to attempt providing a critical and objective summary of current knowledge from basic and clinical research and bring to light the research deficiencies and knowledge gaps that must be addressed in defining the role, if any, of *M. paratuberculosis* in Crohn's disease. As proponents and opponents have their valid points of contention, both sides of the issue are presented and readers are left to draw their own conclusions related to the validity of the issues and claims made. As Medline lists more than 2,580 publications on paratuberculosis and 427 publications on paratuberculosis and Crohn's disease, literature cited is selective rather than exhaustive. References were selected based on relevance and/or historical significance.

As discussed below, while the data supporting the etiologic role of *M. paratuberculosis* in some cases of Crohn's disease are inconclusive, the data also suggest that it is premature to dismiss the notion and that there exists a need to resolve this issue in the interest of patient welfare, food and water safety, and scientific inquiry in general.

The putative agent and ruminant *paratuberculosis*

M. paratuberculosis is a facultative obligate intracellular bacterial pathogen and the causative agent of paratuberculosis or Johne's disease, a chronic progressive granulomatous inflammatory bowel disease primarily of ruminant animals (Chiodini et al., 1984a).

To understand the disease caused by *M. paratuberculosis*, it is important to understand its' relationship to the *M. avium* complex, commonly referred to as MAC. As the term MAC, as well as current taxonomic classifications (Thorel et al., 1990), fails to distinguish between the environmental and host-associated ecotypes, which is problematic for interpretation and comparison between studies, we adapt herein the more recent proposed terminology and classifications (Mijs et al., 2002, Turenne et al., 2007, Radomski et al., 2010).

There are 3 distinct variants or ecotypes of *M. avium*, including *M. avium* subsp. *hominissuis, M. avium* subsp. *avium*, and *M. paratuberculosis*, each of which have their own genetic identities and ecological niches (Table 3) (Mijs et al., 2002, Turenne et al., 2007, Radomski et al., 2010). Another subspecies, *M. avium* subsp. *silvaticum*,

probably should not have subspecies status and should be classified with *M. avium* subsp. *avium* for reasons described below.

M. avium subsp. *hominissuis*, as used herein, refers to environmental strains of *M. avium* that are most commonly associated with disease in human patients with AIDS (*hominis*) and in swine (*suis*) and distinct from the types of *M. avium* commonly affecting birds (avian tuberculosis) or ruminants (Johne's disease). Distinguishing features of *M. avium* subsp. *hominissuis* are the multiple copies of IS*1245* and its lack of the IS*901* and IS*900* insertion sequences (Möbius et al., 2006, Turenne et al., 2007). This is the subspecies commonly found in the environment (Primm et al., 2004) giving rise to the frequent statement that *M. avium* is an environmental organism, which we now know is misleading (Turenne et al., 2007). This subspecies is less virulent than the other *avium* subspecies and is rarely found as the causative agent of disease in animals (Pavlik et al., 2000, Dvorska et al., 2003). As compared with other members of the *M. avium* complex, *M. avium* subsp. *hominissuis* has maintained a large genome with the capacity to survive in diverse ecosystems and conditions.

M. avium subsp. *avium*, as used herein, refers specifically to those strains isolated from birds and one of the causes of avian tuberculosis. It contains the IS*901* insertion element and is unequivocally more virulent than *M. avium* subsp. *hominissuis* (Pavlik et al., 2000) and is considered not to be widely dispersed in the environment. Diseased birds and animals with macroscopic lesions and disease are generally infected with IS*901*-positive mycobacteria (*M. avium* subsp. *avium*) versus those isolated from humans, swine, and other animals without lesions which are generally IS*901*-negative mycobacteria belonging to the *hominissuis* subspecies (Pavlik et al., 2000, Dvorska et al., 2003). It is likely that *M. avium* subsp. *avium* evolved from the more common environmental strains (Turenne et al., 2007) becoming an obligate pathogen of birds.

M. paratuberculosis is the causative agent of ruminant paratuberculosis or Johne's disease (Chiodini et al., 1984a). It is acid-fast, slow-growing (8–16 weeks), and mycobactin dependent. It also contains 15 to 20 copies of a unique species-specific insertion sequence known as IS*900* (Collins et al., 1989, Green et al., 1989). By the use of restriction fragment length polymorphism of the IS*900* sequence, at least 2 primary biovariants of *M. paratuberculosis* have been identified: the cattle-associated (C-type) and the less virulent sheep-associated (S-type) (Collins et al., 1990). Although C and S subtypes appear to have cattle and sheep as their respective host preferences, both strains freely cross-infect cattle and sheep, as well as other ruminants (Chiodini et al., 1984a, Semret et al., 2006). Unlike other members of the *M. avium* group, *M. paratuberculosis* does not appear to contain IS*1245* (Johansen et al., 2005).

M. paratuberculosis (strain K-10; GenBank # 262316 and corrected genome (Wynne et al., 2010)) contains a smaller genome than *M. avium* subsp. *hominissuis* (strain 104; GenBank # 243243) (4.83 Mb vs. 5.48 Mb) but shares similar G+C content (~69%). Although the DNA of *M. paratuberculosis* K-10 and *M. avium* subsp. *hominissuis* 104 are 98–99% identical (Li et al., 2005, Wynne et al., 2010), *M. paratuberculosis* contains at least 27 unique genes as compared with strain 104 and has more genes associated with lipid metabolism (Bannantine et al., 2002). The *mbt*A mycobactin synthesis operon, believed to be the initiator of mycobactin synthesis, is truncated in *M. paratuberculosis* (100, 166) which may account for its mycobactin dependency for *in vitro* growth. Similar to *M. avium* subsp. *avium*, *M. paratuberculosis* is not commonly found in, or generally associated with, the environment *unless directly contaminated by infected animals*. It probably evolved from *M. avium* subsp. *hominissuis,* with genomic downsizing and lateral gene transfer (Stinear et al., 2008, Gordon et al., 2009) to become a specialized pathogen occupying a unique biological niche within the submucosal tissues of the gastrointestinal tract of ruminant animals (Turenne et al., 2007).

The disease caused by *M. paratuberculosis*, clinically known as Johne's disease, was first recognized as a disease of cattle in 1826 (Chiodini et al., 1984a). Since that time, the disease has spread throughout the world's livestock populations causing substantial economic losses in the cattle, sheep, and goat industries. Efforts to control the spread of the disease among domestic livestock populations have been largely ineffective, and the disease is now recognized in every country of the world. In the USA, the disease has gone from rare to endemic over the past century. Currently, 8–34% of all dairy cattle and 22–68% of dairy herds (USDA, 2008, Ferrouillet et al., 2009) are *M. paratuberculosis* infected.

The primary mode of infection is via the fecal-oral route. Adult animals with clinical disease may shed more than 10^8 infectious organisms per gram of feces and, considering an average cow defecates 20–40 kg per day, environmental contamination may be great. Animals are generally infected at an early age (<30 days of age), as an age-dependent resistance appears to develop, but clinical signs do not generally occur until 3–5 years of age (early adolescence to prime of life). The severity and onset of clinical disease is dependent on such factors as level of environmental contamination (exposure dose), genetic susceptibility (inter- and intra-species differences in susceptibility), immunologic responses (maturity: age), and virulence of the *M. paratuberculosis* strain (Chiodini et al., 1984a).

The pathologic and immunologic natural history of the disease most closely resembles that of leprosy with tuberculoid and lepromatoid stages and a bipolar immunologic profile during the course of disease.

Whereas *M. tuberculosis* has a predilection for the lungs and *M. leprae* for the skin and peripheral nerves, *M. paratuberculosis* has it for intestinal tissues. It is possible that *M. paratuberculosis* damages intestinal neurons in the same fashion that *M. leprae* damages peripheral

nerves in leprosy (Gwozdz et al., 2001). Experimental infections have clearly demonstrated that intravenous injection and intra-tracheal inoculation of the organism results, not in a systemic disease, but in a gastrointestinal disease (Kluge et al., 1968, Merkal et al., 1968, Merkal et al., 1968, Chiodini et al., 1984a). Experimental studies have also shown that, although extra-intestinal lesions are common and contain acid-fast bacilli, the organism fails to replicate outside the tissues of the gastrointestinal tract (Kluge et al., 1968, Merkal et al., 1968, Chiodini et al., 1984a). Although infection may occur anywhere from the tonsils to the rectum, the most common site of infection is the terminal ileum, most often near the ileo-cecal valve (Chiodini et al., 1984a). The mechanisms and reasons why *M. paratuberculosis* specifically homes to, and replicates in, the gastrointestinal tissues have not been elucidated.

Although primarily a disease of ruminant animals, under select and undefined conditions, monogastric animals, including nonhuman primates (McClure et al., 1987, Zwick et al., 2002), may also become infected and develop intestinal disease. Despite the wide host range, *M. paratuberculosis* has historically been considered innocuous in humans; it has only recently been specifically classified as a biohazard Level II agent.

Immunologic tests fail to identify most animals during subclinical infection and cultivation of the organism is problematic, requiring specialized media, long incubation periods, and experienced personnel (Chiodini et al., 1984a). *M. paratuberculosis* is an extremely fastidious acid-fast bacillus that requires 8–16 weeks (or longer) for growth on artificial media; some strains fail to grow. Because of an apparent inability to synthesize the iron-chelating siderophore mycobactin, an exogenous source of mycobactin is required for its growth in artificial media. As such, human medical laboratories do not have cultivation capabilities to isolate and/or identify this organism; it fails to grow on standard Lowenstein-Jensen medium as commonly used in medical laboratories. The perception that *M. paratuberculosis* in humans is innocuous may simply reflect the inability of diagnostic and research laboratories to seek or investigate this organism in human tissues.

The disease in animals has been extensively reviewed and the reader is referred elsewhere for additional information (Chiodini et al., 1984a, Harris & Barletta, 2001, Manning & Collins, 2001).

M. paratuberculosis was first reported to have been isolated from several patients with Crohn's disease in 1984 (Chiodini et al., 1984b, 1984d). These studies described the isolation of acid-fast mycobactin-dependent organisms which, based on their physical and biochemical properties (Chiodini et al., 1984b, Chiodini, 1986), were suggested to be biovariants of *M. paratuberculosis*. Inoculation of these organisms into newborn goats produced a granulomatous ileocolitis (Chiodini et al., 1984d, Van Kruiningen et al., 1986), and patients with Crohn's disease were shown to have increased immune responses to antigens of this organism (Thayer et al., 1984a). It was subsequently shown that the organism appeared to exist in a cell-wall deficient state, or a spheroplast, in early culture (Chiodini et al., 1986) and restriction fragment length polymorphisms identified the cultured organisms as *M. paratuberculosis* (Chiodini et al., 1986, McFadden et al., 1987, Chiodini, 1988, 1990a, 1990b).

Shortly after these initial reports, the isolation of *M. paratuberculosis* from the diseased tissues of patients with Crohn's disease was reported from a variety of laboratories around the world (Chiodini, 1989) and a great deal of excitement grew within the gastroenterology and microbiology communities. However, it did not take long before reports of failure to isolate *M. paratuberculosis* from Crohn's disease patients began to surface and the suggestions that *M. paratuberculosis* might be a cause of some cases of Crohn's disease was challenged. These early studies have been previously reviewed and the reader is referred elsewhere for additional details and a comparison between Crohn's disease and mycobacterial infections (Chiodini, 1989).

General similarities between Crohn's disease and Johne's disease

Supporting evidence (protagonist's view)

There have been several reports describing the similarities and dissimilarities between Crohn's disease and the disease in ruminants, commonly known as *paratuberculosis* or *Johne's disease* (Chiodini, 1989, Van Kruiningen, 1999, Mayer, 2010). The remarkable similarity ("as similar as may be") between these two diseases has been noted since the early 1900s (Dalziel, 1913). Although differences do exist (Van Kruiningen, 1999), these differences should be expected when crossing wide species barriers and few animal models present with identical pathologic features. Mycobacterial diseases are, in all practicality, immunologic disorders (it is the host's response which causes disease) and, as such, intra- and inter-species variations in the disease are expected (Verna et al., 2007). For example, some ruminant animals develop tuberculoid-type lesions while others develop a lepromatoid form and caseation necrosis occurs in deer, sheep, and goats but never in cattle (Chiodini et al., 1984a). In addition, comparisons are being made between animals in which the natural course of the disease is allowed to progress unimpeded and a disease course in humans which is altered with a myriad of immune modulators and chemotherapeutic agents. As such, identical clinical and pathological manifestations would not be expected. Most zoonotic diseases of animals cause similar, although not identical, disease in humans.

It is also important to note the subclinical versus overt disease distinction in animals which display signs as opposed to symptoms. Animal have a different innervation of their gastrointestinal tract and as a result a comparatively higher pain threshold and cannot voice complaints of abdominal pain or cramping. Thus, the clinical onset of Crohn's disease would more closely

correspond to subclinical cases in animals rather than animals with overt clinical disease.

The similarities between Johne's disease (paratuberculosis) and Crohn's disease are remarkable (Table 4) and has, since 1914 (Dalziel, 1913), driven the notion that the likelihood of association is obvious; "If it looks like a fish, swims like a fish, and smells like a fish, then it probably is a fish."

Opposing evidence (antagonist's view)

While the proponents suggest that the similarities between Johne's and Crohn's disease are "as similar as may be" (Table 4), these similarities are superficial. The disease in cattle, sheep, goats and other ruminants is most often characterized by a lepromatoid inflammatory response with macrophages containing large numbers of mycobacteria. The disease caused by *M. paratuberculosis* infection in animals is more similar to that of *M. avium* subsp. *hominissuis* infection in patients with acquired-immunodeficiency syndrome (AIDS) than it is to Crohn's disease. Thus, Johne's disease is more of an animal model of MAC infection than it is of Crohn's disease. Even in nonhuman primates (McClure et al., 1987), infection with *M. paratuberculosis* results in a lepromatoid inflammatory reaction with macrophages filled with an abundance of acid-fast bacilli. This is nothing like Crohn's disease.

Aside from the superficial gross pathological similarities, there are a host of features found in Crohn's disease that are never seen in animals infected with *M. paratuberculosis* (Table 5). Considering these major differences, Johne's disease can hardly be considered "as similar as may be" to Crohn's disease and an equivalent or even relevant animal model. While the 100-year-old notion (Dalziel, 1913) that "if it lives in the water, looks like a fish, smells like a fish, and swims like a fish, it probably is a fish" has its legitimacy, in this case, it is likely a porpoise and not a fish.

Proponents claim that they have reproduced a chronic inflammatory bowel disease in newborn goats by inoculation of these animals with *M. paratuberculosis* isolated from patients with Crohn's disease (Chiodini et al., 1984d, Van Kruiningen et al., 1986, Allen et al., 2011). The experimental production of a granulomatous ileocolitis in goats and cows with a human isolate of *M. paratuberculosis* has little meaning and does not support an etiologic role of this agent in Crohn's disease. As the putative agent of Crohn's disease has been identified as *M. paratuberculosis*, by definition, the experimental infection is not like Crohn's disease but only Johne's disease (Graham et al., 1988, Van Kruiningen, 1999). Besides, the lesions produced in these experimental animals contained acid-fast bacilli (Van Kruiningen et al., 1986), a major distinguishing feature from Crohn's disease.

There is little evidence from comparative or animal inoculation/model studies to support the causal role of *M. paratuberculosis* in Crohn's disease (Van Kruiningen, 1999).

Culture of *M. paratuberculosis* from Crohn's disease tissues

Supporting evidence (protagonist's view)

Cultivation of *M. paratuberculosis* is problematic even under the best of circumstances, despite the methodological advances made over the past 100+ years (Chiodini et al., 1984d). In addition to fastidiousness, cultivation of *M. paratuberculosis* is hampered because it is the slowest of all cultivable bacterial pathogens. Compared to a doubling rate of 10–12 hours for *M. avium* and 20 minutes for *Escherichia coli, M. paratuberculosis* has an *in vitro* doubling time of 22–26 hours under ideal conditions (Chiodini, 1986, Bannantine et al., 2003). Although *M. paratuberculosis* can be cultured from approximately 72% of clinically infected cattle (animals with overt Johne's disease), successful cultivation is 30% to 50% in cattle with subclinical disease, depending on the stage of infection (Nielsen & Toft, 2008). Successful cultivation is even less frequent in non-bovine species such as sheep, goats, deer, and other ruminants, and some strains fail to grow and have yet to be cultured. Nevertheless, culture of *M. paratuberculosis* is the "gold standard" for the identification of *M. paratuberculosis* infection and Johne's disease.

Despite the challenges and expected low success rate, a variety of investigators have sought to culture *M. paratuberculosis* from a variety of tissue samples with success rates for Crohn's disease ranging from 0% to 40% for classical culture methods and from 0% to 100% when using polymerase chain reaction (PCR) detection of IS*900* in culture media (Table 6). Few studies have used the best culture methods available or maximized recovery by employing a variety of different culture media (Eamens et al., 2000, Whittington et al., 2011).

Studies employing classical microbiologic approaches, that is, subculture to obtain pure cultures and physiologic and morphologic determinations in addition to IS*900* detection, have only identified *M. paratuberculosis* in Crohn's disease patients and not in any control groups. Many of these human-origin *M. paratuberculosis* strains have been made publically available through the American Type Culture Collection (ATCC 43015, 43544, 43545, 49164) for comparative and confirmatory purposes. Success rates from these studies range from 14% to 40% from Crohn's disease patients which is within the range of the success rates reported for the isolation of *M. paratuberculosis* from subclinically infected cattle (Nielsen & Toft, 2008).

The discordance of results between studies likely results from methodological differences and experience with *M. paratuberculosis* isolation. Culture of *M. paratuberculosis* is difficult for even experienced laboratories with trained personnel. Various investigations used NaOH-N-acetyl-L-cysteine during tissue processing (Rath et al., 2011), commonly used in the isolation of *M. tuberculosis* and *M. avium*, but is known to be detrimental to recovery of *M. paratuberculosis*

Table 4. Comparison of the epidemiological, clinical, and pathological features of Crohn's disease and Johne's disease (ruminant paratuberculosis)[a].

Feature	Johne's disease[b]	Crohn's disease
Epidemiological comparison		
Female predominance	Unknown[c]	30–75%
Ileocecal disease	85–95%	85%
Primary age incidence	3–5 (prime of life)	15–25 (prime of life)
Familial association	Yes	Yes
Clinical comparison		
Intermittent diarrhea	Yes	Yes
Abdominal pain	Assumed[d]	Yes
Weight loss	Yes	Yes
Obstruction	No	Yes
Ileac region mass	No	Yes
Blood in stool	Rare	Rare
Remission/quiescent periods	Yes	Yes
Effect of ant-mycobacterial therapy	Not curative[e]	Not curative
Effect of anti-inflammatory/Anti-suppressive agents	Clinical improvement	Clinical improvement
Pathological comparison		
Segmental distribution (skip lesions)	Yes[f]	Yes
Primary disease site	Ileocecal area[g]	Ileocecal area
Strictures	No	Yes
Perforations	No	Yes
Fibrosis	No	Yes
Ulcerations	Yes	Yes
Transmural inflammation	Yes	Yes
Abdominal edema	Yes	Yes
Fissures & fistulae	No	Yes
Sinus tracts	No	Yes
Lymphoid hyperplasia	Yes	Yes
Pseudopolyps	No	Yes
Granulomas	Yes	Yes
Non-caseating granulomas	Yes	Yes
Non-specific inflammation	Yes	Yes
Giant cells	Yes	Yes
Presence of acid-fast bacilli	Usually[h]	No
Immunologic comparison		
Primary T cell response	Type-1[i]	Type-1
Antibody response	No/Yes[j]	No
NOD2/CARD15 associated	Yes[k]	Yes[k]
Systemic comparison		
Frequency of systemic lesion	Rare	Rare
Amyloidosis	Yes	Yes
Granulomatous hepatitis	Yes	Yes
Nephrolithiasis	Yes	Yes
Oral ulcers	Yes	NK[l]
Ocular lesions	Yes	Yes
Skin lesions	Yes	Yes

[a]Data abstracted in part from Chiodini (Chiodini, 1989).
[b]The comparison between Johne's disease and Crohn's disease is a subjective determination and involves the comparison of a disease which is under therapeutic manipulation at early onset (Crohn's disease) and a bipolar disease that is allowed to progress unimpeded till near death (Johne's disease). Therefore, this table reflects the findings in pre-clinical Johne's disease and not the terminal lepromatoid disease most often used in comparison.
[c]Since the majority of older cattle (>2 years of age) are female, predominance cannot be determined.
[d]Animals do not have symptoms and rarely display pain; therefore pain can only be assumed.
[e]No animal has ever been cured of Johne's disease.
[f]Early lesions are segmental. These segmental lesions may coalesce as the disease advances creating a continuous lesion.
[g]Although the disease may be found anywhere from the mouth to anus, the disease is generally manifested in the terminal ileum/proximal colon area. Other lesions rarely occur in the absence of primary ileocecal disease.
[h]Acid-fast bacilli are generally plentiful in advanced clinical disease, although paucibacillary cases do occur. Acid-fast bacilli may be rare or non-demonstrable in pre-clinical (tuberculoid) cases.
[i]Preclinical disease is Th_1-mediated and paucibacillary (tuberculoid). Advanced clinical disease is Th_2-mediated and multibacillary (lepromatoid).
[j]Antibodies are generally demonstrable only in Th_2-mediated advanced lepromatous disease (animals at or near overt clinical onset and terminal disease stage).
[k]NOD2/CARD15 deficiencies identified in 30% of Caucasian Crohn's disease patients.
[l]NK, not known.

Table 5. Features of Crohn's disease that do not occur in animals infected with *M. paratuberculosis* during subcinical (paucibacillary) or clinical (multibacillary) disease (from Van Kruiningen, 1999).

Aphthoid ulcers	Lymphangiectasia
Muscle coat hyperplasia	Obstruction
Transmural inflammation	Pyloric gland metaplasia
Abdominal mass	Dilatation above constrictions
Fibrosis	Perforation
Fat encroachment	Loop-to-loop adhesions
Stenosis	Focal endarteritis and phlebitis
Extensive ulceration	Arthritis
Bloody stools	Spondylitis
Fissures	Episcleritia
Fibrous thickening of mesentery	Iritis
Fistulas	Erythema nodosum
Angulation of the bowel	Pyoderma gangrenosum
Abscesses	Aphthous stomatitis
Sclerosing cholangitis	Thromboembolic complications

Table 6. Summary of attempts to culture *M. paratuberculosis* from tissues of patients with Crohn's disease and control groups[a].

Type of tissue	Identification methodology	Culture media	Culture (mo)	CD	UC	nIBD	Source
Resected intestine	Classical[b]	HEYM	18	4/28	0/10	0/3	(Chiodini et al., 1986)
Resected intestine	PCR[c]	HEYM	30	6/28	0/10	0/3	(Wall et al., 1993)
Resected intestine	Classical/PCR[d]	HEYM	30	10/28	0/10	0/3	—
Resected and biopsied Intestine	PCR[i]	MG3	2–6 years	6/18	0/5	1/6	(Moss et al., 1992)
Resected and biopsied Intestine	PCR[i]	MGIT[e]	12	10/27	0/14	2/22	(Schwartz et al., 2000)
Blood	PCR[i]	MGIT, BACTEC	2–3	14/28	2/9	0/15	(Naser et al., 2004)
Intestinal biopsy	PCR	MGIT	4	19/30	0/2	5/29	(Sechi et al., 2005)
Blood	PCR[i]	MGIT, HEYM, BACTEC	4.5	0/130	0/0	0/130	(Parrish et al., 2009)
Intestinal biopsy	Classical[a,f]	BACTEC, MGIT	12	4/10	0/2	0/2	(Kirkwood et al., 2009)
Blood	PCR[i]	MGIT	2	30/30	29/29	10/10	(Mendoza et al., 2010)[g]
		MGIT	18	30/30[g]	1/29	0/10	
Intestinal biopsy	PCR[i]	MGIT	12	0/75	0/80	0/135[h]	(Ricanek et al., 2010)
Intestinal biopsy	PCR	MGIT	12	0/14	0/49	0/21	(Rath et al., 2011)

[a]Data are not inclusive and articles in which insufficient information was available to precisely define methodology or the results were inconsistent or unclear are not included. The reader is referred to a previous review for data related to early isolation reports (Chiodini, 1989). CD: Crohn's disease; UC: ulcerative colitis; nIBD: non-inflammatory bowel disease controls; HEYM: Herrold's egg yolk media; MG3: veal heart infusion based medium (Markesich et al., 1988); MGIT: mycobacterial growth indicator tube; BACTEC: Bactec 12B medium; ND: not determined.

[b]Classical identification methodology means the isolation of pure culture, demonstration of mycobactin dependency, culture and microscopic characteristics of *M. paratuberculosis*, and confirmation by IS*900* PCR.

[c]PCR Identification methodology denotes that a positive IS*900* signal was the sole source of identification and no data on culture purity, mycobactin-dependency, or colony/bacillary morphology was obtained.

[d]Represents a composite of the 2 prior publications.

[e]No growth occurred in BACTEC 12B media.

[f]All cultures from liquid media were sub-cultured on HEYM for morphologic and mycobactin-dependency determinations and PCR on pure cultures.

[g]See text for explanation of results.

[h]A single IS900 PCR-positive culture was identified as *M. avium* subsp. *hominissuis*.

[i]Denotes use of the same PCR Primer (P90/P91). See Table 8.

(Chiodini et al., 1984a). In addition, various stringency requirements have been used by different authors to designate cultures as positive for *M. paratuberculosis*. For example, in the study by Mendoza et al. (2010) in which blood samples from all patient populations were positive at 8 weeks by IS*900*-PCR, positive cultures at 18 months were presumably identified solely on the basis of non-specific acridine-orange staining.

In addition to classical microbiological methods, *M. paratuberculosis* has also been detected in liquid cultures by IS*900* PCR in resected tissues from 21% to 63% of Crohn's disease patients, 0% of ulcerative colitis patients, and 9–17% of non-inflammatory bowel disease (nIBD) controls; as well as in 50–60% of blood cultures from Crohn's disease patients, 3–22% of ulcerative colitis patients, and in 0% of nIBD controls (Table 6). These data

are well within the range of expected *M. paratuberculosis* detection rates based on the use of comparable methods in cattle and other animals (Whitlock et al., 2000, Nielsen & Toft, 2008).

These data firmly establish that *M. paratuberculosis* can be cultured and/or identified in culture media from a significant number of patients with Crohn's disease as compared with both ulcerative colitis and nIBD controls. Meta-analysis has revealed that *M. paratuberculosis* IS*900* is seven times (7×) more likely to be detected in Crohn's disease patients as compared with controls (Feller et al., 2007). It is important to note that *M. paratuberculosis* has only been recovered, in pure culture with microbiologic confirmation, from humans with Crohn's disease; the organism has never been isolated in pure culture from ulcerative colitis patients or any other control groups.

Opposing evidence (antagonist's view)

Although it is true that *M. paratuberculosis* has been cultured almost exclusively from Crohn's disease by classical microbiological methods and predominately from Crohn's disease by PCR detection in liquid cultures (Table 6), such findings are more consistent with opportunistic colonization (growth on the luminal mucosal surface) than causality and bona fide infection. *M. paratuberculosis* is widely distributed in the environment (USDA, 2010) and the deep crypts found in Crohn's disease, not found in other disease states, support mucosal surface colonization and replication of *M. paratuberculosis* in Crohn's disease leading to the findings associating *M. paratuberculosis* with this disease. The situation may simply represent mucosal growth in a dysbiotic environment.

There have been too few studies employing classical microbiologic methodologies for the culture of *M. paratuberculosis* from Crohn's disease patients and controls to draw meaningful conclusions about the reported recovery rates. An element of concern regarding these culture findings is the fact that most *M. paratuberculosis* isolates that have been recovered from the Crohn's disease patient tissues were cultivated in veterinary laboratories, where the agent of Johne's disease is commonly grown, thereby raising concerns of sample contamination (Van Kruiningen, 1999). Table 6 does not accurately reflect the actual number of failed attempts to cultivate mycobacteria from Crohn's disease tissues over the years.

In addition, the alleged culture of *M. paratuberculosis* from the blood of patients with Crohn's disease (50–60%), ulcerative colitis (3–22%), and nIBD controls (0%) is directly correlated with the degree of dysbiosis and bacterial translocation and suggests mucosal colonization rather than an invasive etiological role.

Patients with Crohn's disease and ulcerative colitis have increased bacterial translocation and a higher frequency of demonstrable bacteria in their blood (Harada et al., 2008, Schulzke et al., 2009, John et al., 2011) as a result of architectural disruption of the intestinal barrier and increased permeability. The culture of *M. paratuberculosis* from the blood of patients is consistent with mucosal surface colonization and translocation across an altered mucosal barrier. This is more clearly supported by the detection of *M. paratuberculosis* by PCR in Crohn's disease and controls as discussed below.

Although bacteremia may occur at various times during the disease process in animals, it generally occurs late in the disease process with widespread infection or space-occupying multi-bacillary (lepromatoid) lesions as bacteria-laden macrophages are forced out of the intestinal lamina propria and regional lymph nodes into the bloodstream and lymphatics (Chiodini et al., 1984a). Crohn's disease is not a lepromatoid disease with an abundance of *M. paratuberculosis*-laden macrophages and the culture/detection of this organism in the blood of patients is inconsistent with a causal role.

The failure to recover *M. paratuberculosis* by culture or to detect IS*900* in culture media in early Crohn's disease, as opposed to patients with long-term disease (Ricanek et al., 2010) further suggests that the detection of *M. paratuberculosis* is associated with the dysbiosis resulting from long-term disease. Culture data only suggests mucosal surface detection of *M. paratuberculosis* and possible translocation of an environmental organism that is unrelated to causality. There is no evidence that *M. paratuberculosis* is being detected in the submucosal tissues of the gut as opposed to the luminal mucosal surface.

Detection of *M. paratuberculosis* in Crohn's disease intestinal tissues

Supporting evidence (protagonist's view)

With the advent of molecular biology, PCR, and identification of a species-specific insertion element (IS*900*) in *M. paratuberculosis* (Collins et al., 1989, Green et al., 1989), there was a surge in activity seeking to identify IS*900* in tissues from patients with Crohn's and other diseases. As would be expected with a new technology and unique application, results were inconclusive and conflicting; reports ranged from 0% to 100% detection each in Crohn's disease, ulcerative colitis, and controls using a variety of different methodologies and IS*900* target sequences (Table 7) (Chiodini, 1989, Jayarao & Shreekumar, 1999, Feller et al., 2007, Abubakar et al., 2008).

Although PCR is considered to be a routine method commonly employed in research and clinical laboratories, these various studies exemplify the inherent difficulties and variability of PCR assays when methods have not been standardized, that is, in-house methodologies. As a result, data between studies are difficult to compare, analyze, or even find consensus as each study tended to use different patient populations, different types of tissues, different PCR methods and primers (Table 8), different tissues and DNA extraction methods, and the like; each report has its own unique set of weaknesses and strengths. Differences between laboratory detection rates

Table 7. Detection of *M. paratuberculosis* IS*900* by PCR in tissues from patients with Crohn's disease, ulcerative colitis and nIBD controls[a].

Methodology	Tissue type[b]	CD	UC[c]	nIBD	Primers[d]	Source
IS*900* PCR	Resection	26/40	1/23	5/40	P90/P91	(Sanderson et al., 1992)
IS*900* PCR	Biopsy	6/18	0/5	1/6	P90/P91	(Moss et al., 1992)
IS*900* PCR	Biopsy	6/25	0/2	0/3	Wp1/Wp2[e]	(Wall et al., 1993)
IS*900* PCR	Mixed	13/18	1/5	7/24	Is150C/Is921	(Dell'Isola et al., 1994)
Nested IS*900* PCR	Paraffin	4/58	0/55	0/17	PTB1/PTB2	(Lisby et al., 1994)
	Resection	11/24	2/10	3/28		
IS*900* PCR	Paraffin	4/31	0/10	0/20	Wpt1/Wpt2[e]	(Fidler et al., 1994)
IS*900* PCR	Mixed	0/68	0/49	1/26	P90/P91	(Rowbotham et al., 1995)
Nested IS*900* PCR	Biopsy	2/9	2/15	0/11	TDB3/TDB4[f]	(Murray et al., 1995)
IS*900* PCR	Biopsy	10/10	11/18	14/16	P90/p91	(Suenaga et al., 1995)
RT IS*900* PCR	Resection	8/8	0/2	0/2	Wp1/Wp2[e]	(Mishina et al., 1996)
Nested IS*900* PCR	Biopsy	17/36	6/13	13/23	rRNA	(Dumonceau et al., 1996)
		0/36	0/13	0/23	P90/P91	
Nested IS*900* PCR	Paraffin	0/23	0/0	0/11	P90/P91	(Frank & Cook, 1996)
IS*900* PCR	Biopsy	0/10	0/6	0/21[c]	P90/P91	(Al-Shamali et al., 1997)
Nested IS*900* PCR	Paraffin	17/36	2/18	3/20	P90/P91	(Huatian et al., 1997)
IS*900* PCR	Biopsy	1/21	0/5	0/11	P90/P91	(Clarkston et al., 1998)
IS*900* PCR	Resection	16/19	11/13	8/21	rRNA	(Kallinowski et al., 1998)
		0/19	0/13	0/21	P90/P91	
Nested IS*900* PCR	Mixed	0/30	0/14	0/3	P902/P912 & Is150C/Is921	(Chiba et al., 1998)
Nested IS*900* PCR	Mixed	2/47	1/27	2/20	Cp1/Cp2[e,f]	(Cellier et al., 1998)
Nested IS*900* PCR	Resection	0/13	0/14	0/13	P90/P91	(Kanazawa et al., 1999)
Nested IS*900* PCR	Resection	15/79	16/61	3/48	PTB1/PTB2	(Collins et al., 2000)
Nested IS*900* PCR—LCM	Paraffin	6/15	0/0	0/12	P902/P912; Rp1/Rp2	(Ryan et al., 2002)
Taqman IS*900* qPCR	Paraffin	0/16	0/11	0/18	P90/MPARA-R[f]	(Fujita et al., 2002)
IS*900* PCR	Paraffin	0/35	0/36	0/21	Is150C/Is921	(Ellingson et al., 2003)
Nested IS*900* PCR	Biopsy	0/24	0/28	6/28	P902/P912[g]	(Bernstein et al., 2003)
Nested IS*900* PCR	Biopsy	34/37	0/0	9/34	L1/L2 & Tj1/Tj2	(Bull et al., 2003)
Nested IS*900* PCR	Paraffin	8/82	0/20	0/20	P89/P92	(Sechi et al., 2004)
IS*900* PCR	Paraffin	0/18	0/0	0/0	Bp1/Bp2[e,f]	(Baksh et al., 2004)
Nested IS*900* PCR	Blood	13/28	4/15	3/9	P90/P91	(Naser et al., 2004)
IS*900* PCR & Taqman IS*900* qPCR	Biopsy	25/30	0/2	3/29	P89/P92 + Sp1/Sp2[e,f]	(Sechi et al., 2005)
IS*900* PCR	Biopsy	25/37	0/0	7/34	P89/P92	(Sechi et al., 2005)
Nested IS*900* PCR	Resection	52/100	2/100	5/100	L1/L2	(Autschbach et al., 2005)
IS*900* PCR	Blood	0/73	0/0	0/73	P89/92	(Lozano-Leon et al., 2006)
Nested IS*900* PCR	Biopsy	15/63	6/54	7/45	L1/L2	(Clancy et al., 2007)
IS*900* PCR	Blood	122/361	0/0	43/200	SF214/ SR289	(Bentley et al., 2008)
Semi-nested IS*900* PCR	Paraffin	1/20	0/0	0/0	S1/A1	(Toracchio et al., 2008)
Nested IS*900* PCR & IS*900* PCR	Blood	0/130	0/0	1/130	P90/p91;p902/p912[g];Tj1/tj2	(Parrish et al., 2009)
PCR	Mixed Paraffin	0/56	0	0/20	rRNA gene[f]	(Knösel et al., 2009)
Nested IS*900* PCR	Blood	8/50	2/25	0/31	TJ1/TJ2	(Kirkwood et al., 2009)
	Biopsy	39/56	7/22	6/39		
Nested IS*900* PCR	Biopsy	20/23	15/20[c]	3/20	TJ1/TJ2	(Scanu et al., 2007)
Nested IS*900* PCR	Biopsy	0/81	0/0	0/85	P90/p91	(Sasikala et al., 2009)
Nested IS*900* PCR	Blood	30/30	29/29	10/10	P90/P91	(Mendoza et al., 2010)
Nested IS*900* PCR & taqman	Biopsy	1/14	10/49	7/21	TJ1/TJ2	(Rath et al., 2011)
Nested IS*900* PCR	Paraffin	7/20	1/20	1/19	L1/L2 & Tj1/Tj2	(Lee et al., 2011)
Nested IS*900* PCR	Feces	21/31	13/20	11/23	P902/P912[h]	(Tuci et al., 2011)
*q*PCR	Feces	2/6	0/5	16/1282	Various[f]	(Imirzalioglu et al., 2011)

[a]Data are not inclusive and articles in which insufficient information was available to precisely define methodology or the results were inconsistent or unclear are not included. See previous review for older studies (Chiodini, 1989). CD: Crohn's disease; UC: ulcerative colitis; nIBD: non-inflammatory bowel disease.

[b]Type of tissue examined in the study. Resection: resected intestinal tissues; Biopsy: intestinal biopsy; Paraffin: paraffin-embedded tissues; Blood: peripheral blood cells; Mixed: resected intestinal tissues and intestinal biopsy with no defined distinction in results.

[c]Control group consisted of patients diagnosed with irritable bowel syndrome (IBS).

[d]Nickname or other published designation of the PCR primer sets used in the study.

[e]No published or otherwise public primer designation. Primers were arbitrarily named herein for the purpose of comparison.

[f]Unique sequence used only in the corresponding study.

[g]Modified version of P90/P91. See Table 8 for explanation.

[h]Modified version of the P901/P912 primers.

Table 8. PCR Primers used in various studies to detect *M. paratuberculosis* in Crohn's disease[a].

Forward primer	Reverse primer	Nickname	Source
GTTCGGGGCCGTCGCTTAGG	GAGGTCGATCGCCCACGTGA	P90/P91[b]	(Sanderson et al., 1992)
*GAAGGG*TGTTCGGGGCCGTCGCTTAGG	*GGCGTT*GAGGTCGATCGCCCACGTGAC	P902/P912[b]	(Millar et al., 1996)
TGGACAATGACGGTTACGGAGGTGG	TGATCGCAGCGTCTTlGGCGTCGGT	Wp1/Wp2	(Wall et al., 1993)
CCGCTAATTGAGAGATGCGATTGG	AATCAACTCCAGCAGCGCGGCCTCG	Dp1/Dp2	(Vary et al., 1990)
CTGGGCGCTGAGTTCCTCG	CAGCATTGCCCACAGGACGT	Cp1/Cp2	(Cellier et al., 1998)
GTTCGGGGCCGTCGCTTAGG	GCGGGCGGCCAATCTCCTT	P90/MPARA-R	(Fujita et al., 2002)
CTTTCTTGAAGGGTGTTCGG	ACGTGACCTCGCCTCCAT	L1/L2	(Bull et al., 2003)
GCTGATCGCCTTGCTCAT	CGGGAGTTTGGTAGCCAGTA	TJ1/TJ2	(Bull et al., 2003)
CGTCGGGTATGGCTTTCATGTGGTTGCTGTG	CGTCGTTGGCCACCCGCTGCGAGAGCAAT	P89/P92	(Sechi et al., 2001)
CCGACGCGATGATCGAGGAG	GAACTCAGCGCCCAGGATGA	Sp1/Sp2	(Sechi et al., 2005)
ATGACGGTTACGGAGGTGGTT	TGCAGTAATGGTCGGCCTTAC	SF214/SR289	(Bentley et al., 2008)
GATGGAGGCGAGGTCACGT	CTTGCCTTCGCCGCGGTAA	S1/A1	(Toracchio et al., 2008)
GCATGGTTATTAACGACG	CGAAAGTATTCCAGCAGC	PTB1/PTB2	(Lisby et al., 1994)
GGAGCGATTTCGCCGCCA	GTCGGCGGAGGCGAATGC	Bp1/Bp2	(Baksh et al., 2004)
GCCCGGATGCGCCACGACTT	GCGCGGCACGGCTCTTGTTGTA	Rp1/Rp2	(Ryan et al., 2002)
GAGAATTCGTGCT TAACACATGCAAGTCG	ATGGATCCGTGAGATTTCACGAACAACGC	rRNA	(Dumonceau et al., 1996)
GCGCCTGCTACCTGTCGG	GACAGCGTCGTCGCGCAG	Tdb3/Tdb4	(Murray et al., 1995)

[a]May not be inclusive of all primers used and the original source of the primer is referenced even if the primer name was changed in subsequent uses.
[b]Primer pair P90/P91 was originally designed by Sanderson et al. (Sanderson et al., 1992). After related IS*900*-like sequences were discovered that hybridized with this primer pair, it was modified by Millar et al. (Millar et al., 1996) by the addition of nucleotides depicted by italic type; however, Millar et al. maintained the original name P90/P91 for this new primer set. To avoid confusion, the modified P90/P91 primers are designated P902/P912. Most studies continue to use the original P90/P91 primer set.

for *M. paratuberculosis* (range: 0–100%) are also likely related to the facilities and technical skills of each laboratory as multi-centered trials using the same samples and techniques have produced different and conflicting data (Naser et al., 2009).

The results of PCR-detection of *M. paratuberculosis* in Crohn's disease are generally inconsistent and display considerable heterogeneity. However, in-house PCR methods used in the identification of *M. tuberculosis* have also been shown to be error prone because of the many methodological variances among laboratories (Flores et al., 2005). The inherent difficulty in lysing mycobacteria (Singh et al., 2008) and the organisms' buoyancy, which may prevent it from sedimentation during centrifugation, contribute to this variability and heterogeneity of results. Although PCR is extremely sensitive, being theoretically capable of detecting a single genome in a sample, the dilution effect of tissue processing, in addition to other variances, also contribute to assay accuracy as demonstrated by the higher sensitivity of culture in tuberculosis (Greco et al., 2006) and in light shedding dairy cattle (Singh et al., 2008) as compared with PCR.

There have been three publications on the systematic meta-analysis on *M. paratuberculosis* detection in tissues from patients with Crohn's disease by PCR and other molecular techniques (Jayarao & Shreekumar, 1999, Feller et al., 2007, Abubakar et al., 2008).

Jayarao (Jayarao & Shreekumar, 1999) performed a systematic meta-analysis of 20 available peer-reviewed publications representing a total of 458 patients with Crohn's disease and 584 controls between 1990 and 1998. Analysis of available data using PCR-techniques to detect *M. paratuberculosis* in tissues suggested that *M. paratuberculosis* could be detected from a significantly higher number of patients with Crohn's disease (odds ratio 2.35:1) as opposed to controls.

Feller et al. (2007) preformed a systematic review to assess the evidence for an association between *M. paratuberculosis* and Crohn's disease by analyzing 18 case-control studies comparing the detection of *M. paratuberculosis* in patients with Crohn's disease to nIBD controls between 1992 and 2005. The prevalence of *M. paratuberculosis* DNA was found to be higher in patients with Crohn's disease than in nIBD controls in 16 of the 18 studies, resulting in a pooled odds ratio of 7.01 (95% confidence interval [CI], 3.95–12.4). There was no difference whether straight PCR or nested PCR methodologies were used. In the 12 studies which compared the detection of *M. paratuberculosis* in Crohn's disease with nIBD and ulcerative colitis, the odds ratios were similar, 6.88 (3.28–14.4) and 4.13 (1.57–10.9), respectively. Feller et al. concluded that, on the basis of 18 case-control studies, systematic review and meta-analysis shows that detection of *M. paratuberculosis* is substantially more common in patients with Crohn's disease, independent of whether patients with Crohn's disease are compared with nIBD controls or patients with ulcerative colitis.

Abubakar et al. (2008) also performed a systematic review and meta-analysis of 47 studies using nucleic acid-based techniques to detect *M. paratuberculosis* in patients with Crohn's disease as compared with controls. The pooled estimate of risk difference from all included studies between 1996 and 2006 was determined to be 0.23 (95% CI, 0.14–0.32) using a random effects model indicating that *M. paratuberculosis* IS*900* is detected significantly more frequently in Crohn's disease than in controls. Similarly, *M. paratuberculosis* was detected more frequently from patients with Crohn's disease as compared with those with ulcerative colitis (risk difference

0.19, 95% CI, 0.10–0.28). Their data further confirm the observation that *M. paratuberculosis* is detected more frequently among Crohn's disease patients compared with controls and demonstrate that there is a high strength of association with a reasonable level of consistency between *M. paratuberculosis* and Crohn's disease, across many sites, by many investigators, and controlling for a number of factors.

The observation that the detection of *M. paratuberculosis* IS*900* within biopsy material appears to be stable over time during disease recurrence (up to 6 years) further supports an etiologic role of this agent (Wagner et al., 2011).

It is well established, and generally accepted, that *M. paratuberculosis* can be detected in a significant number of patients with Crohn's disease and that an association between *M. paratuberculosis* and Crohn's disease does exist (Nacy & Buckley, 2008).

Opposing evidence (antagonist's view)

The detection of *M. paratuberculosis* or IS*900* in tissues and blood is perhaps the most convincing evidence that *M. paratuberculosis* is widely distributed in the environment, that the human population is commonly exposed to this organism (without any apparent ill-effect) and that *M. paratuberculosis* commonly colonizes the mucosal surfaces of the intestinal lumen.

The recent study by Lee et al. (2011), which sought the presence of IS*900* in pediatric patients, illustrates the general mucosal colonization by *M. paratuberculosis* and suggests that there is simply increased mucosal surface colonization (dysbiosis) in Crohn's disease that is unassociated with causality. In that study, using stringent data interpretation (i.e., all three IS*900* replicates were positive), IS*900* was detected in 35% of patients with Crohn's disease, 5% of patients with ulcerative colitis, and in 5.2% of controls. However, when the presence or absence of IS*900* was used in the interpretation of data (case definition: any one of the three replicates being positive), no significant association with Crohn's disease was found as compared with ulcerative colitis (58% vs. 53% positive, respectively) suggesting dysbiosis and increased mucosal surface colonization in inflammatory bowel disease.

Whether 1 or 3 replicates are positive in an assay relates solely to the sensitivity of the assay (not specificity) and the number of copies of the template (in this case, *M. paratuberculosis* IS*900*). Thus, these data support the notion that *M. paratuberculosis* is present in most individuals as a commensal organism but there are simply more found in Crohn's disease. These findings are consistent with the observation that there are increased organisms of the genus *Bacteroides* (Manichanh et al., 2006, Gillevet et al., 2010) in Crohn's disease resulting from a generalized dysbiotic state of the affected intestinal tissues and supports the mucosal colonization by *M. paratuberculosis* unrelated to causality.

Further suggesting that the detection of *M. paratuberculosis* is an incidental finding is the failure to show a consistent association between the detection of this pathogen and the presence of granulomas, NOD2/CARD15 polymorphisms, length of disease, disease activity, disease location, immunosuppressive therapy, age, sex, or any other clinical parameter (Romero et al., 2005, Bernstein et al., 2007; Bentley et al., 2008).

In addition to intestinal diseases, *M. paratuberculosis* has been found to be "associated" with scrofula and lympadenitis (Hermon-Taylor et al., 1998), sarcoidosis (McFadden & Fidler, 1996), type 1 diabetes (Rosu et al., 2009), Blau Syndrome (Dow & Ellingson, 2011), Hashimoto's thyroiditis (Sisto et al., 2010), multiple sclerosis (Cossu et al., 2011), and others. One may be led to believe that *M. paratuberculosis* may be the cause of all idiopathic diseases of man (Thomas Dow, 2008), and maybe even of dogs (Glanemann et al., 2008). These data all suggest a ubiquitous environmental organism that is, at most, an opportunistic pathogen and not a primary cause of Crohn's disease or any other human disease.

Furthermore, reports suggesting that *M. paratuberculosis* IS*900* can be detected in the blood of 46–50% of patients with Crohn's disease, 22–44% of patients with ulcerative colitis, and 0–20% of controls (Table 7) is higher than that reported in animals chronically infected and/or exposed to *M. paratuberculosis* and further diminishes the importance of the *M. paratuberculosis*–Crohn's disease association.

Bhide et al. (2006) performed *M. paratuberculosis* IS*900* PCR on the blood of 262 cattle and 78 sheep from infected farms by methodologies similar to those applied in humans. Only 30 of 262 (11.45%) cattle and only 1 of 78 (1.28%) sheep from paratuberculosis-endemic herds were found to be positive for IS*900* in blood. Separating cattle into 136 clinically healthy and 126 clinically unhealthy animals, IS*900* detection rates in blood were 5.9% and 17.5%, respectively. Juste et al. (2005) also examined blood from cattle and sheep by IS*900*-PCR and found positive results in 56 of 278 (20%) cattle and in 52 of 496 (10.5%) sheep from endemically infected herds.

It is generally agreed that the primary source of *M. paratuberculosis* is the domestic livestock population, notably cattle, sheep, and goats. It is also generally recognized that the environment of chronically infected herds is heavily contaminated with *M. paratuberculosis* (Raizman et al., 2004). The suggestion that patients with Crohn's disease (and some control groups) have *M. paratuberculosis* circulating in their blood more frequently than animals living in a heavily contaminated area are inexplicable, contrary to conventional wisdom, and greatly diminishes the significance of these findings.

A similar inexplicable finding has been observed with the examination of feces for *M. paratuberculosis* IS*900*. In the study by Tuci et al. (2011), *M. paratuberculosis* IS*900* was detected in 68% of Crohn's disease patients, 65% of patients with ulcerative colitis, and 48% of healthy controls. Such detection rates are higher than those reported for the detection of *M. paratuberculosis* IS*900* in feces of cattle from infected herds (Douarre et al., 2010).

The data support the view that *M. paratuberculosis* is a ubiquitous environmental organism that colonizes the mucosal surfaces of the gut resulting in translocation and increased detection in Crohn's disease patients. The dysbiosis and reduced bacterial diversity of the intestinal microbiome in Crohn's disease (Manichanh et al., 2006, Qin et al., 2010) likely promotes *M. paratuberculosis* mucosal surface growth and detection.

Direct observation of *M. paratuberculosis* in Crohn's disease tissues

Supporting evidence (protagonist's view)

Several studies have attempted to show the presence of *M. paratuberculosis* within the diseased tissues of patients with Crohn's disease by a variety of different methods including light microscopy, immunofluorescense, and *in situ* hybridization (Table 9). Although most studies reported the ability to detect *M. paratuberculosis,* and/or even acid-fast bacilli (Jeyanathan et al., 2006), predominantly in tissues of Crohn's disease patients, these studies have been few.

The difficulties in detecting *M. paratuberculosis* within the diseased tissues may relate to its existence and presence within tissues in an altered cell-wall deficient state (spheroplast) and the paucibacillary infection that ensues. Until these technological limitations are resolved, the demonstration of *M. paratuberculosis* in tissues of patients with Crohn's disease will be challenging.

Nevertheless, in the systematic review and meta-analysis by Abubakar et al. (2008), a number of *in situ* hybridization studies were included. Although there was significant evidence of heterogeneity (chi-square = 301.29, $p < 0.001$), the summary measure of risk difference was 0.43 (95% CI, 0.01–0.84) indicating that the probability of a positive result is significantly higher in Crohn's disease as compared with controls.

Opposing evidence (antagonist's view)

Acid-fast bacilli are not demonstrable within the lesions of Crohn's disease, and *M. paratuberculosis* cannot be reproducibly detected by immunohistochemistry, *in situ* hybridization, or other detection methods (Table 9). Although most of the published studies suggest that *M. paratuberculosis* can be detected in tissues, these studies do not accurately reflect the state of our knowledge or the thousands of failed attempts to demonstrate mycobacteria in Crohn's disease over the past 75 years. It is common knowledge that mycobacteria, that is, acid-fast bacilli, are not present within the tissues of patients with Crohn's disease.

The two explanations offered by proponents for the inability to visually demonstrate mycobacteria within Crohn's disease tissues (i.e., paucibacillary disease and the existence of cell-wall deficient bacterial variants) are inherently flawed.

Although it is well documented in the literature that cell wall deficient forms of mycobacteria and other microbes do exist (Onwuamaegbu et al., 2005, Beran et al., 2006), much of the available information is presumed rather than documented or defined. Many studies presume the existence of cell-wall deficient states based on pleomorphic appearing material within unknown cultures that failed to or stained lightly by acid-fast techniques and the reliance of IS*900*-PCR to identify the material as *M. paratuberculosis* (Naser et al., 2002, Juste et al., 2008, Naser et al., 2009). PCR only detects DNA and does not differentiate between physical and physiological forms. Furthermore, no investigation has demonstrated spheroplast-like bodies by ultrastructure or any other means in tissues from patients with Crohn's disease (Dvorak & Dickersin, 1979, Bataille et al., 2004). To suggest that cell-wall deficient organisms can be identified based only on the detection of IS*900* DNA (Hulten et al., 2000) is erroneous. Without the demonstration of spheroplast forms within tissues, the relevance of their detection in culture must be questioned.

Bacterial infections are generally associated with large numbers of bacteria that are readily demonstrable in tissues—there are no known chronic progressive paucibacillary diseases. Although tuberculoid (paucibacillary) leprosy and paucibacillary paratuberculosis (Johne's

Table 9. Demonstration and observation of *M. paratuberculosis* in Crohn's disease tissues and controls[a].

Method[b]	Detector	Tissue[c]	CD	UC	nIBD	Source
ICC	Ag/Ab	Paraffin	0/16	0/0	0/0	(Cartun et al., 1993)
ISH	IS*900*	Paraffin	7/37	2/21	0/22	(Hulten et al., 2001)
ISH	IS*900*	Paraffin	27/33	0/20	0/20	(Sechi et al., 2001)
IHC	Ag/Ab	Paraffin	0/35	0/36	0/21	(Ellingson et al., 2003)
ISH	IS*900*	Paraffin	73/82	0/20	0/20	(Sechi et al., 2004)
FISH	IS*900*	Resected	8/12	0/0	0/6	(Romero et al., 2005)
ISH	rRNA	Paraffin	15/17	2/5	3/30	(Jeyanathan et al., 2007)
LM/ZN	ZN	Paraffin	10/17	2/5	3/30	

[a]Data are not inclusive and articles in which insufficient information was available to precisely define methodology or the results were inconsistent or unclear are not included. Method: methodology employed in the study; Detector: target of the method; Tissue: type of tissues examined; CD: Crohn's disease; UC: ulcerative colitis; nIBD: non-inflammatory bowel disease control.
[b]ISH: *in situ* hybridization; FISH: fluorescence *in situ* hybridization; IHC: immunohistochemistry; Ag/Ab: antigen/polyclonal antibodies; ICC: immunocytochemistry; LM: light microscopy/Ziehl-Neelsen.
[c]Paraffin: paraffin-embedded tissues of undefined nature (biopsy vs. resected tissues); Resected: fresh resected intestinal tissues.

disease) are often used as examples of paucibacillary disease (Nacy & Buckley, 2008), neither is appropriate. Tuberculoid leprosy is a chronic self-limiting disease and, if not self-limiting, often progresses to the multibacillary form with an abundance of bacteria (Scollard et al., 2006). Paucibacillary paratuberculosis, although it may occur and lead to clinical disease, is poorly understood and not reproducible (Clark et al., 2010). Thus, there is no model of a chronic progressive paucibacillary disease and suggesting this mechanism represents a novel model in bacterial pathogenesis.

It is also alleged that the existence of *M. paratuberculosis* in a cell-wall deficient spheroplast form accounts for the inability to demonstrate the organism in tissues. If *M. paratuberculosis* were present in abundance, even in a cell-wall deficient form, it would be visible by other staining techniques not dependent on the cell wall and/or by electron microscopy; but neither has been demonstrated. Even electron microscopic investigations of tissues from patients with Crohn's disease (Dvorak & Dickersin, 1979, Bataille et al., 2004) do not report unidentified pleomorphic structures or *Mycoplasma*-like organisms within phagocytic cells.

Recent studies on the sensitivity of various detection methods (Jeyanathan et al., 2006) found that standard cell-wall staining methods were no more sensitive than *in situ* hybridization or *in situ* PCR based on the IS*900* sequence and that detection is solely dependent on the bacterial burden of the tissues. Therefore, the existence of spheroplastic forms of *M. paratuberculosis* is irrelevant to its detection and amounts to little more than an excuse for the failure to demonstrate *M. paratuberculosis* within disease tissues of patients with Crohn's disease.

Treatment of Crohn's disease patients with anti-mycobacterial agents

Supporting evidence (protagonist's view)

In cattle and other ruminants infected with *M. paratuberculosis*, a variety of methods, from diet to antimicrobials and probiotics (Merkal & Larsen, 1973, Chiodini et al., 1984a, St-Jean & Jernigan, 1991, Hendrick et al., 2006, Click & Kampen, 2010), have been used in attempts to arrest the disease. Although temporary improvements and/or remission can be achieved in some animals, no animal has ever been cured of an *M. paratuberculosis* infection and all infected animals ultimately succumb to clinical disease (Chiodini et al., 1984a). Even the administration of antimicrobials prior to infection does not prevent infection and the ultimate development of clinical disease (Rankin, 1955, Chiodini et al., 1984a). Despite attempts, no animal has ever been cured of Johne's disease.

A number of case reports and case series have reported the successful treatment of Crohn's disease patients with anti-mycobacterial therapeutic regimes (Chiodini, 1989, Borgaonkar et al., 2000, Borody et al., 2002, Ohkusa & Sato, 2005, Chamberlin et al., 2007), suggesting that treatment with anti-mycobacterial agents may be effective in some patients. Of the more than 19 case reports on the use of anti-mycobacterial agents in Crohn's disease, 14 reported good to dramatic responses.

The systematic review and meta-analysis performed by Borgaonkar et al. (2000) on randomized placebo-controlled trials using anti-mycobacterial therapy from 1966 to 1998 found that anti-mycobacterial therapy to be effective in maintaining disease remission in Crohn's disease. When combined with initial corticosteroid use, the pooled odds ratio of maintenance of Crohn's disease remission was 3.37 (95% CI, 1.38–8.24; $p = 0.013$).

Although meta-analysis suggests beneficial responses to anti-mycobacterial agents, most controlled trials (Table 10), particularly the large Australian trial (Selby et al., 2007), have been considered a failure, results offer various interpretations and are open to debate (Thayer, 1992, Chamberlin, 2007, Gitlin & Biesecker, 2007, Lipton & Barash, 2007, Peyrin-Biroulet et al., 2007, Selby et al., 2007, Behr & Hanley, 2008). There are many different reasons that can be put forth to explain the heterogeneity of trial results (Table 10), including patient selection, antimicrobial choices, single versus multiple drug use, doses used, treatment duration, and the like. In addition, while the evaluations of most therapeutic regimens in Crohn's disease are measured by their ability to induce and/or maintain disease remission, when applied to anti-mycobacterial agents, success/failure is generally judged by the ability of these drugs to produce a curative effect (Peyrin-Biroulet et al., 2007). When more liberal measurements of success are applied to anti-mycobacterial drug use in Crohn's disease, as used in other evaluations, anti-mycobacterial drugs demonstrate efficacy better or equal to currently employed therapeutic practices (Chamberlin, 2007, Gitlin & Biesecker, 2007, Lipton & Barash, 2007, Behr & Hanley, 2008).

In fact, a recent systematic review and meta-analysis on the general use of antimicrobial agents in Crohn's disease concluded that there was a statistically significant effect of antibiotics at inducing remission in active disease, at reducing fistula drainage and in preventing relapse in quiescent disease (Anonymous, 2011, Khan et al., 2011). Interestingly enough, the most effective drugs were rifampicin derivatives and clofazimine, two commonly used anti-mycobacterial agents.

There are various reasons why a curative effect has not been consistently demonstrated in Crohn's disease by the use of anti-mycobacterial therapy. Patients selected for antimicrobial treatment trials are generally refractory to conventional therapies and have undergone years of unsuccessful pharmacologic and/or chemotherapeutic manipulations which produce a host of side-effects which may need to be overcome before achieving a curative response. The concurrent use of corticosteroids, as commonly used in other mycobacterial infections (Thornton, 1970, Golden & Vikram, 2005, Scollard et al., 2006), may be an inappropriate designation of failure. In cattle with *M. paratuberculosis* infection, the administration of immunosuppressive agents has been shown

Table 10. Treatment of Crohn's disease patients with anti-mycobacterial therapeutic agents[a].

Type of study[b]	Patients	Drugs used[c]	Duration (mo)	Remission (%)		Criteria[d]	Source
				Drug	Placebo		
RCT	27	RF + ET	12	38	64	ΔCDAI	(Shaffer et al., 1984)
RCT	24	RB	6	29	38	ΔHBI	(Basilisco et al., 1989)
Open trial	20	RF + ET + IZ + PZ or CL	9	50	NA[e]	Clinical remission	(Hampson et al., 1989)
Open trial	16	RB + ET	6–12	0	NA	Endoscopic healing	(Rutgeerts et al., 1992)
RCT	40	RF + ET + CL + DS	9	84	35	Mucosal healing	(Prantera et al., 1994)
RCT	49	CL	12	64	50	Clinical remission	(Afdhal et al., 1991)
RCT	126	RF + IZ + ET	24	35	38	Clinical remission	(Swift et al., 1994, Thomas et al., 1998)
Open trial	46	RB+CL or AZ	6–35	94	NA	Clinical remission	(Gui et al., 1997)
RCT	31	CM + ET	3	$p = 0.08$ v. placebo		ΔHBI	(Goodgame et al., 2001)
Open trial	25	CM	1–15	32	48	ΔHBI	(Leiper et al., 2000)
Open trial	36[f]	RB + CM	4–17	58	NA	Clinical response	(Shafran et al., 2002)
Open trial	12	RB + CL + CM	24	25	NA	Clinical remission	(Borody et al., 2002)
Retrospective review	39	RB + CL + CM	6–9	52	NA	Mucosal healing	(Borody et al., 2007)
RCT	213	RB + CM + CL	12	61	44	Relapse	(Selby et al., 2007)
	122		24	74	57		
	54		36	42	50		

[a]Case reports and trials in which sufficient data could not be extrapolated are not included.
[b]RCT: randomized controlled trial.
[c]RF: rifampicin; ET: ethambutol; IZ: isoniazid; PZ: pyrazinamide; CL: clofazimine; RB: rifabutin; DS: dapsone; AZ: azithromycin; CM: clarithromycin.
[d]Measurement or criteria used by authors to evaluate efficacy. ΔCDAI: Change in Crohn's disease activity index; ΔHBI: Change in Harvey-Bradshaw index.
[e]Not applicable.
[f]Serologically positive to *M. paratuberculosis* antigens before treatment.

to produce clinical improvement without exacerbation of the disease or infection (Merkal et al., 1970, Chiodini et al., 1984a).

Successful treatment of a mycobacterial infection requires *optimal* intensive therapy *early* during the course of infection (Scollard et al., 2006, Kasperbauer & Daley, 2008). Neither of these conditions has been met in any clinical trial to date. Mycobacterial infections, particularly those caused by the non-tuberculous mycobacteria, may require triple to quintuplicate antimicrobials for periods of 3–5 years (Hornick et al., 1988, Cook, 2010, McGrath et al., 2010). In addition, although current anti-mycobacterial chemotherapeutics are very effective at early bacterial clearance, they are ineffective in tissue sterilization (Roy et al., 2007). Combine the ineffectiveness in tissue sterilization with a deficiency in innate immunity (Cooney & Jewell, 2009, Marks et al., 2010, Mayer, 2010) and effective therapy may require a host of immunomodulators in combination with antimicrobials to achieve long-lasting remission or cure. The use of anti-mycobacterial therapy alone in Crohn's disease may simply be too little too late.

Clinical trials with anti-mycobacterial chemotherapeutic agents in Crohn's disease have not followed American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) guidelines (Griffith et al., 2007) in duration, doses, or drug choices for severe disease. In addition, no clinical trial to date has attempted to use *M. paratuberculosis* detection as a criterion for patient selection or to monitor *M. paratuberculosis* detection during the course of treatment. Until these minimum requirements are met, conclusions based on the apparent failure of anti-mycobacterial therapy to achieve a cure for Crohn's disease may be premature.

Whether it is case reports or controlled trials, antimicrobials are effective in Crohn's disease and there is a subset of patients who respond exceptionally well to anti-mycobacterial therapy, beyond what could be attributed to a placebo or other effect.

Opposing evidence (antagonist's view)

There have been a variety of clinical trials on the use of anti-mycobacterial agents in Crohn's disease and all clinical trials to date have failed to demonstrate a curative or even a long-term clinical benefit (Table 10). Although small uncontrolled studies and meta-analysis of those studies (Borgaonkar et al., 2000) suggest some therapeutic efficacy, the largest long-term, large-scale, randomized, placebo-controlled trial ever conducted in Crohn's disease failed to show a beneficial effect of anti-mycobacterial therapy in remission or maintenance of remission (Selby et al., 2007). The results of that landmark study (Peyrin-Biroulet et al., 2007, Selby et al., 2007), which evaluated the efficacy of 3 anti-mycobacterial drugs over a period of 2-years in 213 patients, illustrate that anti-mycobacterial therapy has no role in the maintenance of Crohn's disease remission, and it does not offer any curative effect either.

As antimicrobial therapy is effective and curative in 80–90% of patients with tuberculosis and non-tuberculous pulmonary disease (Fujikane et al., 2005, Thiam et al., 2007), including infection with organisms of the *M. avium* complex, the failure of similar treatment regimes in Crohn's disease is strong evidence that *M. paratuberculosis* is not a causative agent.

Even if these trials had demonstrated a beneficial effect or if we accept the reports from uncontrolled studies and case reports, results would not specifically support the role of *M. paratuberculosis* in Crohn's disease. Anti-mycobacterial agents are not specific to the genus and have broad spectrum of activity against diverse bacteria, including luminal organisms. The beneficial effects of antimicrobials in some patients (Feller et al., 2010) may simply represent broad antimicrobial activity to luminal bacteria that alter (improve) the dysbiotic state (Khan et al., 2011, Man et al., 2011).

The use of immunosuppressive agents in Crohn's disease

Supporting evidence (protagonist's view)

Immunosuppressive agents are generally effective in minimizing acute disease flare-ups in Crohn's disease (Panés et al., 2007) but are generally contraindicated in mycobacterial infections, causing an exacerbation of the disease. This has generally been considered by gastroenterologists as evidence that *M. paratuberculosis* could not be involved in Crohn's disease.

Thalidimide, methotrexate, prednisolone, cyclosporine, azathioprine and other immunosuppressive drugs are common therapeutic agents used during the treatment of leprosy and extra-pulmonary cases of tuberculosis (Golden & Vikram, 2005, Scollard et al., 2006), and the ATS/IDSA recommends the use of corticosteroids in the treatment of advanced *M. avium* infections (Griffith et al., 2007). There are even early reports on the successful treatment of tuberculosis with corticosteroids alone (Thornton, 1970). In cattle with *M. paratuberculosis* infection, the administration of immunosuppressive agents has been shown to produce clinical improvement without exacerbation of the disease or infection (Merkal et al., 1970, Chiodini et al., 1984a). Thus, the suggestion that the use of immunosuppressive agents in Crohn's disease is evidence against a role of *M. paratuberculosis* in Crohn's disease is erroneous.

It has also been observed that patients with Crohn's disease that are co-infected with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) do not show an exacerbation of intestinal disease, and there is no observed increase in Crohn's disease in areas endemic with HIV/AIDS (Viazis et al., 2010). It has been suggested that if mycobacteria were associated with Crohn's disease, one would expect an exacerbation of intestinal disease with HIV/AIDS co-infection as seen in tuberculosis where infection with the HIV/AIDS causes an exacerbation of latent tuberculosis infection (Barry et al., 2009, Doherty et al., 2009) as well as causing increased susceptibility to non-tuberculous opportunistic mycobacteria such as *M. avium* (Corti & Palmero, 2008).

Such would suggest a paradox, but such a paradox also exists with leprosy. Co-infection with HIV/AIDS does not cause an exacerbation of leprosy nor is there an increase in leprosy observed in areas where HIV/AIDS is endemic (Scollard et al., 2006, Ustianowski et al., 2006). Some leprosy patients may actually improve with HIV co-infection (Talhari et al., 2008, Kar et al., 2009). To address the HIV/AIDS paradox in leprosy, in which the progression and outcome of *M. leprae* infection is not affected by co-infection with HIV even during advanced stages of AIDS, it has been suggested that the slow growth of *M. leprae*, as compared with *M. tuberculosis* and *M. avium*, allows what remaining immunity exists to maintain and control the infection (Scollard et al., 2006). A similar situation may exist for *M. paratuberculosis*.

Anti-TNF-α antibodies could have a beneficial effect by the disruption of granulomata and sequestered bacterial organisms thereby allowing chemotherapeutic agents and effector cells greater access to the infectious agents (Roy et al., 2007).

Opposing evidence (antagonist's view)

Immunosuppressive agents, particularly steroids, play a major role in the treatment and disease remission of Crohn's disease (Peyrin-Biroulet & Lémann, 2011) but are generally contraindicated in mycobacterial infections, causing an exacerbation of the disease. Physicians know that they should screen patients for latent mycobacterial infection or disease prior to the administration of any immunosuppressive agent. Although the use of steroid therapy is commonly used and beneficial in the treatment of various mycobacterial infections (Smego & Ahmed, 2003), they are always used concurrently with antimicrobials and never alone as employed in the treatment of Crohn's disease. Thus, the use and beneficial effects of steroids in the management of Crohn's disease suggests that mycobacteria do not play an etiological role.

In addition to corticosteroids, the efficacy of anti-tumor necrosis factor-α (TNF-α) antibodies in the treatment of Crohn's disease (Lee & Fedorak, 2010) would seem particularly problematic for an etiologic role of mycobacteria in Crohn's disease. Anti-TNF-α treatment in patients with underlying tuberculosis, leprosy, and non-tuberculous pulmonary disease all cause an exacerbation of the mycobacterial disease (Scollard et al., 2006, Winthrop et al., 2009), yet such does not occur in Crohn's disease. One would expect the proliferation of *M. paratuberculosis* and exacerbation of disease under anti-TNF-α conditions (Quesniaux et al., 2010). The fact that this does not occur suggests that *M. paratuberculosis* is not etiologically related to the causation of Crohn's disease.

Immunologic responses to *M. paratuberculosis* antigens in Crohn's disease patients

Supporting evidence (protagonist's view)

As a complex disease with tuberculoid and lepromatoid phases, immunologic responses to *M. paratuberculosis* in infected animals are often variable and unpredictable. A variety of immunologic-based assays, both cellular and humoral, have been evaluated for their usefulness as diagnostic tools in animals (Chiodini et al., 1984a, Stevenson, 2010). As a general rule, assays based on cellular immunity, including skin testing, blastogenesis, cytokine assays, and others are of low sensitivity and specificity, suffer from cross-reactivity, and have limited diagnostic value (Chiodini et al., 1984a, Nielsen & Toft, 2008, Stevenson, 2010). Assays of humoral immunity have been found to have more utility in the detection of infectious animals (animals actively shedding *M. paratuberculosis* in feces), although the reliability of methods varies between species. There are also differences in the antigens recognized by different species of animals (Chiodini et al., 1984a, Nielsen & Toft, 2008).

In cattle, the preferred diagnostic immunologic assay is the enzyme-linked immunosorbent assay (ELISA), which has a reported sensitivity and specificity of 76–98% and 96–99%, respectively, in detecting infectious and clinically diseased cattle. However, although these sensitivities and specificities are impressive, they are based on fecal culture (the "gold standard") which has a sensitivity of 40–60% (Chiodini et al., 1984a, Nielsen & Toft, 2008, Stevenson, 2010). When used on subclinically infected animals or animals shedding *M. paratuberculosis* in feces below culture detection limits, the sensitivity of the ELISA falls to 15–37% (Whitlock et al., 2000, Stevenson, 2010). Sensitivity of the ELISA is even less in non-cattle species such as goats, sheep, and deer (Nielsen & Toft, 2008) and specificity may be affected by environmental and other micro-organisms (Dunn et al., 2005, Osterstock et al., 2007). Detection of infectious animals (fecal shedders) is good and many commercial tests are available, but the detection of all *M. paratuberculosis*-infected animals, particularly subclinical cases, is problematic.

A variety of studies have sought the presence of *M. paratuberculosis* antibodies in Crohn's disease and controls with conflicting results (Table 11). Most of these studies used different methodologies and *M. paratuberculosis* antigens, which may account for the heterogeneity of results reported.

In the systematic review and meta-analysis of the detection of *M. paratuberculosis* antibodies in Crohn's disease patients between the period 1966–2006 (Feller et al., 2007), 10 of the 13 studies included in the meta-analysis found the prevalence of antibodies against *M. paratuberculosis* antigens was higher in patients with Crohn's disease as compared with controls with a pooled odds ratio of 1.72 (1.02–2.90). Pooled odds ratios were similar for comparisons between Crohn's disease and ulcerative colitis patients (1.88 [1.26–2.81]).

These various studies convincingly establish that Crohn's disease patients have elevated antibodies to *M. paratuberculosis* antigens and that responses are similar to those expected in both subclinical Johne's disease in animals (Whitlock et al., 2000, Stevenson, 2010) and in human tuberculosis (Steingart et al., 2007, Verma & Jain, 2007).

In addition to humoral immune studies, a few studies have examined the more relevant cellular immune responses but most of these studies have relied on peripheral blood mononuclear cells with variable results (Table 11). Since peripheral blood mononuclear cells express different antigen receptors than compartmentalized mononuclear cells (Juarez et al., 2010), it is important to rely more on reactivity of cells within compartmentalized tissues such as the gastrointestinal tract.

Clancy et al. (2007) established gut mucosal organ cultures from 63 patients with Crohn's disease 53 with ulcerative colitis, 45 with irritable bowel disease, and 74 normal controls to determine TNF-α production in response to *M. paratuberculosis* antigens. They found a significantly higher production of TNF-α in Crohn's disease patients as compared with other patient groups and controls. In addition, by incorporating *M. paratuberculosis* IS*900*-PCR detection, they observed significantly higher TNF-α production in PCR positive samples from Crohn's disease patients as compared with PCR positive samples from other patient groups and controls.

Olsen et al. (2009) established T cell lines without *ex vivo* stimulation from biopsies of 11 patients with Crohn's disease, 13 patients with ulcerative colitis, and 10 controls. Generated cell lines were tested for reactivity to *M. paratuberculosis* and commensal bacteria. Patients with Crohn's disease were found to have a higher frequency of *M. paratuberculosis*-reactive T cells (71%) than other groups examined. The 3 Crohn's disease patients that failed to have a detectable response to *M. paratuberculosis* had colon involvement only. These *M. paratuberculosis*-reactive T cells produced higher levels of IL-17 and IFN-γ compared with commensal bacteria, ulcerative colitis and nIBD controls. The *M. paratuberculosis*-reactive T cells from Crohn's disease patients were Th_1 or Th_1/Th_{17} mixed phenotypes.

These studies suggest that the cellular immune response within intestinal tissues (the gut-associated lymphoid tissue or GALT) in patients with Crohn's disease have increased cellular reactivity and TNF-α production in response to *M. paratuberculosis* antigens greater than that to commensal bacteria or controls.

Thus, the predominance of data suggests that there exists a higher frequency of serum antibodies and reactive intestinal T cells to *M. paratuberculosis* in Crohn's disease patients compared with controls—findings comparable with those seen with other mycobacterial diseases.

Table 11. Detection of antibodies and cell-mediated immune reactivity to *M. paratuberculosis* antigens in Crohn's disease[a].

Humoral antibody studies							
Year	Method[b]	Antigen[c]	CD[d]	UC[d]	nIBD[d]	Association[e]	Source
1984	ELISA	PPA	56	34	67	$p < 0.0005$	(Thayer et al., 1984)
1986	ELISA	disrupted	33	21	12	NS	(Cho et al., 1986)
1991	ELISA	PPA	52	15	41	NS	(Tanaka et al., 1991)
1991	ELISA	PPA	4/108	2/40	3/149	NS	(Brunello et al., 1991)
1992	Wblot	38-kDa	16/28	0/20	0/18	ND	(Elsaghier et al., 1992)
	cELISA	24-kDa	15/28	0/20	0/18	ND	
	ELISA	18-kDa	15/28	2/20	0/18	ND	
1993	ELISA	Sonicate	38	15	30	NS	(Stainsby et al., 1993)
1996	ELISA	18 kDa	40	15	21	NS	(Walmsley et al., 1996)
1999	ELISA	PPA	13	20	0	$p < 0.05$	(Suenaga et al., 1999)
2000	ELISA	PPA[h]	272	167	275	$p < 0.005$	(Collins et al., 2000)
2000	ImmBot	35 kDa	40/53	1/10	5/35	$p < 0.0001$	(Naser et al., 2000)
		36 kDa	79/89	4/27	7/50	$p < 0.0001$	
		35K & 36K	39/53	0/10	0/35	$p < 0.0001$	
2001	ELISA	14-kDa	10	10	0	$p < 0.05$	(Olsen et al., 2001)
2004	ELISA	PPA	14/42	0	3/34	ND	(Barta et al., 2004)
2004	ELISA	PPA	283	144	402	NS	(Bernstein et al., 2004)
006	ELISA	IS*900*-GST	23/50	0/40	0/44	$p < 0.005$	(Nakase et al., 2006)
2009	ELISA	PPA-3	124	90	80	$p < 0.0001$	(Juste et al., 2009)
2010	ELISA	Cf	48	0	46	$p < 0.001$	(Shin et al., 2010)
2010	ELISA	PtpA / PknG	20	0	20	$p < 0.01$	(Bach et al., 2011)

Cell-mediated immunity studies							
Year	Method[b]	Tissue/antigen[f]	CD[d]	UC[d]	nIBD[d]	Association[e]	Source
1990	MIFA	PBMC/Sonicate	35	28	25	NS	(Seldenrijk et al., 1990)
1991	BLAST	PBMC/Sonicate	33	18	23	$p < 0.001$[h]	(Ebert et al., 1991)
1992	BLAST	PBMC /ND	32	5	21	NS	(Ibbotson et al., 1992)
1992	BLAST	PBMC/PPD	10	9	10	$p < 0.05$[h]	(Dalton et al., 1992)
1995	Skin test	*In situ*/PPD	0/10	0/9	0/10	ND	(Rowbotham et al., 1995)
2000	BLAST	PBMC/Sonicate	13/13	17/17	17/17	NS	(Collins et al., 2000)
2001	IFN-γ[g]	PBMC/PPD	125	101	107	$p < 0.05$	(Olsen et al., 2001)
	IL-5[g]	PBMC/PPD	125	101	107	NS	(Clancy et al., 2007)
	IFN-γ/IL-10[g]	PBMC/14-kDa-PPD	10	10	0	NS	
	TNF-α	Mucosal organ culture/ND	63	53	74	$p < 0.0001$	
2007	IFN-γ, IL-2, IL-10,IL-12[g]					NS	
2008	IL-4, TNF-α, IFN-γ, IL-2[g]	PBMC	46	30	18	NS	(Ren et al., 2008)
2009	IL-17/IFN-γ[g]	Mucosal T cells/PPD	11	13	10	$p < 0.025$	(Olsen et al., 2009)
2009	IFN-γ[g]	PBMC/PPA-3	124	90	80	$p < 0.001$[h]	(Juste et al., 2009)

[a]Data are not inclusive and articles in which insufficient information was available to precisely define methodology or the results were inconsistent or unclear are not included. See previous review for older studies (Chiodini, 1989).
[b]ELISA: Enzyme-linked immunosorbent assay; Wblot: Western Blot; cELISA: Competitive ELISA; ImmBot: Immunoblot; MIFA; macrophage inhibition factor assay; BLAST: Blastogenesis or proliferative assay.
[c]PPA: Purified protoplasmic antigen; disrupted: disrupted whole cells; sonicate: sonicated whole cells; PtpA: protein tyrosine phosphatase A; PknG: protein kinase G; Cf: culture filtrates
[d]CD: Crohn's disease; UC: ulcerative colitis; nIBD: non-inflammatory bowel disease controls; number positive/total number of patients when data available or total number of patients.
[e]Crohn's disease versus control groups; ND: not defined; NS: not significant.
[f]PBMC: peripheral blood mononuclear cells; PPD: purified protein derivative.
[g]Proliferative/blastogenesis assay followed by ELISA for cytokine detection; IFN-γ: gamma interferon; IL-5: Interleukin-5; IL-10: Interleulin-10; IL-4: Interleulin-4; IL-2: Interleulin-2; TNF-α: Tumor necrosis factor alpha.
[h]Suppression.

Opposing evidence (antagonist's view)

The intestinal lumen and mucosal microbiome in Crohn's disease is dysbiotic (Packey & Sartor, 2009, Gillevet et al., 2010), and there is increased bacterial translocation (Adams et al., 2008, Baker et al., 2009, Lewis et al., 2010). As a result, there are increased humoral and cellular reactivity to enteric and non-enteric organisms in Crohn's disease. Numerous studies have demonstrated that patients with Crohn's disease have increased antibody titers against *Escherichia coli*, aerobes, anaerobes, and enteric bacterial pathogens at both the systemic and mucosal level (Tabaqchali et al., 1978, Blaser et al., 1984, Macpherson et al., 1996, Wen & Fiocchi, 2004). These studies provide supporting and convincing evidence of a generalized sensitization to bacterial antigens in patients with Crohn's disease.

It is therefore not surprising that increased humoral and cellular activity against *M. paratuberculosis* is demonstrable in Crohn's disease (Table 11), but in light of the generalized bacterial sensitization in Crohn's disease, it does not add support for a causal role.

In addition, most antigens of *M. paratuberculosis* are shared with other environmental organisms and there is broad cross-reactivity between species. In the absence of *M. paratuberculosis*-specific antigens, there is insufficient evidence to suggest that the observed immunologic responses are actually against *M. paratuberculosis* specifically and not some other environmental organism or that the observed response is not simply the result of a generalized increased reactivity to commensal and environmental organisms.

Genetic susceptibility to Johne's disease and Crohn's disease

Supporting evidence (protagonist's view)

Heritability of disease concordance and immune responses to bacteria have been clearly shown in Crohn's disease (Cooney & Jewell, 2009, Lees & Satsangi, 2009) and mycobacterial diseases (Zhang et al., 2009, Misch et al., 2010, Modlin, 2010, Möller & Hoal, 2010, Yim & Selvaraj, 2010) and have provided early evidence for the importance of gene–gene interactions in regulating resistance and exacerbation of disease.

Bacteria clearly play a major role in the etiopathogenesis of Crohn's disease. Whether it is a specific infectious agent or commensal bacteria that drives the initial inflammatory reaction or secondary bacterial colonization that perpetuates the process, there exists a localized dysregulation of immune responsiveness to bacterial antigens that is, at least partially, reflected in the individual's genetic susceptibility (Packey & Sartor, 2009).

Although there are at present more than 30–71 susceptibility genes and loci associated with Crohn's disease (Barrett et al., 2008, Franke et al., 2010), the strongest associations are with polymorphisms observed in the NOD2/CARD15 and the autophagy-associated ATG16L1 and IRGM genes (Cooney & Jewell, 2009, Lees & Satsangi, 2009). These genes are associated with innate immunity and the ability to process bacterial antigens and regulate intracellular killing. These same deficiencies have been identified as susceptibility factors in both tuberculosis and leprosy (Schurr & Gros, 2009, Modlin, 2010, Möller & Hoal, 2010) and comparable deficiencies are being identified in susceptibility to *M. paratuberculosis* infection in animals (Pinedo et al., 2009).

Genetic susceptibility to *M. paratuberculosis* infection in cattle and other ruminants is currently in its infancy with 51 possible susceptibility loci identified (Kirkpatrick et al., 2010). Although most identified susceptibility loci have not been confirmed or corroborated, the association of NOD2/CARD15 with *M. paratuberculosis* infection in cattle has been well established (Pinedo et al., 2009, Ruiz-Larrañaga et al., 2010a). Other susceptibility loci associated with *M. paratuberculosis* infection in ruminants include SLC11A1 (solute carrier family 11 member A1) (Korou et al., 2010, Ruiz-Larrañaga et al., 2010b), toll-like receptor (TLP) genes (Bhide et al., 2009, Koets et al., 2010), Interleukin-10 (IL-10) (Verschoor et al., 2010c) and SP110 nuclear body protein (Ruiz-Larrañaga et al., 2010). Undefined loci on chromosomes 9, 11 and 12 have also been suggested (Minozzi et al., 2010). Most of these susceptibility loci were identified based on the *M. paratuberculosis* infection phenotype of antibody production alone and require further examination and corroboration.

In addition to common genotypic profiles between Johne's and Crohn's disease, a direct correlation between the detection of *M. paratuberculosis* and NOD2/CARD15 polymorphisms has been reported (Sechi et al., 2005) as well as a strong association between Crohn's disease and SLC11A1 (NRAMP1) polymorphisms (Sechi et al., 2006). These findings firmly establish a common genetic fingerprint between Crohn's disease, *M. paratuberculosis* infection and other mycobacterial diseases (Schurr & Gros, 2009).

Opposing evidence (antagonist's view)

Crohn's disease has been found to be associated with NOD2/CARD15 and the autophagy-associated ATG16L1 and IRGM genes (Cooney & Jewell, 2009, Lees & Satsangi, 2009) and, although some of these genes are also found associated with mycobacterial diseases, NOD2/CARD15 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection found in all Gram-negative and Gram-positive bacteria (Kawai & Akira, 2009). It can be equally correlated with mycobacteria as it can for other bacterial infections such as *E. coli*, *Streptococcus pneumonia* and *Listeria monocytogenes* (Franchi et al., 2009).

In addition, epidemiological and clinical data of NOD2/CARD15 and other deficiencies in Crohn's disease do not correlate with those found associated with mycobacterial disease. For instance, in leprosy, NOD2/CARD15 is associated with lepromatous leprosy in Chinese patients and not tuberculoid (paucibacillary) disease (Zhang et al., 2009). In contrast, there is no correlation between

NOD2 polymorphisms and Crohn's disease in patients of Chinese descent (Hume & Radford-Smith, 2002, Leong et al., 2003, Zhang et al., 2008) and Crohn's disease (if mycobacterial) is a paucibacillary disease, not lepromatous. In tuberculosis, leprosy, paratuberculosis and in experimental animals, NOD2 deficiencies are associated with increased bacterial proliferation (Möller & Hoal, 2010, Montoya & Modlin, 2010, Ruiz-Larrañaga et al., 2010, Purdie et al., 2011), not paucibacillary disease. As such, although a common genetic fingerprint may exist between Crohn's disease and mycobacterial disease (Schurr & Gros, 2009), such commonality does not support the role of *M. paratuberculosis* in Crohn's disease.

Although Sechi et al. (2005) reported an association between the detection of *M. paratuberculosis* and NOD2/CARD15 polymorphism in 70% of the Crohn's disease patients examined, these findings are not supported by other studies (Bentley et al., 2008). Likewise, there is no apparent correlation between NOD2/CARD15 deficiencies and serologic responsiveness to *M. paratuberculosis* in patients with Crohn's disease (Bernstein et al., 2004, 2007). While SLC11A1 polymorphisms are associated with *M. paratuberculosis* infection in animals (Stewart et al., 2010, Purdie et al., 2011), there is no correlation between SLC11A1 polymorphisms and Crohn's disease (Crawford et al., 2005, Chermesh et al., 2007), or the detection of *M. paratuberculosis* and Crohn's disease (Stewart et al., 2010).

While a superficial genetic commonality may exist between Johne's disease and Crohn's disease, there is no reproducible association between the detection of *M. paratuberculosis* and any of the recognized susceptibility genes in Crohn's disease.

Environmental distribution of *M. paratuberculosis* and human sources of infection

Supporting evidence (protagonist's view)

There has been a great deal of effort devoted to the examination of *M. paratuberculosis* in the environment, particularly in milk and other products for human consumption, to identify possible public sources of *M. paratuberculosis* exposure. *M. paratuberculosis* has been detected in milk, cheese, river water, tap water, and a host of other products and sources (Eltholth et al., 2009). For example, by IS*900* PCR, *M. paratuberculosis* has been detected in 2.8% of retail milk samples (Ellingson et al., 2005), 5% of retail cheese curds (Clark et al., 2006), 4.2% of raw milk cheese samples (Stephan et al., 2007), 8% of water samples (Whan et al., 2005), 32.3% of river water samples (Pickup et al., 2005), and in 81% of drinking water and biofilm samples (Pinedo et al., 2009). Although suggestive of a widespread environmental distribution of *M. paratuberculosis*, these data may simply reflect an episodic distribution (Chiodini et al., 2011).

In addition, a variety of studies, using cultural and genetic-based methodologies, have shown that *M. paratuberculosis* is shed in the milk of infected animals (Chiodini et al., 1984a, 2010) and can survive routine pasteurization methods (Chiodini & Hermon-Taylor, 1993, Lund et al., 2002), resulting in the presence of viable *M. paratuberculosis* and its antigenic components in retail milk (Cerf et al., 2007, Eltholth et al., 2009). Meat may also be a source of infection (Jaravata et al., 2007, Eltholth et al., 2009, Klanicova et al., 2011).

These various studies conclusively establish that *M. paratuberculosis* is present within the human food supply, particularly dairy-associated products, and provides a source of human exposure that simulates the primary infectious route observed in ruminant animals (i.e., oral route of exposure). This episodic exposure to *M. paratuberculosis*, coupled with an age-dependent exposure (Chiodini et al., 1984a, Windsor & Whittington, 2010) and a deficiency in intestinal innate immunity (Marks et al., 2010, Uematsu & Fujimoto, 2010), could establish the conditions for infection leading to disease in susceptible hosts.

Opposing evidence (antagonist's view)

As previously discussed, *M. paratuberculosis* appears to be widely distributed in the environment and present in our food supply (USDA, 2010), thereby creating widespread exposure of the general population. Under such circumstances, *M. paratuberculosis* must be considered a ubiquitous environmental organism and an opportunistic pathogen. As an opportunistic organism, *M. paratuberculosis* is not likely to be a primary causative agent in Crohn's disease; at most, it would constitute an opportunistic infection in an already compromised individual.

Milk and other dairy products are not sterile and common nonpathogenic environmental organisms are expected. The presence of *M. paratuberculosis* in dairy products and the environment has no public health significance.

Epidemiological parameters

Supporting evidence (protagonist's view)

Crohn's disease is considered predominately an urban disease, having a higher frequency in developing urban areas as opposed to rural areas (Economou et al., 2009). Individuals living in rural areas are presumed to have an increased exposure to domestic and wild ruminants (and hence *M. paratuberculosis*) and as infection within domestic livestock populations increase, so should theoretically the incidence of Crohn's disease within those rural areas.

However, limited survey-based epidemiological investigations have failed to establish any correlation between Crohn's disease and the exposure to known *M. paratuberculosis*-infected cattle or identify raw milk consumption, cattle ownership or other parameters as risk factors (Jones et al., 2006, Qual et al., 2010). These studies compared agricultural workers with known and unknown exposure to infected cattle and found no

increased prevalence between these populations thereby concluding that there was no association between Crohn's disease and the exposure to infected cattle. However, the authors overlooked an important parameter in their data. These studies found the prevalence of Crohn's disease within the study populations to be as high as 474 per 100,000 (Qual et al., 2010). This is a very high prevalence rate among agricultural workers that is several fold higher than the national average of 174–200 per 100,000 (Loftus et al., 1998, Kappelman et al., 2007). Such high prevalence suggests increased incidence of Crohn's disease among agricultural workers as opposed to non-agricultural workers and the general population and the risk of developing Crohn's disease appears greater among populations exposed to animals potentially infected with *M. paratuberculosis*. This is strong evidence for a causative role of the agent in Crohn's disease.

The epidemiology of zoonotic diseases has dramatically changed for various sanitary, socioeconomic and political reasons, as well as international travel and the global distribution of food and increased contact with potential sources of infection. As a result, epidemiological studies often fail to uncover associations even where they are known to exist.

This is evident in recent studies on the epidemiology of zoonotic bovine tuberculosis (*M. bovis*) and the exposure of human populations to reactor cattle (cattle tested positive for *M. bovis* infection). There is no statistically significant association between reactor cattle and human cases of *M. bovis* infection in farm households or their contacts (Cleaveland et al., 2007). In a recent review of zoonotic *M. bovis* infections in the UK, where both human and bovine tuberculosis are on the increase, showed no correlation of higher numbers of zoonotic tuberculosis cases in areas where the incidence of bovine tuberculosis in cattle is highest (de la Rua-Domenech, 2006). There was also little strain correlation between human and bovine strains of *M. bovis* from the same geographical area.

The fact that epidemiologic studies show a high prevalence of Crohn's disease among agricultural workers (Qual et al., 2010) as opposed to the normal population suggests an etiological link between Johne's disease and Crohn's disease.

Opposing evidence (antagonist's view)

The most compelling evidence that Johne's disease and Crohn's disease do not share a common etiology is the observation that there is not an increased incidence of Crohn's disease among farmers and their family at farms that are heavily infected and contaminated with *M. paratuberculosis* (Jones et al., 2006, Qual et al., 2010). Given the poor sanitation at most dairy farms, the itinerant and uneducated help commonly employed on the farm and the propensity for farm families and workers to drink unpasteurized milk, the failure to show increased incidences of Crohn's disease in farm and farm workers exposed to infected cattle is important testimony to the lack of correlation between Crohn's disease and Johne's disease (paratuberculosis) (Van Kruiningen, 1999).

When cattle, sheep, or goats shed *Brucella, Leptospira, Yersinia, Campylobacter* or *Salmonella*, human contacts frequently develop disease (Collins & Wall, 2004). Even though *M. paratuberculosis* is widely shed by animals with Johne's disease, there is no epidemiologic evidence to support its recognition as a zoonotic agent or even an opportunistic pathogen.

Caution needs to be used when interpreting and reaching conclusions from epidemiological observations because they are generally based on operational data which reflect the intensity of ongoing work more than the extent of any given problem.

Conclusions

Supporting evidence (protagonist's view)

While the protagonists recognize that there is insufficient evidence to firmly establish that *M. paratuberculosis* is the causative agent of Crohn's disease, there is sufficient evidence to strongly support the notions that *M. paratuberculosis* is etiologically related and associated with some cases of Crohn's disease.

As detailed in the above discussions, there is a remarkable similarity between Johne's disease and Crohn's disease including gross and histologic lesions and genetic susceptibility loci that suggests more than mere coincidence. *M. paratuberculosis* has been isolated and detected in Crohn's disease at a higher frequency than in ulcerative colitis or nIBD controls; patients with Crohn's disease have increased humoral and cellular reactivity to *M. paratuberculosis* antigens that is greater than responses to commensal bacteria and to responses in ulcerative colitis and controls; and treatment with antimycobacterial agents can have a profound beneficial effect in some cases of Crohn's disease (Table 12).

Koch's postulates, as originally proposed by Robert Koch and Friedrich Loeffler in 1884 (Koch, 1884), and the various modifications made thereafter by Koch and others recognizing the limitations imposed by these postulates, remains a valid guideline to establish the causal relationship between an organism and a specific disease. A recent example of the use of Koch's postulates in gastroenterology was in defining the role of *Helicobacter pylori* in peptic ulcer disease. To fulfill Koch's postulates, in the absence of an animal model, Marshall ingested the organism and demonstrated the production of an erosive digestive process (Marshall et al., 1985, Marshall, 2002).

Technical and mechanistic advances in genomics, proteomics and techniques of molecular detection have expanded our understanding of the scope and pathogenesis of infectious diseases, and it has become increasingly clear that Koch's original postulates are often too rigid and inadequate to explain the occurrence, course and outcome of many serious infections. In addition, the absence of a suitable animal models (e.g., *Helicobacter pylori*), the inability to grow an

infectious agent (e.g., *M. leprae*), the recognition of asymptomatic infections and ethical issues of infecting normal individuals with a pathogen, to name just a few, hinder the application and the ability to fulfill Koch's postulates in modern medical research. Several accepted causes of disease (e.g., *Tropheryma whipplei* and Whipple's disease) have failed to fulfill the rigid criteria in Koch's postulates.

Despite these limitations, as illustrated in Table 13, Koch's postulates have essentially been fulfilled for *M. paratuberculosis* as a causative agent in Crohn's disease and that *M. paratuberculosis* establishes a true infection and is not a simple commensal. Furthermore, studies have conclusively established that Crohn's disease patients have significantly higher levels of antibodies to *M. paratuberculosis* as compared with controls and nIBD patients and patients with Crohn's disease have increased Th_1 and Th_{17} cellular responsiveness of gut associated lymphoid tissue to *M. paratuberculosis* that is greater than that detected in controls and to commensal bacterial species.

In addition to Koch's postulates, epidemiologists have developed their own list of criteria for causality known as the Hill's criteria (Table 14) (Hill, 1965, Rothman & Greenland, 2005). Although best suited for non-infectious diseases with long latent periods, such as smoking and cancer, and retrospective epidemiological studies, these criteria can be applied to pathogenic organisms and infectious diseases. As illustrated in Table 14, most of the standards and principles established in the Hill's criteria can be met for *M. paratuberculosis* and Crohn's disease within the context of animal models.

Although there are many yet unanswered questions related to the association of *M. paratuberculosis* and

Table 12. Summary of evidence for a causal role of *M. paratuberculosis* in some cases of Crohn's disease.

Similarity to inflammatory bowel disease of ruminant animals.
Human isolates of *M. paratuberculosis* produce a granulomatous ileocolitis in experimental animals.
Crohn's disease and mycobacterial diseases share common genetic susceptibilities.
Culture of *M. paratuberculosis* from the tissues of 15–40% of Crohn's disease patients and not from any control populations.
Detection of *M. paratuberculosis* IS*900* in the tissues of a significant proportion of Crohn's disease patients (7 times more frequently) as compared with control populations.
Visualization of *M. paratuberculosis* IS*900* and/or antigens within the tissues of patients with Crohn's disease by light microscopic methods.
A significant proportion of Crohn's disease patients have elevated antibodies against *M. paratuberculosis* as compared to controls.
A significant proportion of Crohn's disease patients have increased TH_1 and TH_{17} reactive intestinal T cells to *M. paratuberculosis* as compared with commensal bacteria and controls.
Human sources of exposure to *M. paratuberculosis* have been identified within the food chain.
Antibiotics are effective in the treatment of Crohn's disease and subpopulations of patients respond exceptionally well to anti-mycobacterial agents.
There is an increased prevalence of Crohn's disease within animal industry workers as opposed to the general population.

Table 13. Modified Koch's postulates as applied to the role of *M. paratuberculosis* in Crohn's disease.

Classical Koch's postulates	
The specific causative agent must be found predominately in persons suffering from the disease.	*M. paratuberculosis* is found in Crohn's disease at a statistically significant higher frequency than in ulcerative colitis and nIBD controls by cultural and DNA-based methods with a odds ratio of 6.88–7.01 (Feller et al., 2007, Abubakar et al., 2008).
The disease organism must be isolated from diseased persons and grown in pure culture.	*M. paratuberculosis* has been isolated in pure culture with microbiologic confirmation only in cases of Crohn's disease.
Inoculation of a sample of the culture into a susceptible animal must produce the same disease.	Inoculation of *M. paratuberculosis* strains from humans into susceptible ruminants (goats) produces a granulomatous ileocolitis resembling Crohn's disease (Chiodini et al., 1984, Van Kruiningen et al., 1986).
The organism must be reisolated from the experimentally infected animal.	*M. paratuberculosis* has been re-isolated in pure culture from susceptible animals that developed intestinal disease following infection with human isolates (Chiodini et al., 1984d, Van Kruiningen et al., 1986).
Host-dependent Koch's postulates	
The agent is associated with a host-defined immunod eficiency.	Infection with *M. paratuberculosis* and Crohn's disease are associated with deficiencies in NOD2/CARD15 and the autophagy ATG16L1 and IRGM genes.
The organism is isolated from the diseased host and grown in pure culture.	Pure cultures of *M. paratuberculosis* with microbiologic confirmation have only been obtained from patients with Crohn's disease.
Inoculation of a sample of the culture into an animal produces the same disease only in a host that shares the same immunodeficiency.	Susceptible animals inoculated with human isolates of *M. paratuberculosis* develop a similar granulomatous ileocolitis. Non-susceptible animals (rats, mice, etc.) fail to develop disease.
The organism is then re-isolated from the experimentally infected animal.	*M. paratuberculosis* can be re-isolated from infected animals that develop granulomatous ileocolitis following inoculation of *M. paratuberculosis*.

Table 14. Hill's Criteria for establishing causality as related to *M. paratuberculosis* and Crohn's disease (Hill, 1965, Rothman & Greenland, 2005).

Criterion[a]	Question to be answered	Answer[b]
Biological plausibility	Does the suggested cause make sense in the context of current knowledge?	Yes. *M. paratuberculosis* causes a similar disease in susceptible ruminant animals and humans are known to react violently to *M. paratuberculosis* antigens. Evidence Level: Strong
Dose response		
Natural	Does disease occur more in individuals closer to the source?	Yes. There is a higher incidence of Crohn's disease among agricultural workers with a prevalence 2x that of the normal population. Evidence Level: Moderate
Interventional	Does the disease recede with anti-mycobacterial treatment?	Yes. There is a statistically significant effect of antibiotics at inducing remission in active Crohn's disease. Evidence Level: Conflicting
Strength of Association	What is the risk of disease after infection?	Strong. In ruminant animals, inoculation with *M. paratuberculosis* consistently and reproducibly results in intestinal disease. Evidence Level: Strong
Specificity of Association	Is the agent associated with only one clinical syndrome?	Yes. *M. paratuberculosis* is only associated with intestinal Johne's disease in ruminants and is not known to cause any other disease. Evidence Level: Strong
Consistency	Do studies by different groups consistently arrive at the same findings?	No. There is conflicting results between laboratories using different methodologies, reagents, and procedures. However, meta-analysis of high quality studies supports an association. Evidence Level: Conflicting
Temporality	Does infection precede disease?	Yes. Infection of susceptible experimental animals results in subsequent disease.Evidence Level: Strong
Coherency	Are there any serious conflicts with generally known facts of the natural history and biology of the disease?	No. *M. paratuberculosis* as a cause of Crohn's disease does not conflict with the natural history or biology of Crohn's disease. Evidence Level: Strong
Experimental verification	Are there any animal studies that support the hypothesis?	Yes. Pure culture strains of *M. paratuberculosis* isolated from patients produce a similar granulomatous ileocolitis in experimental animals. Evidence Level: Strong
Analogy	Is there any analogy with other causal relationships?	Yes. Johne's and Crohn's disease are similar diseases with similar natural histories. Evidence Level: Strong

[a]An association need not satisfy all criterion to be considered causal; however, the more that are, the better the case that is made. Some criterion may not be relevant to particular diseases.
[b]Strength at an evidence level refers to how strong the evidence supporting a conclusion is. Requirements for levels of evidence (Roffey et al., 2010):
Strong: 2 or more high-quality studies with consistent multivariate results
Moderate: 1 high-quality study or 2 low-quality studies with consistent multivariate results
Limited: 1 low-quality study or unadjusted results
Conflicting: Inconsistent studies of same quality (consistent high quality > inconsistent low quality)

Crohn's disease, the data clearly establish *M. paratuberculosis* as a probable cause of at least some cases of Crohn's disease (Table 12). Many of the unanswered questions will be addressed and resolved as improved research tools with increased specificity, sensitivity and reproducibility are developed and applied in carefully designed large-scale studies.

It would be a mistake, based on the evidence presented herein, to discount *M. paratuberculosis* as a possible causative agent in some cases of Crohn's disease. It may also be inappropriate to suggest that *M. paratuberculosis* may be a causative agent in only a few cases and therefore not worth investigating (Chassaing & Darfeuille-Michaud, 2011). Suffering patients may disagree with such a conclusion.

After 25+ years of effort, there is sufficient evidence to warrant a concerted effort into resolving the role of *M. paratuberculosis* in Crohn's disease. There can be little justification to allow this issue to continue to linger—the well-being of thousands of patients are at stake.

Opposing evidence (antagonist's view)

The protagonists present an interesting, intriguing and compelling argument for *M. paratuberculosis* as a cause of some cases of Crohn's disease but fall short in establishing this organism as a primary pathogen and leave the biological significance of *M. paratuberculosis* in Crohn's disease in question (Table 15). Association is not the same as causation and the imputation of guilt by simple association is unsupported in modern medicine. After 25+ years of unsuccessful efforts to establish a causal relationship, the time may be ripe to finally lay this hypothesis to rest.

Association is not the same as causation. There are many things associated with Crohn's disease including such things as smoking (Thia et al., 2010) and sunlight exposure (Nerich et al., 2011), neither of which are likely the cause of the disease. If there were more than a simple association, investigators would have shown a temporal or causal relationship. This issue has continued to linger because you cannot establish a causal link when one does

Table 15. Summary of evidence that refutes or negates the role of *M. paratuberculosis* as a causal agent in Crohn's disease.

The similarities between Crohn's disease and Johne's disease are superficial at best and do not add support for a causal role.
The inoculation of animals with *M. paratuberculosis* produces Johne's disease, not Crohn's disease.
The genetic susceptibilities between Crohn's disease and the mycobacterioses are general similarities and there is no correlation between the detection of *M. paratuberculosis* and any recognized susceptibility gene in Crohn's disease.
M. paratuberculosis can be cultured from Crohn's disease as well as controls suggesting an opportunistic pathogen at best and mucosal colonization in a dysbiotic environment at the least.
Because of the dysbiotic state in Crohn's disease and the environmental distribution of *M. paratuberculosis*, increased detection of the organism in Crohn's disease supports mucosal surface colonization, not causality.
Acid-fast bacilli or any other evidence of mycobacteria cannot be generally found in the tissues of patients with Crohn's disease.
Patients with Crohn's disease have increased antibodies and cellular responsiveness to most commensal bacteria as a result of dysbiosis, impaired mucosal barrier, and bacterial mucosal translocation. Responsiveness to *M. paratuberculosis* is similar to the increased responses to other commensal bacteria.
All double-blinded control trials, including the largest trial ever conducted in Crohn's disease, have failed to show a sustained curative or beneficial effect of anti-mycobacterial therapy. The apparent beneficial effect in some patients is likely the result of the broad spectrum activity of antimicrobials used.
The beneficial use of steroids and other anti-inflammatory agents, and in particular anti-TNF-α treatments, which are all contraindicated in mycobacterial infections, strongly suggests that mycobacteria are not involved in Crohn's disease.
Epidemiological studies have failed to show any association between Crohn's disease and the exposure to animals infected with *M. paratuberculosis*. Available data suggests that *M. paratuberculosis* is a ubiquitous environmental organism with no clinical significance in humans.

not exist and a negative can never be proven. Although there may be an association between *M. paratuberculosis* and Crohn's disease, it is a simple association, nothing more. The continued pursuit of an etiologic role of *M. paratuberculosis* in Crohn's disease is like beating a dead horse—It is time to let it die an honorable death.

The suggestion that Koch's postulates (Table 13) and Hill's criteria (Table 14) have been met is a stretch of the imagination and based almost exclusively on limited animal model studies that are flawed. Crohn's disease is a granulomatous intestinal disease without visibly demonstrable bacteria, viruses, or other microbial pathogens. In contract, the animal model in which Koch's postulates and Hill's criteria are based, produces a granulomatous intestinal disease with visible acid-fast bacilli (Van Kruiningen et al., 1986) as seen in Johne's disease. These experiments fulfilled Koch's postulates for Johne's disease (known to be caused by *M. paratuberculosis*) and not Crohn's disease. When this simple concept is applied to Koch's postulates and Hill's Criteria, *M. paratuberculosis* as a causative agent in Crohn's disease fails: Neither Koch's Postulates or Hill's Criteria have been met.

The data presented by protagonists are straightforward but do not suggest causation. *M. paratuberculosis* is a ubiquitous organism, present in a variety of environments (Nacy & Buckley, 2008, Gill et al., 2011). To single out milk and other dairy products, because of its affiliation with Johne's disease and cattle, would seem inappropriate when *M. paratuberculosis* is just as commonly detected, if not more so, in tap water (Beumer et al., 2010) than it is in milk. It is clear from available data that *M. paratuberculosis* can be detected within intestinal tissues of a variety of normal and diseased tissues, expected for a ubiquitous organism, and that the only real difference between Crohn's disease and controls appears to be the number of organisms present (Lee et al., 2011). It is likely that assay sensitivity causes the increased detection of *M. paratuberculosis* in Crohn's disease. This does not suggest causation. Crohn's disease is characterized by dysbiosis and the loss of the protective mucosal microbiome (Manichanh et al., 2006, Packey & Sartor, 2009) which allows increased mucosal surface colonization by environmental organisms, such as *M. paratuberculosis*. As there is an increase in the number of organisms of the phylum *Firmicutes* (Manichanh et al., 2006, Frank & Pace, 2008), there is simply an increase in the number of *M. paratuberculosis* organisms detected. Importantly, the level of detection is far below the number of organisms generally associated with bacterial infections and may simply represent background "noise."

There is also no evidence that the detection of *M. paratuberculosis* is within the diseased intestinal tissues as opposed to the mucosal surfaces. This is a major distinction since one suggests infection (submucosal) and the other suggests colonization (superficial mucosal surfaces). Biopsy material is predominately superficial mucosal tissue and resected tissues are likewise over representative of the bacteria present on the mucosal surface; that is, the large number of bacteria colonizing the mucosal surface will dominate and obscure anything within the submucosal tissues. As a result, the detection of *M. paratuberculosis* in resected tissues and/or biopsy material may simply be detecting mucosal surface colonization with little contribution to causation.

All other data reported likewise suggests an incidental finding unrelated to causality (Table 15). There is no consistent immune response to *M. paratuberculosis* in Crohn's disease patients and no evidence that detectable responses are specific to *M. paratuberculosis* or that the observed response is greater than the general increased responsiveness and sensitization in Crohn's disease to all environmental and commensal bacteria. There is no evidence of an increased incidence of Crohn's disease in individuals exposed to cattle infected with

M. paratuberculosis (Jones et al., 2006, Qual et al., 2010) and epidemiological data does not support a zoonosis or any link between Crohn's disease and *M. paratuberculosis* exposure.

As antimicrobial therapy is effective and curative in essentially all mycobacterial infections, the failure of targeted therapy against *M. paratuberculosis* to be curative, or even beneficial in most patients, strongly suggests that *M. paratuberculosis* does not play a causative or even propagating role. The apparent beneficial effect observed in some patients is likely due to the broad spectrum of activity of the antimicrobials used. The beneficial effect of immunosuppressive agents, particularly anti-TNF-α (Quesniaux et al., 2010), is further evidence against a role of *M. paratuberculosis* in Crohn's disease.

Considering available evidence, proponents rely almost exclusively on the detection of *M. paratuberculosis* in a greater proportion of Crohn's disease patients as opposed to control groups as the sole basis of suggesting causality without any other supporting evidence and in the presence of substantial conflicting and inconsistent data. Although this association may exist, recent evidence suggests that the association is more related to assay sensitivities that create the artifact of association rather than suggestive of causality. The continued pursuit of this research area serves only to detract effort and resources from solving the etiology of Crohn's disease.

After 100+ years of futile efforts to establish a link between *M. paratuberculosis*, Crohn's disease, and Johne's disease, it is time to stop beating the old dead horse and devote investigations to more productive avenues of pursuit. Available data do not support continued efforts.

Author's Conclusions

Significant advances have been achieved in the understanding of Crohn's disease. A number of genetic variations, termed susceptibility genes, have been detected by large genome wide screening approaches and, although we do not completely understand the function of most of these genetic variants, there is good evidence that most of them are associated with the recognition, detection and defense against intracellular bacteria. There is also a recognized dysbiosis in Crohn's disease whereby the protective bacterial layers of the mucosal surface, that is, the mucosal microbiota, has been lost and there is impairment of mucosal barrier function leading to increased bacterial translocation. Although these new understandings are generally considered important to the pathogenesis of Crohn's disease, there is little evidence that they are the cause of disease and not simply incidental or the result of the disease process (Schirbel & Fiocchi, 2010).

Genome-wide association studies (GWAS) are based on the theoretical framework of the "common disease-common variants hypothesis" which consists of many common alleles of small effect. Much of the data generated from GWAS has been disappointing (Daly, 2010) and most variants identified confer only a small proportion of heritability. For example, in Crohn's disease the NOD2/CARD15 polymorphism, although highly associated with Crohn's disease, is only found in about 30% of Crohn's disease cases, appears to be associated predominately with ileal disease and patients of European descent (Zhang et al., 2008, Marks et al., 2010) and is apparently not associated with the formation of granulomas (Shaoul et al., 2004). In addition, about 20% of the normal population has this identical NOD2 phenotype, which means, in absolute terms, that for every Crohn's disease patient that carries this deficiency, there are about 500 healthy individuals with the same genotype that do not have any evidence of bowel or other disease.

Genetics alone are insufficient to explain the development and pathogenesis of Crohn's disease. These "common traits" identified may provide insight into general concepts but the variable results of single nucleotide variants (SNVs) in different populations suggest that susceptibility to Crohn's disease is multigenic, with a high degree of heterogeneity among the different populations studied. The complexity of Crohn's disease likely requires multiple molecular mechanisms, including genomic altercations, acting in a cascade of events that would lead to the final clinical outcome. Different gene polymorphism and the balance among these pathways may ultimately lead to infection, disease development, progression, or immunity. It is likely that gene–gene interactions and their cumulative effects play a far more important role in an individual's susceptibility to complex disease than a single polymorphism would on their own. To conclude that Crohn's disease *results* from a defect in innate immunity (Subramanian et al., 2006) based on the "common disease-common variants hypothesis" may be premature; susceptibilities exist for all diseases and the manifestation of most disease can be interpreted as an insufficiency in immunity. These susceptibility loci are rarely the actual cause of the disease.

The intestinal bacterial population in Crohn's disease is abnormal (dysbiotic), being more pronounced within affected segments of the intestinal tract and in ileal disease. The dysbiotic state in Crohn's disease is characterized by an overall decrease in biodiversity of commensal bacteria associated with an increase in organisms of the genus *Bacteriodes* and reduced numbers and lower diversity of organisms in the phylum *Firmicutes* (Manichanh et al., 2006, Gillevet et al., 2010, Qin et al., 2010). In addition, there appears also to be at least two distinct microbiota in healthy individuals: a luminal microbiome (which is dynamic (Turnbaugh et al., 2009, Gillevet et al., 2010)) and a mucosal bacterial microbiome or mucosal biofilm which forms a synergistic and stable interaction with the host immune system. The distinction between the luminal and mucosal microbiome may have been lost in Crohn's disease (Gillevet et al., 2010).

This dysbiotic state, in conjunction with recent findings in genetic susceptibility loci, has lead to the current etiologic hypotheses that Crohn's disease is caused by an

overly aggressive immune response to normal commensal enteric bacteria; that is, the complex microbiome of the distal ileum and colon provide a constant source of antigens that stimulate chronic inflammation in genetically susceptible hosts. While dysbiosis may be responsible for many of the clinical features of Crohn's disease, it is unlikely the cause.

Dysbiosis is not unique to Crohn's disease but rather occurs under a variety of conditions that alter the microenvironment of the intestinal tract and other mucosal tissues. Dysbiosis of the gastrointestinal microbiome has been described in obesity (Cani & Delzenne, 2009), coeliac disease (De Palma et al., 2010), irritable bowel syndrome (Cremon et al., 2010, Salonen et al., 2010) and colorectal cancer (Pagnini et al., 2011, Sobhani et al., 2011). It can become dysbiotic in response to diet (Turnbaugh et al., 2009), chemotherapy (van Vliet et al., 2010), cigarette smoking (Charlson et al., 2010), changes to the cellular tissue composition (Fraune et al., 2009) and many other conditions and circumstances. It is generally the result of some other condition, extrinsic or intrinsic, that alters the normal microenvironment of the gastrointestinal tract. Anything that disrupts the homeostasis of the gastrointestinal tract will result in dysbiosis, mucosal barrier disturbance and increased bacterial translocation. There is likely dysbiosis in animals suffering from Johne's disease.

Although dysbiosis is a significant clinical entity, data would suggest that the dysbiosis observed in Crohn's disease is the result of some other precipitating or propagating factor (trigger) rather than the etiology of the disease. Although many of the clinical manifestations of Crohn's disease may be manifested by the dysbiotic state, to suggest that Crohn's disease is caused by dysbiosis implies etiology and may not be accurate. There is something in Crohn's disease that not only is acting as a "trigger" but also maintains the homeostatic imbalance that perpetuates the clinical syndrome (Schirbel & Fiocchi, 2010).

The common genetic traits, the dysbiotic state, and even the association of *M. paratuberculosis* could all be epiphenomena unrelated to the actual initiating cause of the disease. However, such does not detract from their clinical significance or importance of these findings to general disease pathogenesis, disease course, chemotherapeutic approaches, or clinical outcomes. At present, there are only two avenues of investigation exploring the possible underlying causes of Crohn's disease and both are based on the persistent bacterial infection theory: *M. paratuberculosis* and adherent-invasive *E. coli* (Packey & Sartor, 2009, Hansen et al., 2010, Chassaing et al., 2011). These investigations need to be pursued and focused on specific subpopulations of patients.

The clinical manifestations of Crohn's disease, as well as disease course and response to treatments, are so diverse as to strongly suggest that Crohn's disease is a clinical syndrome rather than a disease caused by a single etiologic agent or factor. Rather, Crohn's disease is best viewed as a common clinical syndrome which may have multiple etiologies. To study Crohn's disease as a single disease entity using "Crohn's disease" as the sole criteria for the selection of study populations is akin to studying "cancer" as a single disease—the progress made in carcinogenesis would not have been accomplished without first recognizing each type of malignancy as a unique and separate disease. Until Crohn's disease patients are segregated into clearly defined objective subpopulations, the actual etiology of Crohn's disease is unlikely to be resolved.

There is evidence that suggests a role of *M. paratuberculosis* in the etiopathogenesis of Crohn's disease that warrant the continued investigation and resolution of this controversy. Crohn's disease is one of, if not the most, important disease in gastroenterology, and Johne's disease is one of the most important diseases affecting the world's food animal industries. The tools and knowledge are available to resolve this issue and determine if a causal link exists between these two globally important illnesses.

A variety of investigators have put forth hypotheses to explain the mechanism by which *M. paratuberculosis* may cause disease in a select subpopulation of patients (Behr & Schurr, 2006, Uzoigwe et al., 2007, Behr & Kapur, 2008). Although many of these theories introduce new concepts in bacterial pathogenesis, most do have a basis in fact. Paucibacillary (tuberculoid) leprosy, although generally considered a self-limiting disease, can be chronic and persist for years. These lesions can become super-infected with a host of other microorganisms, like the leprosy-associated corynebacteria (Hottat et al., 1988, Baelden et al., 1994) for example, that may obscure the true causative agent.

Available data support the observation that *M. paratuberculosis* homes to, penetrates, and invades the submucosal layers of the gastrointestinal tract through M cells and enterocytes in cattle and goats (Momotani et al., 1988, Sigurdardóttir et al., 2005), mice (Bermudez et al., 2010), and humans (Golan et al., 2009). As with other pathogenic mycobacteria, the fate of *M. paratuberculosis* will depend on the host response to the lipid-rich antigenic constituents of the organism and whether that response is protective or destructive to the host.

M. paratuberculosis possesses potent immunomodulating antigens that elicit vigorous immunologic reactivity in human and nonhuman primates. The accidental human inoculation of the killed whole cell paratuberculosis vaccine used in animals requires surgical excision or amputation of the infected tissues (Patterson et al., 1988, Windsor et al., 2005, Kennedy & Tetlow, 2008). The paratuberculosis vaccine administered to nonhuman primates ultimately required euthanasia of the animals because of a chronic progressive granulomatous disease which spread from the injection site and became life threatening (Chiodini, unpublished observation, McClure et al., 1987). The lower temperature of humans as opposed to the natural hosts of *M. paratuberculosis*

(ruminants: 38°C) may further alter pathogenicity and metabolic pathways of the agent in the human host (Lamont & Sreevatsan, 2010).

Although *M. paratuberculosis* appears to have the ability to infect a variety of hosts under certain circumstances (Chiodini et al., 1984a), its ability to infect and replicate in normal monogastric animals may be limited. In certain breeds of mice, such as C57/B6 and C57/B10 for example, animals are capable of spontaneously reducing infection with *M. paratuberculosis* as evidenced by decreasing tissue bacterial counts over a 6-month period (Chiodini & Buergelt, 1993). In contrast, a steady-state infection occurs in Balb/C mice whereby infection is maintained for periods of up to 6 months. In these animals, bacterial tissue counts neither increased nor decreased over the 6-month study period suggesting that either bacterial proliferation was equal to bacterial death or that the organism was neither growing nor dying. The ability to alter this steady-state infection with antimicrobial agents suggests that the organism is metabolically active and not in a stagnant stationary phase of growth (Chiodini et al., 1993). These findings suggest that *M. paratuberculosis* can establish a low-level infection in non-immunocompromised animals that may be persistent and unaffected by therapeutic doses of antimicrobial agents. Furthermore, it has been established that a low-level persistent and asymptomatic infection with *M. paratuberculosis* in normal Balb/C mice augments acute intestinal injury. Mice with low-level intestinal infection with *M. paratuberculosis* have an increased and exaggerated response to acute dextran sulfate sodium (DSS)–mediated intestinal inflammation with increased weight loss, increased frequency of rectal blood, and an exacerbation of gross and histologic lesions (Johnson et al., 2011).

These observations firmly establish the ability of *M. paratuberculosis* to cause a chronic low-level persistent infection in normal monogastric animals that sensitizes the host to enhanced intestinal injury and inflammation. Such a property is consistent with disease caused by an environmental organism in susceptible hosts (e.g., Balb/C vs. C57 mice) or infection with a primary pathogen.

The research now required is to move the established association between *M. paratuberculosis* and Crohn's disease to causality.

Establishing causality, as opposed to association, is not an easy or straightforward path. Although a variety of approaches could be used to indirectly support a causative role (Hill, 1965, Lowe et al., 2008), few would offer convincing or conclusive evidence that would be widely accepted. Since *M. paratuberculosis* has never been conclusively shown to cause disease in humans, establishing causality is problematic. Defining modes of transmission to humans and/or development of diagnostic tests (as opposed to investigative tools) (Nacy & Buckley, 2008) or the development of human vaccines would seem premature. Defining the role of *M. paratuberculosis* in a single case is more important that attempting to illustrate an association with many cases.

Future direction

The most straightforward approach to establishing a significant causal (as opposed to secondary or opportunistic) role for *M. paratuberculosis* in the etiopathogenesis of Crohn's disease would be through a properly organized treatment trial. Establishing a correlation between the disappearance of *M. paratuberculosis* and clinical/pathologic improvement in the disease and a correlation between the re-emergence of *M. paratuberculosis* and the recurrence of disease would be strong evidence for a causal role; that is, disappearance of the organism is associated with disease improvement or resolution and recurrence of disease is associated with recurrence of the organism. However, no clinical trial or individual case study has attempted to make this correlation. Neither has any trial attempted to select patients for anti-mycobacterial treatment that have tested positive for *M. paratuberculosis* or to monitor *M. paratuberculosis* detection during treatment.

Although the antimicrobial susceptibility of *M. paratuberculosis* has been defined for a host of different antimicrobials in a few strains (Chiodini et al., 1984a, 1984c, Chiodini, 1990a, 1990b, 1991, Krishnan et al., 2009), these data unlikely represent the heterogeneity of wild-type strains or *in vivo* conditions. Small genetic mutations can have a great impact on the susceptibility of mycobacteria to chemotherapeutic agents (Beckler et al., 2008, Chiang et al., 2010). More importantly, *in vitro* antimicrobial activity may not correlate with *in vivo* activity. In mice systemically infected with a rifabutin-susceptible strain of *M. paratuberculosis*, high doses of rifabutin (50 mg/kg), far exceeding human doses, over a 6-month period reduced but did not eliminate infection (Chiodini et al., 1993). Doses lower than 50 mg/kg failed to reduce tissue bacterial counts or arrest infection over the 6 months of treatment.

The choice of rifabutin as the primary drug in the treatment of Crohn's disease is empirical and not based on sound clinical data. In the 1980s, rifabutin was being developed by Pharmatalia (Milan, Italy) as an anti–*M. avium* drug for use in AIDS patients because of its anti-reverse transcriptase activity. Subhuman primates, at the time presumed to have simian AIDS and gastrointestinal *M. avium* infection (McClure et al., 1987), were treated with some success using rifabutin and amikacin. From these observations, rifabutin was chosen as the primary agent in early experimental trials (Chiodini, 1989). However, rifabutin is associated with several serious side effects, including a "flu-like" syndrome with severe leukopenia, which has affected clinical trials because of the dropout rate associated with rifabutin side effects. Although the *in vitro* activity of rifabutin is greater than that of rifampin (Chiodini et al., 1984c, Chiodini, 1990a, 1990b, 1991), there is little to no evidence to suggest greater clinical efficacy (Griffith et al., 2007) and its continued use in Crohn's disease should be questioned.

The American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) recommends that treatment for *M. avium* complex pulmonary disease (Table 16) should include a macrolide plus rifamycin or rifampicin and ethambutol (Griffith et al., 2007). For nodular/bronchiectatic disease, clarithromycin or azithromycin, rifampicin, and ethambutol is recommended, whereas for fibrocavitary or severe disease, a daily regimen of clarithromycin or azithromycin, rifampicin or rifabutin, and ethambutol with the addition of amikacin/streptomycin early in therapy is recommended. In cases of severe disease, prednisolone over a course of 4–8 weeks is also recommended (Griffith et al., 2007). Standard treatment duration for pulmonary non-tuberculous mycobacterial disease is 18–24 months of therapy (Cook, 2010) with treatment continuing for 12 months *after* sputum culture conversion (Glassroth, 2008, McGrath et al., 2010).

Clinical trials with anti-mycobacterial chemotherapeutic agents in Crohn's disease have not followed ATS/IDSA guidelines (Griffith et al., 2007) in duration, doses, or drug choices for severe disease. Under suboptimal treatment protocols, as employed in Crohn's disease, a curative effect or long-lasting remission would not be an expected outcome. Recognizing that successful treatment of a mycobacterial infection requires *optimal* intensive therapy *early* during the course of infection (Scollard et al., 2006, Kasperbauer & Daley, 2008) and that most Crohn's disease patients subjected to anti-mycobacterial therapy are in advanced late-stage disease, the use of anti-mycobacterial therapy alone in Crohn's disease may simply be too little too late. Anti-tuberculous drugs alone are rarely effective in advanced primary or hypertrophic intestinal tuberculosis (Cattell & Mosely, 1946, Chiodini, 1998). Effective therapy may require a host of other chemotherapeutics, such as corticosteroids or Vitamin D (Martineau et al., 2007, Nerich et al., 2011) in combination with antimicrobials to achieve long-lasting remission or cure.

In addition to appropriate drug choices and duration, trials should be based on a curative therapy design and have different objectives, study design, data analysis, and end points than a supportive approach (Chiodini, 1998). Study designs and data interpretation need to consider possible treatment complications and pitfalls of prolonged therapy, irrespective of subjective data such as the Crohn's Disease Activity Index (CDAI), in determining endpoints and failures. Healing of severely inflamed tissue may lead to fibrotic healing resulting in fibrostenotic obstruction and the need for surgery. As surgery is often a required adjunct therapy for primary hypertrophic intestinal tuberculosis (Cattell & Mosely, 1946), surgery may not be an appropriate endpoint or indication of therapeutic failure. Subjective data, such as the CDAI, may show a detrimental effect of treatment while the patient may be improving objectively. Absent objective data, a curative therapy designed trial may prematurely be designated a failure.

If Crohn's disease is not a homogeneous disease (Arnott & Satsangi, 2003) and only a subset of patients are infected with *M. paratuberculosis*, treating all patients without rigid patient selection is unlikely to yield a statically significant benefit even if the treatment regimen is effective in that subset. No trial to date has attempted to select patients for anti-mycobacterial treatment that have tested positive for *M. paratuberculosis* or to monitor *M. paratuberculosis* detection during treatment. Without this information, the efficacy or failure of anti-mycobacterial therapy and the relevance of *M. paratuberculosis* in Crohn's disease will not be resolved.

The mandate for further trials of antibiotic therapy in Crohn's disease (Khan et al., 2011) should justify the selection of patients based on the consistent detection of *M. paratuberculosis* and the treatment of these patients employing ATS/IDSA antimicrobial guidelines for severe atypical mycobacterial disease for durations of up to 1-year *after M. paratuberculosis* is no longer detected in that patient.

However, before these trials or other causality related issues are addressed, there are a few questions that have arisen in basic microbiology that need to be resolved. These basic questions fall to the heart of the issue and developing controversies.

What is *M. paratuberculosis*?

Historically, for more than 100 years, *M. paratuberculosis* has been identified as a small (0.5 × 1.5 micron) Gram-positive facultative acid-fast bacillus and obligate pathogen that requires an exogenous source of mycobactin for *in vitro* growth and produces small (1–5 mm) firm, glistening, white rough-smooth colonies after 8–12 weeks

Table 16. American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) guidelines for the treatment of non-tuberculous mycobacterial disease (Griffith et al., 2007).

Drug	Procedure[a]	Dose[b]
Clarithromycin (azithromycin)	Recommended for most infections	Clarithromycin (500 mg–1 g), Azithromycin (250–500 mg)
Ethambutol	Add as "enhancing" drug	Ethambutol (15–25 mg/kg)
Rifampin or rifabutin	Add as third drug	Rifampicin (600 mg), Rifabutin (150–300 mg)
Parenteral aminoglycoside	Add as 4th drug for severe illness. 3× weekly for 1–3 months	Amikacin (25 mg/kg), Streptomycin (25 mg/kg)
Corticosteroids	Tapered over 4–8 weeks in severe illness	Prednisolone (1–2 mg/kg/day)

[a]Treatment duration: 18–24 months or 12-month therapy *beyond* sputum conversion to smear negative.
[b]Dose dependent on disease severity with lower ranges being standard and higher ranges recommended for severe disease. Dosing frequency: 3× weekly or daily depending on disease severity.

incubation (Chiodini et al., 1984a). With the discovery of IS*900* as a species-specific insertion sequence (Collins et al., 1989, Green et al., 1989), the identification of IS*900* alone has become *prima facie* evidence of *M. paratuberculosis*. As a result, over the years, other characteristics have been eroded and it is becoming increasingly unclear exactly what are the current identifying characteristics of *M. paratuberculosis* (Chiodini et al., 2011) (Table 17). The identification of *M. paratuberculosis* based exclusively on IS*900*, as used in many studies, may be problematic.

It must be questioned whether the detection of a specific sequence in bulk-extracted DNA actually represents a microbe, an *in situ* microbe, and a member of the microbial community without establishing a direct link between the molecular sequence and an actual *in situ* active microbe. It may be a leap of faith to assume that it does (Chiodini et al., 2011).

Although IS*900* has proved to be species-specific and reliable for the detection and confirmation of *M. paratuberculosis* infection in animals with Johne's disease (Benedictus & Kalis, 2003, Taddei et al., 2004, Sidoti et al., 2011, Zhang & Zhang, 2011), that specificity in other biological ecosystems such as human tissues and the environment is unknown.

The species-specific insertion sequence of *M. paratuberculosis* (IS*900*) is only species-specific within the context of organisms examined. Recent studies on the human gut microflora by metagenomic sequencing suggests that there are at least 160 distinct bacterial species in each individual (total species = 1,000–1,105) and as many as 80% of these bacteria may be unknown (Qin et al., 2010). The opportunities for similar or identical sequences occurring in currently unknown microbes are numerous. In domestic livestock, where IS*900* PCR detection of *M. paratuberculosis* occurs on a daily basis throughout the world, false-positive reactions and cross-reactivity with other organisms have been known to occur (Cousins et al., 1999, EngLund et al., 2002, Tasara et al., 2005). Combined with the knowledge that "species-specific" insertion sequences have been shown to cross-species barriers and exist in other species (Keller et al., 2002, da Silva Rabello et al., 2010), the *sole* reliance on IS*900* as a means of identifying *M. paratuberculosis* would seem to be misguided when applied to remote ecosystems.

Several studies have shown that the *M. paratuberculosis* "species-specific" IS*900* sequence is not that specific and may react strongly with identical or related sequences in *M. avium,* including those strains recovered from AIDS patients. In three separate studies, with the commonly used P90/P91 primer sequences, IS*900* was shown to provide positive signals in 57.6% (Naser et al., 1999), 25% (Roiz et al., 1995) and 73% (Hampson et al., 1989) of *M. avium* strains (assumed to be *M. avium* subsp. *hominissuis*) isolated from patients with HIV infection. Are these strains of *M. avium* subsp. *hominissuis* possessing IS*900* or strains of *M. paratuberculosis* that are not mycobactin-dependent and not slow growing? In our studies, we have detected IS*900* (confirmed by sequencing) within normal human tissues in the absence of IS*1311*, 251F, and F57; all of which should be present in *M. paratuberculosis* (Bannantine et al., 2002, Li et al., 2005). We have also detected 251F in Crohn's disease tissues in the absence of IS*900* and IS*1311*. Can either of these be identified as *M. paratuberculosis* simply because available databases suggest the sequences only exists within this known species?

Sequencing of PCR products is an important step in the right direction, but it does not exclude the existence of IS*900* in other unidentified microbes. In addition, with polymorphisms occurring within the IS*900* sequence (Möbius et al., 2008, Sohal et al., 2010), at what point do they represent different insertion elements as opposed to strain genomic variability. The IS*901*-IS*902* sequence is an example on point (Ahrens et al., 1995, Turenne et al., 2007, Turenne et al., 2008).

Insertion sequence elements (also known as *jumping genes* or transposable elements) are by nature mobile elements, and so there is risk that identical or similar elements are found in unrelated as well as related bacteria because of mobility to and from other strains and species (lateral and recombinant gene transfer). Thus, although studies may report on the specificity of an element across *M. paratuberculosis* strains, the degree of specificity is not assured in diagnostic or investigative settings classifying

Table 17. Erosion of *M. paratuberculosis* identification when IS*900* is used as sole basis of identification.

Characteristic[a]	Recent observations
Slow growing (8–12 weeks)	Grows similarly to environmental *M. avium* strains and does not require extensively long incubation periods as evidenced by the detection of IS*900* in *M. avium* strains isolated from AIDS patients (Hampson et al., 1989, Roiz et al., 1995, Naser et al., 1999).
Acid-fast bacillus	May exist as a cell-wall deficient variant that is variable or non-acid-fast and coccoid in shape (Naser et al., 2004, Sechi et al., 2005, Mendoza et al., 2010).
Mycobactin dependency	Grows on standard media without an exogenous source of mycobactin (Hampson et al., 1989, Naser et al., 1999).
Small size (0.5 × 1.5 μm)	Pleomorphic in appearance with no defined size (Naser et al., 2004, Sechi et al., 2005, Mendoza et al., 2010).
Colony morphology	Not discernable in liquid cultures.
Obligate pathogen	Widely distributed environmental organism and, by definition, an opportunistic pathogen (Nacy & Buckley, 2008).

[a]Original description: A small (0.5 × 1.5 micron) Gram-positive facultative acid-fast bacillus and obligate pathogen that requires an exogenous source of mycobactin for *in vitro* growth and produces small (1–5 mm) firm, glistening, white rough-smooth colonies after 8–12 weeks incubation (Chiodini et al., 1984a).

unknown isolates unless the organism has first been shown to be *M. paratuberculosis* by other methods. Until it can be ascertained that this element (IS*900*), or something genetically similar, is not found among the more than 130 mycobacteria (Euzéby, 2011) and related microbes, and their respective biovariants and strains that may be present in an unknown sample, a positive PCR element should not on its own be considered sufficient evidence to state that *M. paratuberculosis* has been detected.

The use of PCR should be limited to its appropriate application as a confirmatory tool and not used as the sole basis of identification. The detection of IS*900* is, at most, only suggestive of a specific microbe and is not direct evidence of an active *in situ* organism or that the detected sequence actually represents a member of the microbial community (Chiodini et al., 2011).

The question arises in these various studies using IS*900* as the sole method for the identification of *M. paratuberculosis* in tissues and culture material actually detecting *M. paratuberculosis* alone or *M. paratuberculosis and* some yet-to-be-identified environmental microbe. This could explain the widespread detection of IS*900* in some control groups and in the environment that do not coincide with known biological properties of *M. paratuberculosis*.

Recognizing the genomic heterogeneity of populations, reliance on a single genomic sequence as the sole means of identification without support from other characteristics would seem misguided in modern medicine. Research is needed to define the specificity of IS*900* in remote ecosystems to insure that the detection of IS*900* is a reliable marker for *M. paratuberculosis* and no other known or unknown microbe that may exist in remote ecosystems.

Obligate pathogen or environmental opportunist?

Data from PCR detection of IS*900* suggest that *M. paratuberculosis* is a ubiquitous environmental organism that can be found in dairy products, river waters, and even domestic tap water (Beumer et al., 2010, Gill et al., 2011) and that the human (and animal) populations are routinely exposed to this organism. This is similar to the environmental distribution of *M. avium* subsp. *hominissuis* (Turenne et al., 2007), suggesting that *M. paratuberculosis* is an opportunistic pathogen rather than an obligate pathogen. Such a property does not coincide with other known biological properties of the organism.

Bacterial species that have a wide distribution in the environment are generally capable of replication in the environment; without environmental replication, they become diluted below detectable and infectious levels. A variety of studies have documented the ability of *M. paratuberculosis* to survive in the environment for extended periods (Chiodini et al., 1984a, Whittington et al., 2004, Cook et al., 2010), but none have demonstrated its ability to replicate outside the gastrointestinal tissues of the host. In fact, experimental studies have shown that *M. paratuberculosis* does not replicate in the environment and that certain conditions destroy viability (such as urine, sunlight, high pH, etc.) (Chiodini et al., 1984a). The inability of *M. paratuberculosis* to produce mycobactin, and thus compete for iron, would also preclude its ability to replicate in the environment.

If the primary biomass of *M. paratuberculosis* was the environment as suggested by various environmental studies based on IS*900* detection, the prevalence of *M. paratuberculosis* infection (Johne's disease) in domestic and wild ruminants would be far more widespread than reported and the direct correlation between herd infectivity and the introduction of an infected animal (Chiodini et al., 1984a) would not be so firmly established. There are few, if any, documented cases of *M. paratuberculosis* infection or herd infectivity associated with drinking pond or river water, or drinking tap water during the winter months or any other environmental source (absent direct contamination by infected animals), all of which presumably contain *M. paratuberculosis*. The only known mode of infection is the direct or indirect exposure to feces from an infected animal.

If *M. paratuberculosis* exists in an environmental reservoir as suggested, the classification of *M. paratuberculosis* as an obligate pathogen may be erroneous and the organism may need to be reclassified as an environmental opportunistic pathogen. Considering the presumed predominance of this organism in the environment and the routine exposure of wild and domestic ruminants to this agent, its role as a primary etiologic agent of Johne's disease may need to be questioned as well.

Concluding remarks

There is little doubt that Crohn's disease is a complex disorder involving the interaction of a variety of extrinsic and intrinsic factors that, in combination, result in the clinical manifestations of Crohn's disease. The disease is often considered to be caused by a complex inter-relationship between the environment, genetic susceptibility, and immunologic responses. However, is Crohn's disease really a complex disease or just have the appearance of complexity (Norman, 2011)? All diseases involve the interplay of environment, genetic susceptibility, and immunologic responses, and all biological systems and metabolic pathways can be considered complex adaptive systems regardless of their etiology, if we choose to look at them that way. The appearance of complexity often becomes an excuse that may blind us to the simplicity.

Although Crohn's disease and the gastrointestinal environments appear to be complex and multi-dimensional, involving many facets of biology with many competing and changing biological parameters and interests, this does not preclude the possibility that the simple involvement of an infectious agent or other straightforward and deterministic factor is the causative driving force behind the disease. Apparently complex situations often hide simple relationships (Norman, 2011). The collective

cognitive bias within the field of gastroenterology may cause science to miss or dismiss an obvious relationship.

Medicine, and in particular the field of gastroenterology, does not change quickly and is slow to accept or implement new developments and findings. Despite overwhelming evidence, it took 12 years and the National Institutes of Health Consensus Development Conference to recognize the role of *H. pylori* in peptic ulcer disease and to make recommendations for antimicrobial therapy (NIH Consensus Conference, 1994). Despite being highly publicized, it took years for gastroenterologists to implement these recommendations (Thamer et al., 1998).

In Crohn's disease, it has long been recognized that antimicrobial agents are beneficial in the treatment and maintaining disease remission (Chiodini, 1998). However, it took almost 15-years for a consensus recognizing the beneficial effects of antimicrobials in Crohn's disease and the mandate for further investigations on the therapeutic benefits of antimicrobials in Crohn's disease (Khan et al., 2011). However, as part of that same journal issue, the American College of Gastroenterology published a Continuing Medical Education Supplement on medical therapies for inflammatory bowel diseases in which all sections referring to the use of antibiotics in Crohn's disease is prefaced by the statement that "A pooled analysis of antibiotic therapies shows a statistically significant effect at … but these are *not* recommended . . ." (emphasis in original) (Anonymous, 2011). Will it be another 10–15 years or longer before antimicrobials become an accepted treatment in Crohn's disease and will patients continue to be treated symptomatically without regard to etiologies?

The suggestion that *M. paratuberculosis* may be the cause of some cases of Crohn's disease has, since its first suggestion in 1984, been a controversial subject, particularly within the field of gastroenterology. Both sides of the issue may fail to recognize that the desire to prove or disprove often blinds people on both sides. How much longer will this controversy continue to linger without resolution?

If *M. paratuberculosis* is ultimately shown to be one of the causes of Crohn's disease, the effects would have overwhelming consequences to the medical, pharmaceutical, agricultural, and veterinary industries, in addition to affecting the lives of thousands of suffering patients. New and more effective chemotherapeutic agents and standardized diagnostic procedures and methodologies to identify the affected subpopulation of patients would need to be developed. The reclassification of Johne's disease, which is now widespread and out of control in the world's animal industries, as a zoonotic disease would be devastating.

One is again reminded that transposable elements (Barbara McClintock; Nobel Laureate, 1983) and the bacterial etiology of peptic ulcers (Barry Marshall; Nobel Laureate, 2005) were once controversial and polarizing issues. It is time to resolve this controversial and polarizing issue and resolve the role, if any, of *M. paratuberculosis* in Crohn's disease.

This review has attempted to present both sides of the issue to bring to light the strengths and weaknesses of the proponents and opponents views related to the role of *M. paratuberculosis* in Crohn's disease, without interpretation, and provide the reader the opportunity to judge these strengths and weaknesses for themselves. This review should also be considered a mandate for further research to resolve this lingering issue. Patients cannot afford to wait another 25 years.

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Declaration of interest

The authors report no declarations of interest.

References

Abubakar I, Myhill D, Aliyu SH, Hunter PR. (2008). Detection of *Mycobacterium avium* subspecies *paratuberculosis* from patients with Crohn's disease using nucleic acid-based techniques: a systematic review and meta-analysis. Inflamm Bowel Dis, 14, 401–410.

Adams RJ, Heazlewood SP, Gilshenan KS, O'Brien M, McGuckin MA, Florin TH. (2008). IgG antibodies against common gut bacteria are more diagnostic for Crohn's disease than IgG against mannan or flagellin. Am J Gastroenterol, 103, 386–396.

Afdhal NH, Long A, Lennon J, Crowe J, O'Donoghue DP. (1991). Controlled trial of antimycobacterial therapy in Crohn's disease. Clofazimine versus placebo. Dig Dis Sci, 36, 449–453.

Ahrens P, Giese SB, Klausen J, Inglis NF. (1995). Two markers, IS901-IS902 and p40, identified by PCR and by using monoclonal antibodies in *Mycobacterium avium* strains. J Clin Microbiol, 33, 1049–1053.

Al-Shamali M, Khan I, Al-Nakib B, Al-Hassan F, Mustafa AS. (1997). A multiplex polymerase chain reaction assay for the detection of *Mycobacterium paratuberculosis* DNA in Crohn's disease tissue. Scand J Gastroenterol, 32, 819–823.

Allen AJ, Park KT, Barrington GM, Lahmers KK, Abdellrazeq GS, Rihan HM, Sreevatsan S, Davies C, Hamilton MJ, Davis WC. (2011). Experimental infection of a bovine model with human isolates of *Mycobacterium avium* subsp. *paratuberculosis*. Vet Immunol Immunopathol, 141, 258–266.

Anonymous. (2011). Continuing medical education: supplement 1, April 2011. An evidence-based systematic review on medical therapies for inflammatory bowel diseases. Am J Gastroenterol, 106 Suppl 1, S1.

Arnott ID, Satsangi J. (2003). Crohn's disease or Crohn's diseases? Gut, 52, 460–461.

Autschbach F, Eisold S, Hinz U, Zinser S, Linnebacher M, Giese T, Löffler T, Büchler MW, Schmidt J. (2005). High prevalence of *Mycobacterium avium* subspecies *paratuberculosis* IS900 DNA in gut tissues from individuals with Crohn's disease. Gut, 54, 944–949.

Bach H, Ko HH, Raizman EA, Attarian R, Cho B, Biet F, Enns R, Bressler B. (2011). Immunogenicity of *Mycobacterium avium* subsp. *paratuberculosis* proteins in Crohn's disease patients. Scand J Gastroenterol, 46, 30–39.

Baelden MC, Bouckaert AE, Grégoire D, van de Poele M, Coene M. (1994). Comparison of thermostable macromolecular antigens from leprosy-associated bacteria. Can J Microbiol, 40, 508–512.

Baker PI, Love DR, Ferguson LR. (2009). Role of gut microbiota in Crohn's disease. Expert Rev Gastroenterol Hepatol, 3, 535–546.

Baksh FK, Finkelstein SD, Ariyanayagam-Baksh SM, Swalsky PA, Klein EC, Dunn JC. (2004). Absence of *Mycobacterium avium* subsp. *paratuberculosis* in the microdissected granulomas of Crohn's disease. Mod Pathol, 17, 1289–1294.

Bannantine JP, Zhang Q, Li LL, Kapur V. (2003). Genomic homogeneity between *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *paratuberculosis* belies their divergent growth rates. BMC Microbiol, 3, 10.

Bannantine JP, Baechler E, Zhang Q, Li L, Kapur V. (2002). Genome scale comparison of *Mycobacterium avium* subsp. *paratuberculosis* with *Mycobacterium avium* subsp. *avium* reveals potential diagnostic sequences. J Clin Microbiol, 40, 1303–1310.

Barnich N, Darfeuille-Michaud A. (2007). Adherent-invasive Escherichia coli and Crohn's disease. Curr Opin Gastroenterol, 23, 16–20.

Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ; NIDDK IBD Genetics Consortium; Belgian-French IBD Consortium; Wellcome Trust Case Control Consortium. (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet, 40, 955–962.

Barry CE 3rd, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, Schnappinger D, Wilkinson RJ, Young D. (2009). The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol, 7, 845–855.

Barta Z, Csipo I, Mekkel G, Zeher M, Majoros L. (2004). Seroprevalence of *Mycobacterium paratuberculosis* in patients with Crohn's Disease. J Clin Microbiol, 42, 5432; author reply 5432–5432; author reply 5433.

Basilisco G, Ranzi T, Campanini C, Piodi L, Bianci PA. (1989). Controlled trial of rifabutin in Crohn's disease. Curr Ther Res, 6, 245–250.

Bataille F, Klebl F, Rümmele P, Schroeder J, Farkas S, Wild PJ, Fürst A, Hofstädter F, Schölmerich J, Herfarth H, Rogler G. (2004). Morphological characterisation of Crohn's disease fistulae. Gut, 53, 1314–1321.

Beckler DR, Elwasila S, Ghobrial G, Valentine JF, Naser SA. (2008). Correlation between rpoB gene mutation in *Mycobacterium avium* subspecies *paratuberculosis* and clinical rifabutin and rifampicin resistance for treatment of Crohn's disease. World J Gastroenterol, 14, 2723–2730.

Behr MA, Schurr E. (2006). Mycobacteria in Crohn's disease: a persistent hypothesis. Inflamm Bowel Dis, 12, 1000–1004.

Behr MA, Kapur V. (2008). The evidence for *Mycobacterium paratuberculosis* in Crohn's disease. Curr Opin Gastroenterol, 24, 17–21.

Behr MA, Hanley J. (2008). Antimycobacterial therapy for Crohn's disease: a reanalysis. Lancet Infect Dis, 8, 344.

Belsheim MR, Darwish RZ, Watson WC, Schieven B. (1983). Bacterial L-form isolation from inflammatory bowel disease patients. Gastroenterology, 85, 364–369.

Benedictus G, Kalis CJ. (2003). Paratuberculosis: eradication, control and diagnostic methods. Acta Vet Scand, 44, 231–241.

Bentley RW, Keenan JI, Gearry RB, Kennedy MA, Barclay ML, Roberts RL. (2008). Incidence of *Mycobacterium avium* subspecies *paratuberculosis* in a population-based cohort of patients with Crohn's disease and control subjects. Am J Gastroenterol, 103, 1168–1172.

Beran V, Havelkova M, Kaustova J, Dvorska L, Pavlik I. (2006). Cell wall deficient forms of mycobacteria: a review. Veterinarni Medicina, 51, 365–389.

Bermudez LE, Petrofsky M, Sommer S, Barletta RG. (2010). Peyer's patch-deficient mice demonstrate that *Mycobacterium avium* subsp. *paratuberculosis* translocates across the mucosal barrier via both M cells and enterocytes but has inefficient dissemination. Infect Immun, 78, 3570–3577.

Bernstein CN, Nayar G, Hamel A, Blanchard JF. (2003). Study of animal-borne infections in the mucosas of patients with inflammatory bowel disease and population-based controls. J Clin Microbiol, 41, 4986–4990.

Bernstein CN, Blanchard JF, Rawsthorne P, Collins MT. (2004). Population-based case control study of seroprevalence of *Mycobacterium paratuberculosis* in patients with Crohn's disease and ulcerative colitis. J Clin Microbiol, 42, 1129–1135.

Bernstein CN, Wang MH, Sargent M, Brant SR, Collins MT. (2007). Testing the interaction between NOD-2 status and serological response to *Mycobacterium paratuberculosis* in cases of inflammatory bowel disease. J Clin Microbiol, 45, 968–971.

Beumer A, King D, Donohue M, Mistry J, Covert T, Pfaller S. (2010). Detection of *Mycobacterium avium* subsp. *paratuberculosis* in drinking water and biofilms by quantitative PCR. Appl Environ Microbiol, 76, 7367–7370.

Bhide M, Chakurkar E, Tkacikova L, Barbuddhe S, Novak M, Mikula I. (2006). IS900-PCR-based detection and characterization of *Mycobacterium avium* subsp. *paratuberculosis* from buffy coat of cattle and sheep. Vet Microbiol, 112, 33–41.

Bhide MR, Mucha R, Mikula I Jr, Kisova L, Skrabana R, Novak M, Mikula I Sr. (2009). Novel mutations in TLR genes cause hyporesponsiveness to *Mycobacterium avium* subsp. *paratuberculosis* infection. BMC Genet, 10, 21.

Blaser MJ, Miller RA, Lacher J, Singleton JW. (1984). Patients with active Crohn's disease have elevated serum antibodies to antigens of seven enteric bacterial pathogens. Gastroenterology, 87, 888–894.

Borgaonkar MR, MacIntosh DG, Fardy JM. (2000). A meta-analysis of antimycobacterial therapy for Crohn's disease. Am J Gastroenterol, 95, 725–729.

Borody TJ, Leis S, Warren EF, Surace R. (2002). Treatment of severe Crohn's disease using antimycobacterial triple therapy–approaching a cure? Dig Liver Dis, 34, 29–38.

Borody TJ, Bilkey S, Wettstein AR, Leis S, Pang G, Tye S. (2007). Anti-mycobacterial therapy in Crohn's disease heals mucosa with longitudinal scars. Dig Liver Dis, 39, 438–444.

Brunello F, Pera A, Martini S, Marino L, Astegiano M, Barletti C, Gastaldi P, Verme G, Emanuelli G. (1991). Antibodies to *Mycobacterium paratuberculosis* in patients with Crohn's disease. Dig Dis Sci, 36, 1741–1745.

Bull TJ, McMinn EJ, 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. J Clin Microbiol, 41, 2915–2923.

Burnham WR, Lennard-Jones JE, Stanford JL, Bird RG. (1978). Mycobacteria as a possible cause of inflammatory bowel disease. Lancet, 2, 693–696.

Cani PD, Delzenne NM. (2009). Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. Curr Opin Pharmacol, 9, 737–743.

Cartun RW, Van Kruiningen HJ, Pedersen CA, Berman MM. (1993). An immunocytochemical search for infectious agents in Crohn's disease. Mod Pathol, 6, 212–219.

Cattell RB, Mosely CH. (1946). The surgical treatment of tuberculosis of the bowel. Lahey Clin Bull, 5, 6–9.

Cellier C, De Beenhouwer H, Berger A, Penna C, Carbonnel F, Parc R, Cugnenc PH, Le Quintrec Y, Gendre JP, Barbier JP, Portaels F. (1998). *Mycobacterium paratuberculosis* and *Mycobacterium*

avium subsp. *silvaticum* DNA cannot be detected by PCR in Crohn's disease tissue. Gastroenterol Clin Biol, 22, 675–678.

Cerf O, Griffiths M, Aziza F. (2007). Assessment of the prevalence of *Mycobacterium avium* subsp. *paratuberculosis* in commercially pasteurized milk. Foodborne Pathog Dis, 4, 433–447.

Chamberlin W. (2007). Importance of the Australian Crohn's disease antibiotic study. Gastroenterology, 133, 1744–5; author reply 1745.

Chamberlin W, Ghobrial G, Chehtane M, Naser SA. (2007). Successful treatment of a Crohn's disease patient infected with bacteremic *Mycobacterium paratuberculosis*. Am J Gastroenterol, 102, 689–691.

Charlson ES, Chen J, Custers-Allen R, Bittinger K, Li H, Sinha R, Hwang J, Bushman FD, Collman RG. (2010). Disordered microbial communities in the upper respiratory tract of cigarette smokers. PLoS ONE, 5, e15216.

Chassaing B, Darfeuille-Michaud A. (2011). The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. Gastroenterology, 140, 1720–1728.

Chassaing B, Rolhion N, de Vallée A, Salim SY, Prorok-Hamon M, Neut C, Campbell BJ, Söderholm JD, Hugot JP, Colombel JF, Darfeuille-Michaud A. (2011). Crohn disease–associated adherent-invasive E. coli bacteria target mouse and human Peyer's patches via long polar fimbriae. J Clin Invest, 121, 966–975.

Chen W, Li D, Paulus B, Wilson I, Chadwick VS. (2000). Detection of Listeria monocytogenes by polymerase chain reaction in intestinal mucosal biopsies from patients with inflammatory bowel disease and controls. J Gastroenterol Hepatol, 15, 1145–1150.

Chermesh I, Azriel A, Alter-Koltunoff M, Eliakim R, Karban A, Levi BZ. (2007). Crohn's disease and SLC11A1 promoter polymorphism. Dig Dis Sci, 52, 1632–1635.

Chiang CY, Centis R, Migliori GB. (2010). Drug-resistant tuberculosis: past, present, future. Respirology, 15, 413–432.

Chiba M, Fukushima T, Horie Y, Iizuka M, Masamune O. (1998). No *Mycobacterium paratuberculosis* detected in intestinal tissue, including Peyer's patches and lymph follicles, of Crohn's disease. J Gastroenterol, 33, 482–487.

Chiodini RJ. (1986). Biochemical characteristics of various strains of *Mycobacterium paratuberculosis*. Am J Vet Res, 47, 1442–1445.

Chiodini RJ. (1988). Identification of mycobacteria from Crohn's disease by restriction polymorphism of the 5s ribosomal DNA genes. In: MacDermott RP, ed. Inflammatory bowel disease. Current status and future approach. Amsterdam: Elsevier Science Publishing, Inc, 509–514.

Chiodini RJ. (1989). Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. Clin Microbiol Rev, 2, 90–117.

Chiodini RJ. (1990a). Bactericidal activities of various antimicrobial agents against human and animal isolates of *Mycobacterium paratuberculosis*. Antimicrob Agents Chemother, 34, 366–367.

Chiodini RJ. (1990b). Characterization of *Mycobacterium paratuberculosis* and organisms of the *Mycobacterium avium* complex by restriction polymorphism of the rRNA gene region. J Clin Microbiol, 28, 489–494.

Chiodini RJ. (1991). Antimicrobial activity of rifabutin in combination with two and three other antimicrobial agents against strains of *Mycobacterium paratuberculosis*. J Antimicrob Chemother, 27, 171–176.

Chiodini RJ. (1998). Antimicrobial agents and Crohn's disease: do they have a therapeutic role? Ital J Gastroenterol Hepatol, 30, 593–598.

Chiodini RJ, Buergelt CD. (1993). Susceptibility of Balb/c, C57/B6 and C57/B10 mice to infection with *Mycobacterium paratuberculosis*. J Comp Pathol, 109, 309–319.

Chiodini RJ, Hermon-Taylor J. (1993). The thermal resistance of *Mycobacterium paratuberculosis* in raw milk under conditions simulating pasteurization. J Vet Diagn Invest, 5, 629–631.

Chiodini RJ, Chamberlin WM, Pfaller S. (2011). What is *Mycobacterium avium* subsp. *paratuberculosis*? Appl Environ Microbiol, 77, 1923–4; author reply 1923.

Chiodini RJ, Van Kruiningen HJ, Merkal RS. (1984a). Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet, 74, 218–262.

Chiodini RJ, Kreeger JM, Thayer WR. (1993). Use of rifabutin in treatment of systemic *Mycobacterium paratuberculosis* infection in mice. Antimicrob Agents Chemother, 37, 1645–1648.

Chiodini RJ, Van Kruiningen HJ, Thayer WR, Coutu JA. (1986). Spheroplastic phase of mycobacteria isolated from patients with Crohn's disease. J Clin Microbiol, 24, 357–363.

Chiodini RJ, Van Kruiningen HJ, Merkal RS, Thayer WR Jr, Coutu JA. (1984b). Characteristics of an unclassified *Mycobacterium* species isolated from patients with Crohn's disease. J Clin Microbiol, 20, 966–971.

Chiodini RJ, Van Kruiningen HJ, Thayer WR, Coutu JA, Merkal RS. (1984c). *In vitro* antimicrobial susceptibility of a *Mycobacterium* sp. isolated from patients with Crohn's disease. Antimicrob Agents Chemother, 26, 930–932.

Chiodini RJ, Van Kruiningen HJ, Thayer WR, Merkal RS, Coutu JA. (1984d). Possible role of mycobacteria in inflammatory bowel disease. I. An unclassified *Mycobacterium* species isolated from patients with Crohn's disease. Dig Dis Sci, 29, 1073–1079.

Cho SN, Brennan PJ, Yoshimura HH, Korelitz BI, Graham DY. (1986). Mycobacterial aetiology of Crohn's disease: serologic study using common mycobacterial antigens and a species-specific glycolipid antigen from *Mycobacterium paratuberculosis*. Gut, 27, 1353–1356.

Clancy R, Ren Z, Turton J, Pang G, Wettstein A. (2007). Molecular evidence for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in Crohn's disease correlates with enhanced TNF-alpha secretion. Dig Liver Dis, 39, 445–451.

Clark DL Jr, Anderson JL, Koziczkowski JJ, Ellingson JL. (2006). Detection of *Mycobacterium avium* subspecies *paratuberculosis* genetic components in retail cheese curds purchased in Wisconsin and Minnesota by PCR. Mol Cell Probes, 20, 197–202.

Clark RG, Griffin JF, Mackintosh CG. (2010). Johne's disease caused by *Mycobacterium avium* subsp. *paratuberculosis* infection in red deer (Cervus elaphus): an histopathological grading system, and comparison of paucibacillary and multibacillary disease. N Z Vet J, 58, 90–97.

Clarkston WK, Presti ME, Petersen PF, Zachary PE Jr, Fan WX, Leonardi CL, Vernava AM 3rd, Longo WE, Kreeger JM. (1998). Role of *Mycobacterium paratuberculosis* in Crohn's disease: a prospective, controlled study using polymerase chain reaction. Dis Colon Rectum, 41, 195–199.

Cleaveland S, Shaw DJ, Mfinanga SG, Shirima G, Kazwala RR, Eblate E, Sharp M. (2007). *Mycobacterium bovis* in rural Tanzania: risk factors for infection in human and cattle populations. Tuberculosis (Edinb), 87, 30–43.

Click RE, Van Kampen CL. (2010). Assessment of Dietzia subsp. C79793-74 for treatment of cattle with evidence of paratuberculosis. Virulence, 1, 145–155.

Collins DM, Gabric DM, De Lisle GW. (1989). Identification of a repetitive DNA sequence specific to *Mycobacterium paratuberculosis*. FEMS Microbiol Lett, 51, 175–178.

Collins DM, Gabric DM, de Lisle GW. (1990). Identification of two groups of *Mycobacterium paratuberculosis* strains by restriction endonuclease analysis and DNA hybridization. J Clin Microbiol, 28, 1591–1596.

Collins JD, Wall PG. (2004). Food safety and animal production systems: controlling zoonoses at farm level. Rev – Off Int Epizoot, 23, 685–700.

Collins MT, Lisby G, Moser C, Chicks D, Christensen S, Reichelderfer M, Høiby N, Harms BA, Thomsen OO, Skibsted U, Binder V. (2000). Results of multiple diagnostic tests for *Mycobacterium avium* subsp. *paratuberculosis* in patients with inflammatory bowel disease and in controls. J Clin Microbiol, 38, 4373–4381.

Cook JL. (2010). Nontuberculous mycobacteria: opportunistic environmental pathogens for predisposed hosts. Br Med Bull, 96, 45–59.

Cook KL, Britt JS, Bolster CH. (2010). Survival of *Mycobacterium avium* subsp. *paratuberculosis* in biofilms on livestock watering trough materials. Vet Microbiol, 141, 103–109.

Cooney R, Jewell D. (2009). The genetic basis of inflammatory bowel disease. Dig Dis, 27, 428–442.

Corti M, Palmero D. (2008). *Mycobacterium avium* complex infection in HIV/AIDS patients. Expert Rev Anti Infect Ther, 6, 351–363.

Cossu D, Cocco E, Paccagnini D, Masala S, Ahmed N, Frau J, Marrosu MG, Sechi LA. (2011). Association of *Mycobacterium avium* subsp. *paratuberculosis* with multiple sclerosis in Sardinian patients. PLoS ONE, 6, e18482.

Cousins DV, Whittington R, Marsh I, Masters A, Evans RJ, Kluver P. (1999). Mycobacteria distenct from *Mycobacterium avium* subsp. *paratuberculosis* isolated from the faeces of ruminants possess IS900-like sequences detectable IS900 polymerase chain reaction: implications for diagnosis. Mol Cell Probes, 13, 431–442.

Crawford NP, Eichenberger MR, Colliver DW, Lewis RK, Cobbs GA, Petras RE, Galandiuk S. (2005). Evaluation of SLC11A1 as an inflammatory bowel disease candidate gene. BMC Med Genet, 6, 10.

Cremon C, Carini G, De Giorgio R, Stanghellini V, Corinaldesi R, Barbara G. (2010). Intestinal dysbiosis in irritable bowel syndrome: etiological factor or epiphenomenon? Expert Rev Mol Diagn, 10, 389–393.

da Silva Rabello MC, de Oliveira RS, Silva RM, Leao SC. (2010). Natural occurrence of horizontal transfer of *Mycobacterium avium*–specific insertion sequence IS1245 to *Mycobacterium kansasii*. J Clin Microbiol, 48, 2257–2259.

Dalton HR, Hoang P, Jewell DP. (1992). Antigen induced suppression in peripheral blood and lamina propria mononuclear cells in inflammatory bowel disease. Gut, 33, 324–330.

Daly AK. (2010). Genome-wide association studies in pharmacogenomics. Nat Rev Genet, 11, 241–246.

Dalziel TK. (1913). Chronic interstitial enteritis. Br Med J, 2, 1068–1070.

de la Rua-Domenech R. (2006). Human *Mycobacterium bovis* infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. Tuberculosis (Edinb), 86, 77–109.

De Palma G, Nadal I, Medina M, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. (2010). Intestinal dysbiosis and reduced immunoglobulin-coated bacteria associated with coeliac disease in children. BMC Microbiol, 10, 63.

Dell'Isola B, Poyart C, Goulet O, Mougenot JF, Sadoun-Journo E, Brousse N, Schmitz J, Ricour C, Berche P. (1994). Detection of *Mycobacterium paratuberculosis* by polymerase chain reaction in children with Crohn's disease. J Infect Dis, 169, 449–451.

Doherty TM, Wallis RS, Zumla A. (2009). Biomarkers of disease activity, cure, and relapse in tuberculosis. Clin Chest Med, 30, 783–96, x.

Douarre PE, Cashman W, Buckley J, Coffey A, O'Mahony JM. (2010). Isolation and detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from cattle in Ireland using both traditional culture and molecular based methods. Gut Pathog, 2, 11.

Dow CT, Ellingson JL. (2011). Detection of *Mycobacterium avium* ss. *paratuberculosis* in Blau Syndrome tissues. Autoimmune Dis, 2011, 127692.

Dumonceau JM, Van Gossum A, Adler M, Fonteyne PA, Van Vooren JP, Deviere J, Portaels F. (1996). No *Mycobacterium paratuberculosis* found in Crohn's disease using polymerase chain reaction. Dig Dis Sci, 41, 421–426.

Dunn JR, Kaneene JB, Grooms DL, Bolin SR, Bolin CA, Bruning-Fann CS. (2005). Effects of positive results for *Mycobacterium avium* subsp *paratuberculosis* as determined by microbial culture of feces or antibody ELISA on results of caudal fold tuberculin test and interferon-gamma assay for tuberculosis in cattle. J Am Vet Med Assoc, 226, 429–435.

Dvorak AM, Dickersin GR. (1979). Crohn's disease: electron microscopic studies. Pathol Annu, 14 Pt 2, 259–306.

Dvorska L, Bull TJ, Bartos M, Matlova L, Svastova P, Weston RT, Kintr J, Parmova I, Van Soolingen D, Pavlik I. (2003). A standardised restriction fragment length polymorphism (RFLP) method for typing *Mycobacterium avium* isolates links IS901 with virulence for birds. J Microbiol Methods, 55, 11–27.

Eamens GJ, Whittington RJ, Marsh IB, Turner MJ, Saunders V, Kemsley PD, Rayward D. (2000). Comparative sensitivity of various faecal culture methods and ELISA in dairy cattle herds with endemic Johne's disease. Vet Microbiol, 77, 357–367.

Ebert EC, Bhatt BD, Liu S, Das KM. (1991). Induction of suppressor cells by *Mycobacterium paratuberculosis* antigen in inflammatory bowel disease. Clin Exp Immunol, 83, 320–325.

Economou M, Zambeli E, Michopoulos S. (2009). Incidence and prevalence of Crohn's disease and its etiological influences. Ann Gastroenterol, 22, 158–167.

Ellingson JL, Cheville JC, Brees D, Miller JM, Cheville NF. (2003). Absence of *Mycobacterium avium* subspecies *paratuberculosis* components from Crohn's disease intestinal biopsy tissues. Clin Med Res, 1, 217–226.

Ellingson JL, Anderson JL, Koziczkowski JJ, Radcliff RP, Sloan SJ, Allen SE, Sullivan NM. (2005). Detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in retail pasteurized whole milk by two culture methods and PCR. J Food Prot, 68, 966–972.

Elsaghier A, Prantera C, Moreno C, Ivanyi J. (1992). Antibodies to *Mycobacterium paratuberculosis*–specific protein antigens in Crohn's disease. Clin Exp Immunol, 90, 503–508.

Eltholth MM, Marsh VR, Van Winden S, Guitian FJ. (2009). Contamination of food products with *Mycobacterium avium paratuberculosis*: a systematic review. J Appl Microbiol, 107, 1061–1071.

Engel MA, Neurath MF. (2010). New pathophysiological insights and modern treatment of IBD. J Gastroenterol, 45, 571–583.

Englund S, Bölske G, Johansson KE. (2002). An IS900-like sequence found in a *Mycobacterium* sp. other than *Mycobacterium avium* subsp. *paratuberculosis*. FEMS Microbiol Lett, 209, 267–271.

Euzéby JP. (2011). List of prokaryotic names with standing in nomenclature. Available at: http://www.bacterio.cict.fr/

Feller M, Huwiler K, Schoepfer A, Shang A, Furrer H, Egger M. (2010). Long-term antibiotic treatment for Crohn's disease: systematic review and meta-analysis of placebo-controlled trials. Clin Infect Dis, 50, 473–480.

Feller M, Huwiler K, Stephan R, Altpeter E, Shang A, Furrer H, Pfyffer GE, Jemmi T, Baumgartner A, Egger M. (2007). *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease: a systematic review and meta-analysis. Lancet Infect Dis, 7, 607–613.

Ferrouillet C, Wells SJ, Hartmann WL, Godden SM, Carrier J. (2009). Decrease of Johne's disease prevalence and incidence in six Minnesota, USA, dairy cattle herds on a long-term management program. Prev Vet Med, 88, 128–137.

Fidler HM, Thurrell W, Johnson NM, Rook GA, McFadden JJ. (1994). Specific detection of *Mycobacterium paratuberculosis* DNA associated with granulomatous tissue in Crohn's disease. Gut, 35, 506–510.

Flores LL, Pai M, Colford JM Jr, Riley LW. (2005). In-house nucleic acid amplification tests for the detection of *Mycobacterium tuberculosis* in sputum specimens: meta-analysis and meta-regression. BMC Microbiol, 5, 55.

Franchi L, Warner N, Viani K, Nuñez G. (2009). Function of Nod-like receptors in microbial recognition and host defense. Immunol Rev, 227, 106–128.

Frank DN, Pace NR. (2008). Gastrointestinal microbiology enters the metagenomics era. Curr Opin Gastroenterol, 24, 4–10.

Frank TS, Cook SM. (1996). Analysis of paraffin sections of Crohn's disease for *Mycobacterium paratuberculosis* using polymerase chain reaction. Mod Pathol, 9, 32–35.

Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellinghaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latiano A, Mathew CG, Montgomery GW, Prescott NJ, Raychaudhuri S, Rotter JI, Schumm P, Sharma Y, Simms LA, Taylor KD, Whiteman D, Wijmenga C, Baldassano RN,

Barclay M, Bayless TM, Brand S, Büning C, Cohen A, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D'Inca R, Dubinsky M, Edwards C, Florin T, Franchimont D, Gearry R, Glas J, Van Gossum A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, Mowat C, Newman W, Panés J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinhart AH, Stokkers PC, Torkvist L, Kullak-Ublick G, Wilson D, Walters T, Targan SR, Brant SR, Rioux JD, D'Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annese V, Hakonarson H, Daly MJ, Parkes M. (2010). Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet, 42, 1118–1125.

Fraune S, Abe Y, Bosch TC. (2009). Disturbing epithelial homeostasis in the metazoan Hydra leads to drastic changes in associated microbiota. Environ Microbiol, 11, 2361–2369.

Friswell M, Campbell B, Rhodes J. (2010). The role of bacteria in the pathogenesis of inflammatory bowel disease. Gut Liver, 4, 295–306.

Fujikane T, Fujiuchi S, Yamazaki Y, Sato M, Yamamoto Y, Takeda A, Nishigaki Y, Fujita Y, Shimizu T. (2005). Efficacy and outcomes of clarithromycin treatment for pulmonary MAC disease. Int J Tuberc Lung Dis, 9, 1281–1287.

Fujita H, Eishi Y, Ishige I, Saitoh K, Takizawa T, Arima T, Koike M. (2002). Quantitative analysis of bacterial DNA from *Mycobacteria* spp., *Bacteroides vulgatus*, and *Escherichia coli* in tissue samples from patients with inflammatory bowel diseases. J Gastroenterol, 37, 509–516.

Gill CO, Saucier L, Meadus WJ. (2011). *Mycobacterium avium* subsp. *paratuberculosis* in dairy products, meat, and drinking water. J Food Prot, 74, 480–499.

Gillevet P, Sikaroodi M, Keshavarzian A, Mutlu EA. (2010). Quantitative assessment of the human gut microbiome using multitag pyrosequencing. Chem Biodivers, 7, 1065–1075.

Gitlin L, Biesecker J. (2007). Australian Crohn's antibiotic study opens new horizons. Gastroenterology, 133, 1743–4; author reply 1745.

Glanemann B, Schönenbrücher H, Bridger N, Abdulmawjood A, Neiger R, Bülte M. (2008). Detection of *Mycobacterium avium* subspecies *paratuberculosis*-specific DNA by PCR in intestinal biopsies of dogs. J Vet Intern Med, 22, 1090–1094.

Glassroth J. (2008). Pulmonary disease due to nontuberculous mycobacteria. Chest, 133, 243–251.

Golan L, Livneh-Kol A, Gonen E, Yagel S, Rosenshine I, Shpigel NY. (2009). *Mycobacterium avium paratuberculosis* invades human small-intestinal goblet cells and elicits inflammation. J Infect Dis, 199, 350–354.

Golden MP, Vikram HR. (2005). Extrapulmonary tuberculosis: an overview. Am Fam Physician, 72, 1761–1768.

Goodgame RW, Kimball K, Akram S, Ike E, Ou CN, Sutton F, Graham D. (2001). Randomized controlled trial of clarithromycin and ethambutol in the treatment of Crohn's disease. Aliment Pharmacol Ther, 15, 1861–1866.

Gordon SV, Bottai D, Simeone R, Stinear TP, Brosch R. (2009). Pathogenicity in the tubercle bacillus: molecular and evolutionary determinants. Bioessays, 31, 378–388.

Graham DY, Markesich DC, Yoshimura HH. (1988). Letter. Digestive Diseases and Sciences, 33.

Grant IR. (2005). Zoonotic potential of *Mycobacterium avium* ssp. *paratuberculosis*: the current position. J Appl Microbiol, 98, 1282–1293.

Greco S, Girardi E, Navarra A, Saltini C. (2006). Current evidence on diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis. Thorax, 61, 783–790.

Green EP, Tizard ML, Moss MT, Thompson J, Winterbourne DJ, McFadden JJ, Hermon-Taylor J. (1989). Sequence and characteristics of IS900, an insertion element identified in a human Crohn's disease isolate of *Mycobacterium paratuberculosis*. Nucleic Acids Res, 17, 9063–9073.

Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ Jr, Winthrop K; ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. (2007). An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med, 175, 367–416.

Gui GP, Thomas PR, Tizard ML, Lake J, Sanderson JD, Hermon-Taylor J. (1997). Two-year-outcomes analysis of Crohn's disease treated with rifabutin and macrolide antibiotics. J Antimicrob Chemother, 39, 393–400.

Gwozdz JM, Thompson KG, Manktelow BW. (2001). Lymphocytic neuritis of the ileum in sheep with naturally acquired and experimental paratuberculosis. J Comp Pathol, 124, 317–320.

Hampson SJ, Parker MC, Saverymuttu SH, Joseph AE, McFadden JJ, Hermon-Taylor J. (1989). Quadruple antimycobacterial chemotherapy in Crohn's disease: results at 9 months of a pilot study in 20 patients. Aliment Pharmacol Ther, 3, 343–352.

Hampson SJ, Portaels F, Thompson J, Green EP, Moss MT, Hermon-Taylor J, McFadden JJ. (1989). DNA probes demonstrate a single highly conserved strain of *Mycobacterium avium* infecting AIDS patients. Lancet, 1, 65–68.

Hansen R, Thomson JM, El-Omar EM, Hold GL. (2010). The role of infection in the aetiology of inflammatory bowel disease. J Gastroenterol, 45, 266–276.

Harada A, Ohkusa T, Kushima K, Sakamoto M, Benno Y, Beppu K, Shibuya T, Sakamoto N, Watanabe S. (2008). Identification of bacteria from blood in febrile patients with ulcerative colitis by terminal restriction fragment length polymorphism profile analysis of 16S rRNA gene. Scand J Gastroenterol, 43, 423–430.

Harris NB, Barletta RG. (2001). *Mycobacterium avium* subsp. *paratuberculosis* in veterinary medicine. Clin Microbiol Rev, 14, 489–512.

Hendrick SH, Kelton DF, Leslie KE, Lissemore KD, Archambault M, Bagg R, Dick P, Duffield TF. (2006). Efficacy of monensin sodium for the reduction of fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in infected dairy cattle. Prev Vet Med, 75, 206–220.

Hermon-Taylor J, Barnes N, Clarke C, Finlayson C. (1998). *Mycobacterium paratuberculosis* cervical lymphadenitis, followed five years later by terminal ileitis similar to Crohn's disease. BMJ, 316, 449–453.

Hill AB. (1965). The environment and disease: association or causation? Proc R Soc Med, 58, 295–300.

Hornick DB, Dayton CS, Bedell GN, Fick RB Jr. (1988). Nontuberculous mycobacterial lung disease. Substantiation of a less aggressive approach. Chest, 93, 550–555.

Hottat F, Coene M, Cocito C. (1988). DNA methylation in leprosy-associated bacteria: *Mycobacterium leprae* and *Corynebacterium tuberculostearicum*. Med Microbiol Immunol, 177, 33–45.

Huatian G, Qin O, Hong B. (1997). *Mycobacterium paratuberculosis* in the intestine of patients with Crohn's disease. Chin J Intern Med, 36, 228–230.

Hulten K, Karttunen TJ, El-Zimaity HM, Naser SA, Collins MT, Graham DY, El-Zaatari FA. (2000). Identification of cell wall deficient forms of *M. avium* subsp. *paratuberculosis* in paraffin embedded tissues from animals with Johne's disease by in situ hybridization. J Microbiol Methods, 42, 185–195.

Hulten K, Karttunen TJ, El-Zimaity HM, Naser SA, Almashhrawi A, Graham DY, El-Zaatari FA. (2000). *In situ* hybridization method for studies of cell wall deficient *M. paratuberculosis* in tissue samples. Vet Microbiol, 77, 513–518.

Hulten K, El-Zimaity HM, Karttunen TJ, Almashhrawi A, Schwartz MR, Graham DY, El-Zaatari FA. (2001). Detection of *Mycobacterium avium* subspecies *paratuberculosis* in Crohn's diseased tissues by in situ hybridization. Am J Gastroenterol, 96, 1529–1535.

Hume G, Radford-Smith GL. (2002). The pathogenesis of Crohn's disease in the 21st century. Pathology, 34, 561–567.

Ibbotson JP, Lowes JR, Chahal H, Gaston JS, Life P, Kumararatne DS, Sharif H, Alexander-Williams J, Allan RN. (1992). Mucosal cell-mediated immunity to mycobacterial, enterobacterial and other microbial antigens in inflammatory bowel disease. Clin Exp Immunol, 87, 224–230.

Imirzalioglu C, Dahmen H, Hain T, Billion A, Kuenne C, Chakraborty T, Domann E. (2011). Highly specific and quick detection of *Mycobacterium avium* subsp. *paratuberculosis* in feces and gut tissue of cattle and humans by multiple real-time PCR assays. J Clin Microbiol, 49, 1843–1852.

Jaravata CV, Smith WL, Rensen GJ, Ruzante J, Cullor JS. (2007). Survey of ground beef for the detection of *Mycobacterium avium paratuberculosis*. Foodborne Pathog Dis, 4, 103–106.

Jayarao B, Shreekumar P. (1999). A meta-analytic study of the detection of *Mycobacterium avium* subsp. *paratuberculosis* from patients with Crohn's disease. Internet Med J, Available at: http://www.medjournal.com/archives/1999/19990726-crohns.htm

Jeyanathan M, Alexander DC, Turenne CY, Girard C, Behr MA. (2006). Evaluation of in situ methods used to detect *Mycobacterium avium* subsp. *paratuberculosis* in samples from patients with Crohn's disease. J Clin Microbiol, 44, 2942–2950.

Jeyanathan M, Boutros-Tadros O, Radhi J, Semret M, Bitton A, Behr MA. (2007). Visualization of *Mycobacterium avium* in Crohn's tissue by oil-immersion microscopy. Microbes Infect, 9, 1567–1573.

Johansen TB, Djønne B, Jensen MR, Olsen I. (2005). Distribution of IS1311 and IS1245 in *Mycobacterium avium* subspecies revisited. J Clin Microbiol, 43, 2500–2502.

John LJ, Fromm M, Schulzke JD. (2011). Epithelial barriers in intestinal inflammation. Antioxid Redox Signal, 15, 1255–1270.

Johnson CS, Wannemuehler MJ, Hostetter JM. (2011). Persistent enteric mycobacterial infection enhances sensitivity to acute mucosal injury. Exp Biol Med (Maywood), 236, 36–43.

Jones PH, Farver TB, Beaman B, Cetinkaya B, Morgan KL. (2006). Crohn's disease in people exposed to clinical cases of bovine paratuberculosis. Epidemiol Infect, 134, 49–56.

Juarez E, Nuñez C, Sada E, Ellner JJ, Schwander SK, Torres M. (2010). Differential expression of Toll-like receptors on human alveolar macrophages and autologous peripheral monocytes. Respir Res, 11, 2.

Juste RA, Garrido JM, Geijo M, Elguezabal N, Aduriz G, Atxaerandio R, Sevilla I. (2005). Comparison of blood polymerase chain reaction and enzyme-linked immunosorbent assay for detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in cattle and sheep. J Vet Diagn Invest, 17, 354–359.

Juste RA, Elguezabal N, Pavón A, Garrido JM, Geijo M, Sevilla I, Cabriada JL, Tejada A, García-Campos F, Casado R, Ochotorena I, Izeta A. (2009). Association between *Mycobacterium avium* subsp. *paratuberculosis* DNA in blood and cellular and humoral immune response in inflammatory bowel disease patients and controls. Int J Infect Dis, 13, 247–254.

Juste RA, Elguezabal N, Garrido JM, Pavon A, Geijo MV, Sevilla I, Cabriada JL, Tejada A, García-Campos F, Casado R, Ochotorena I, Izeta A, Greenstein RJ. (2008). On the prevalence of *M. avium* subspecies *paratuberculosis* DNA in the blood of healthy individuals and patients with inflammatory bowel disease. PLoS ONE, 3, e2537.

Kallinowski F, Wassmer A, Hofmann MA, Harmsen D, Heesemann J, Karch H, Herfarth C, Buhr HJ. (1998). Prevalence of enteropathogenic bacteria in surgically treated chronic inflammatory bowel disease. Hepatogastroenterology, 45, 1552–1558.

Kanazawa K, Haga Y, Funakoshi O, Nakajima H, Munakata A, Yoshida Y. (1999). Absence of *Mycobacterium paratuberculosis* DNA in intestinal tissues from Crohn's disease by nested polymerase chain reaction. J Gastroenterol, 34, 200–206.

Kappelman MD, Rifas-Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, Finkelstein JA. (2007). The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. Clin Gastroenterol Hepatol, 5, 1424–1429.

Kappelman MD, Porter CQ, Galanko JA, Rifas-Shiman SL, Ollendorf DA, Sandler RS, Finkelstein JA. (2011). Utilization of healthcare resources by U.S. children and adults with inflammatory bowel disease. Inflamm Bowel Dis, 17, 62–68.

Kar HK, Sharma P, Bhardwaj M. (2009). Borderline tuberculoid leprosy with upgrading Type 1 reaction in a HIV seropositive patient, after antiretroviral therapy: an immune reconstitution inflammatory syndrome. Lepr Rev, 80, 85–88.

Kasperbauer SH, Daley CL. (2008). Diagnosis and treatment of infections due to *Mycobacterium avium* complex. Semin Respir Crit Care Med, 29, 569–576.

Kawai T, Akira S. (2009). The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol, 21, 317–337.

Keller AP, Beggs ML, Amthor B, Bruns F, Meissner P, Haas WH. (2002). Evidence of the presence of IS1245 and IS1311 or closely related insertion elements in nontuberculous mycobacteria outside of the *Mycobacterium avium* complex. J Clin Microbiol, 40, 1869–1872.

Kennedy KJ, Tetlow L. (2008). Test and teach. Chronic soft tissue inflammation following accidental inoculation with a sheep vaccine. Diagnosis: Immune response to mineral oil and *Mycobacterium* antigens in vaccine strain (Gudair) of *Mycobacterium avium* subsp. *paratuberculosis*. Pathology, 40, 711–713.

Khan KJ, Ullman TA, Ford AC, Abreu MT, Abadir A, Abadir A, Marshall JK, Talley NJ, Moayyedi P. (2011). Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol, 106, 661–673.

Kirkpatrick BW, Shi X, Shook GE, Collins MT. (2010). Whole-Genome association analysis of susceptibility to paratuberculosis in holstein cattle. Anim Genet, 42, 149–160.

Kirkwood CD, Wagner J, Boniface K, Vaughan J, Michalski WP, Catto-Smith AG, Cameron DJ, Bishop RF. (2009). *Mycobacterium avium* subspecies *paratuberculosis* in children with early-onset Crohn's disease. Inflamm Bowel Dis, 15, 1643–1655.

Klanicova B, Slana I, Vondruskova H, Kaevska M, Pavlik I. (2011). Real-time quantitative PCR detection of *Mycobacterium avium* subspecies in meat products. J Food Prot, 74, 636–640.

Kluge JP, Merkal RS, Monlux WS, Larsen AB, Kopecky KE, Ramsey FK, Lehmann RP. (1968). Experimental paratuberculosis in sheep after oral, intratracheal, or intravenous inoculation lesions and demonstration of etiologic agent. Am J Vet Res, 29, 953–962.

Knösel T, Schewe C, Petersen N, Dietel M, Petersen I. (2009). Prevalence of infectious pathogens in Crohn's disease. Pathol Res Pract, 205, 223–230.

Koch R. (1884). Die Aetiologie der Tuberkulose. Mittheilungen aus den Kaiserlichen Gesundheitsamte, 2, 1–88.

Koets A, Santema W, Mertens H, Oostenrijk D, Keestra M, Overdijk M, Labouriau R, Franken P, Frijters A, Nielen M, Rutten V. (2010). Susceptibility to paratuberculosis infection in cattle is associated with single nucleotide polymorphisms in Toll-like receptor 2 which modulate immune responses against *Mycobacterium avium* subspecies *paratuberculosis*. Prev Vet Med, 93, 305–315.

Korou LM, Liandris E, Gazouli M, Ikonomopoulos J. (2010). Investigation of the association of the SLC11A1 gene with resistance/sensitivity of goats (Capra hircus) to paratuberculosis. Vet Microbiol, 144, 353–358.

Krishnan MY, Manning EJ, Collins MT. (2009). Comparison of three methods for susceptibility testing of *Mycobacterium avium* subsp. *paratuberculosis* to 11 antimicrobial drugs. J Antimicrob Chemother, 64, 310–316.

Laffineur G, Lescut D, Vincent P, Quandalle P, Wurtz A, Colombel JF. (1992). [Bacterial translocation in Crohn disease]. Gastroenterol Clin Biol, 16, 777–781.

Lamont EA, Sreevatsan S. (2010). Paradigm redux-*Mycobacterium avium* subspecies *paratuberculosis*-macrophage interactions show clear variations between bovine and human physiological body temperatures. Microb Pathog, 48, 143–149.

Lawlor G, Moss AC. (2010). Cytomegalovirus in inflammatory bowel disease: pathogen or innocent bystander? Inflamm Bowel Dis, 16, 1620–1627.

Lee A, Griffiths TA, Parab RS, King RK, Dubinsky MC, Urbanski SJ, Wrobel I, Rioux KP. (2011). Association of *Mycobacterium avium*

subspecies *paratuberculosis* with Crohn Disease in pediatric patients. J Pediatr Gastroenterol Nutr, 52, 170–174.

Lee TW, Fedorak RN. (2010). Tumor necrosis factor-a monoclonal antibodies in the treatment of inflammatory bowel disease: clinical practice pharmacology. Gastroenterol Clin North Am, 39, 543–557.

Lees CW, Satsangi J. (2009). Genetics of inflammatory bowel disease: implications for disease pathogenesis and natural history. Expert Rev Gastroenterol Hepatol, 3, 513–534.

Leiper K, Morris AI, Rhodes JM. (2000). Open label trial of oral clarithromycin in active Crohn's disease. Aliment Pharmacol Ther, 14, 801–806.

Leong RW, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, Sung JJ. (2003). NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. Aliment Pharmacol Ther, 17, 1465–1470.

Levesque BG, Pai R, Cartwright CA. (2009). Crohn's colitis complicated by cytomegalovirus infection. Dig Dis Sci, 54, 1864–1867.

Lewis K, Lutgendorff F, Phan V, Söderholm JD, Sherman PM, McKay DM. (2010). Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate. Inflamm Bowel Dis, 16, 1138–1148.

Li L, Bannantine JP, Zhang Q, Amonsin A, May BJ, Alt D, Banerji N, Kanjilal S, Kapur V. (2005). The complete genome sequence of *Mycobacterium avium* subspecies *paratuberculosis*. Proc Natl Acad Sci USA, 102, 12344–12349.

Lidar M, Langevitz P, Shoenfeld Y. (2009). The role of infection in inflammatory bowel disease: initiation, exacerbation and protection. Isr Med Assoc J, 11, 558–563.

Lipton JE, Barash DP. (2007). Flawed Australian CD study does not end MAP controversy. Gastroenterology, 133, 1742; author reply 1745–1742; author reply 1746.

Lisby G, Andersen J, Engbaek K, Binder V. (1994). *Mycobacterium paratuberculosis* in intestinal tissue from patients with Crohn's disease demonstrated by a nested primer polymerase chain reaction. Scand J Gastroenterol, 29, 923–929.

Liu Y, van Kruiningen HJ, West AB, Cartun RW, Cortot A, Colombel JF. (1995). Immunocytochemical evidence of Listeria, Escherichia coli, and Streptococcus antigens in Crohn's disease. Gastroenterology, 108, 1396–1404.

Loftus EV Jr, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. (1998). Crohn's disease in Olmsted County, Minnesota, 1940–1993: incidence, prevalence, and survival. Gastroenterology, 114, 1161–1168.

Lowe AM, Yansouni CP, Behr MA. (2008). Causality and gastrointestinal infections: Koch, Hill, and Crohn's. Lancet Infect Dis, 8, 720–726.

Lozano-Leon A, Barreiro-de Acosta M, Domínguez-Munoz JE. (2006). Absence of *Mycobacterium avium* subspecies *paratuberculosis* in Crohn's disease patients. Inflamm Bowel Dis, 12, 1190–1192.

Lund BM, Gould GW, Rampling AM. (2002). Pasteurization of milk and the heat resistance of *Mycobacterium avium* subsp. *paratuberculosis*: a critical review of the data. Int J Food Microbiol, 77, 135–145.

Macpherson A, Khoo UY, Forgacs I, Philpott-Howard J, Bjarnason I. (1996). Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. Gut, 38, 365–375.

Man SM, Kaakoush NO, Mitchell HM. (2011). The role of bacteria and pattern-recognition receptors in Crohn's disease. Nat Rev Gastroenterol Hepatol, 8, 152–168.

Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, Nalin R, Jarrin C, Chardon P, Marteau P, Roca J, Dore J. (2006). Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut, 55, 205–211.

Manning EJ, Collins MT. (2001). *Mycobacterium avium* subsp. *paratuberculosis*: pathogen, pathogenesis and diagnosis. Rev - Off Int Epizoot, 20, 133–150.

Markesich DC, Graham DY, Yoshimura HH. (1988). Progress in culture and subculture of spheroplasts and fastidious acid-fast bacilli isolated from intestinal tissues. J Clin Microbiol, 26, 1600–1603.

Marks DJ, Rahman FZ, Sewell GW, Segal AW. (2010). Crohn's disease: an immune deficiency state. Clin Rev Allergy Immunol, 38, 20–31.

Marshall BJ. (2002). The discovery that Helicobacter pylori, a spiral bacterium, caused peptic ulcer disease. Carlton, Victoria, Australia: Blackwell Science Asia.

Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. (1985). Attempt to fulfil Koch's postulates for pyloric Campylobacter. Med J Aust, 142, 436–439.

Marteau P. (2009). Bacterial flora in inflammatory bowel disease. Dig Dis, 27 Suppl 1, 99–103.

Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ. (2007). Vitamin D in the treatment of pulmonary tuberculosis. J Steroid Biochem Mol Biol, 103, 793–798.

Mayer L. (2010). Evolving paradigms in the pathogenesis of IBD. J Gastroenterol, 45, 9–16.

McClure HM, Chiodini RJ, Anderson DC, Swenson RB, Thayer WR, Coutu JA. (1987). *Mycobacterium paratuberculosis* infection in a colony of stumptail macaques (Macaca arctoides). J Infect Dis, 155, 1011–1019.

McFadden JJ, Fidler HM. (1996). Mycobacteria as possible causes of sarcoidosis and Crohn's disease. Soc Appl Bacteriol Symp Ser, 25, 47S–52S.

McFadden JJ, Butcher PD, Chiodini R, Hermon-Taylor J. (1987). Crohn's disease-isolated mycobacteria are identical to *Mycobacterium paratuberculosis*, as determined by DNA probes that distinguish between mycobacterial species. J Clin Microbiol, 25, 796–801.

McGrath EE, Blades Z, McCabe J, Jarry H, Anderson PB. (2010). Nontuberculous mycobacteria and the lung: from suspicion to treatment. Lung, 188, 269–282.

McGuckin MA, Eri R, Simms LA, Florin TH, Radford-Smith G. (2009). Intestinal barrier dysfunction in inflammatory bowel diseases. Inflamm Bowel Dis, 15, 100–113.

Mendoza JL, San-Pedro A, Culebras E, Cíes R, Taxonera C, Lana R, Urcelay E, de la Torre F, Picazo JJ, Díaz-Rubio M. (2010). High prevalence of viable *Mycobacterium avium* subspecies *paratuberculosis* in Crohn's disease. World J Gastroenterol, 16, 4558–4563.

Merkal RS, Larsen AB. (1973). Clofazimine treatment of cows naturally infected with *Mycobacterium paratuberculosis*. Am J Vet Res, 34, 27–28.

Merkal RS, Kopecky KE, Larsen AB. (1970). Immunologic mechanisms in bovine paratuberculosis. Am J Vet Res, 31, 475–485.

Merkal RS, Kluge JP, Monlux WS, Larsen AB, Kopecky KE, Quinn LY, Lehmann RP. (1968). Experimental paratuberculosis in sheep after oral, intratracheal, or intravenous inoculation: histochemical localization of hydrolase activities. Am J Vet Res, 29, 985–994.

Merkal RS, Larsen AB, Kopecky KE, Kluge JP, Monlux WS, Lehmann RP, Quinn LY. (1968). Experimental paratuberculosis in sheep after oral, intratracheal, or intravenous inoculation: serologic and intradermal tests. Am J Vet Res, 29, 963–969.

Mijs W, de Haas P, Rossau R, Van der Laan T, Rigouts L, Portaels F, van Soolingen D. (2002). Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp. *avium* for bird-type isolates and '*M. avium* subsp. *hominissuis*' for the human/porcine type of *M. avium*. Int J Syst Evol Microbiol, 52, 1505–1518.

Mikula C. (2000). Measles vaccination a risk factor for inflammatory bowel disease? Gastroenterol Nurs, 23, 168–171.

Millar D, Ford J, Sanderson J, Withey S, Tizard M, Doran T, Hermon-Taylor J. (1996). IS900 PCR to detect *Mycobacterium paratuberculosis* in retail supplies of whole pasteurized cows' milk in England and Wales. Appl Environ Microbiol, 62, 3446–3452.

Minozzi G, Buggiotti L, Stella A, Strozzi F, Luini M, Williams JL. (2010). Genetic loci involved in antibody response to *Mycobacterium avium* ssp. *paratuberculosis* in cattle. PLoS ONE, 5, e11117.

Misch EA, Berrington WR, Vary JC Jr, Hawn TR. (2010). Leprosy and the human genome. Microbiol Mol Biol Rev, 74, 589–620.

Mishina D, Katsel P, Brown ST, Gilberts EC, Greenstein RJ. (1996). On the etiology of Crohn disease. Proc Natl Acad Sci USA, 93, 9816–9820.

Möbius P, Luyven G, Hotzel H, Köhler H. (2008). High genetic diversity among *Mycobacterium avium* subsp. *paratuberculosis* strains from German cattle herds shown by combination of IS900 restriction fragment length polymorphism analysis and mycobacterial interspersed repetitive unit-variable-number tandem-repeat typing. J Clin Microbiol, 46, 972–981.

Möbius P, Lentzsch P, Moser I, Naumann L, Martin G, Köhler H. (2006). Comparative macrorestriction and RFLP analysis of *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *hominissuis* isolates from man, pig, and cattle. Vet Microbiol, 117, 284–291.

Modlin RL. (2010). The innate immune response in leprosy. Curr Opin Immunol, 22, 48–54.

Möller M, Hoal EG. (2010). Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. Tuberculosis (Edinb), 90, 71–83.

Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. (2011). Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases with Time, Based on Systematic Review. Gastroenterology [Epub ahead of print]. DOI: 10.1053/j.gastr.2011.10.001

Momotani E, Whipple DL, Thiermann AB, Cheville NF. (1988). Role of M cells and macrophages in the entrance of *Mycobacterium paratuberculosis* into domes of ileal Peyer's patches in calves. Vet Pathol, 25, 131–137.

Montoya D, Modlin RL. (2010). Learning from leprosy: insight into the human innate immune response. Adv Immunol, 105, 1–24.

Moss MT, Sanderson JD, Tizard ML, Hermon-Taylor J, el-Zaatari FA, Markesich DC, Graham DY. (1992). Polymerase chain reaction detection of *Mycobacterium paratuberculosis* and *Mycobacterium avium* subsp *silvaticum* in long term cultures from Crohn's disease and control tissues. Gut, 33, 1209–1213.

Müller S, Arni S, Varga L, Balsiger B, Hersberger M, Maly F, Seibold F. (2006). Serological and DNA-based evaluation of *Chlamydia pneumoniae* infection in inflammatory bowel disease. Eur J Gastroenterol Hepatol, 18, 889–894.

Murray A, Oliaro J, Schlup MM, Chadwick VS. (1995). *Mycobacterium paratuberculosis* and inflammatory bowel disease: frequency distribution in serial colonoscopic biopsies using the polymerase chain reaction. Microbios, 83, 217–228.

Nacy C, Buckley M. (2008). A report from the American Academy of Microbiology. *Mycobacterium avium* paratuberculosis: Infrequent Human Pathogen or Public Health Threat? Available at: http://academy.asm.org/images/stories/documents/mycobacteriumaviumparatuberculosis.pdf

Nakase H, Nishio A, Tamaki H, Matsuura M, Asada M, Chiba T, Okazaki K. (2006). Specific antibodies against recombinant protein of insertion element 900 of *Mycobacterium avium* subspecies *paratuberculosis* in Japanese patients with Crohn's disease. Inflamm Bowel Dis, 12, 62–69.

Naser SA, Ghobrial G, Romero C, Valentine JF. (2004). Culture of *Mycobacterium avium* subspecies *paratuberculosis* from the blood of patients with Crohn's disease. Lancet, 364, 1039–1044.

Naser SA, Collins MT, Crawford JT, Valentine JF. (2009). Culture of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from the Blood of Patients with Crohn's disease: A Follow-Up Blind Multi Center Investigation. Open Inflamm J, 2, 22–23.

Naser SA, Hulten K, Shafran I, Graham DY, El-Zaatari FA. (2000). Specific seroreactivity of Crohn's disease patients against p35 and p36 antigens of *M. avium* subsp. *paratuberculosis*. Vet Microbiol, 77, 497–504.

Naser SA, Shafran I, Schwartz D, El-Zaatari F, Biggerstaff J. (2002). In situ identification of mycobacteria in Crohn's disease patient tissue using confocal scanning laser microscopy. Mol Cell Probes, 16, 41–48.

Naser SA, Felix J, Liping H, Romero C, Naser N, Walsh A, Safranek W. (1999). Occurrence of the IS900 gene in *Mycobacterium avium* complex derived from HIV patients. Mol Cell Probes, 13, 367–372.

Nerich V, Jantchou P, Boutron-Ruault MC, Monnet E, Weill A, Vanbockstael V, Auleley GR, Balaire C, Dubost P, Rican S, Allemand H, Carbonnel F. (2011). Low exposure to sunlight is a risk factor for Crohn's disease. Aliment Pharmacol Ther, 33, 940–945.

Nielsen SS, Toft N. (2008). Ante mortem diagnosis of paratuberculosis: a review of accuracies of ELISA, interferon-gamma assay and faecal culture techniques. Vet Microbiol, 129, 217–235.

NIH Consensus Conference. (1994). *Helicobacter pylori* in peptic ulcer disease. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease. JAMA, 272, 65–69.

Norman G. (2011). Chaos, complexity and complicatedness: lessons from rocket science. Med Educ, 45, 549–559.

Ohkusa T, Sato N. (2005). Antibacterial and antimycobacterial treatment for inflammatory bowel disease. J Gastroenterol Hepatol, 20, 340–351.

Olsen I, Wiker HG, Johnson E, Langeggen H, Reitan LJ. (2001). Elevated antibody responses in patients with Crohn's disease against a 14-kDa secreted protein purified from *Mycobacterium avium* subsp. *paratuberculosis*. Scand J Immunol, 53, 198–203.

Olsen I, Tollefsen S, Aagaard C, Reitan LJ, Bannantine JP, Andersen P, Sollid LM, Lundin KE. (2009). Isolation of *Mycobacterium avium* subspecies *paratuberculosis* reactive CD4 T cells from intestinal biopsies of Crohn's disease patients. PLoS ONE, 4, e5641.

Onwuamaegbu ME, Belcher RA, Soare C. (2005). Cell wall-deficient bacteria as a cause of infections: a review of the clinical significance. J Int Med Res, 33, 1–20.

Osterstock JB, Fosgate GT, Norby B, Manning EJ, Collins MT, Roussel AJ. (2007). Contribution of environmental mycobacteria to false-positive serum ELISA results for paratuberculosis. J Am Vet Med Assoc, 230, 896–901.

Packey CD, Sartor RB. (2009). Commensal bacteria, traditional and opportunistic pathogens, dysbiosis and bacterial killing in inflammatory bowel diseases. Curr Opin Infect Dis, 22, 292–301.

Pagnini C, Corleto VD, Mangoni ML, Pilozzi E, Torre MS, Marchese R, Carnuccio A, Giulio ED, Delle Fave G. (2011). Alteration of local microflora and a-defensins hyper-production in colonic adenoma mucosa. J Clin Gastroenterol, 45, 602–610.

Panés J, Gomollón F, Taxonera C, Hinojosa J, Clofent J, Nos P. (2007). Crohn's disease: a review of current treatment with a focus on biologics. Drugs, 67, 2511–2537.

Parent K, Mitchell PD. (1976). Bacterial variants: etiologic agent in Crohn's disease? Gastroenterology, 71, 365–368.

Parrish NM, Radcliff RP, Brey BJ, Anderson JL, Clark DL Jr, Koziczkowski JJ, Ko CG, Goldberg ND, Brinker DA, Carlson RA, Dick JD, Ellingson JL. (2009). Absence of *mycobacterium avium* subsp. *paratuberculosis* in Crohn's patients. Inflamm Bowel Dis, 15, 558–565.

Patterson CJ, LaVenture M, Hurley SS, Davis JP. (1988). Accidental self-inoculation with *Mycobacterium paratuberculosis* bacterin (Johne's bacterin) by veterinarians in Wisconsin. J Am Vet Med Assoc, 192, 1197–1199.

Pavlik I, Svastova P, Bartl J, Dvorska L, Rychlik I. (2000). Relationship between IS901 in the *Mycobacterium avium* complex strains isolated from birds, animals, humans, and the environment and virulence for poultry. Clin Diagn Lab Immunol, 7, 212–217.

Persson S, Danielsson D. (1979). On the occurrence of serum antibodies to *Bacteroides fragilis* and serogroups of *E. coli* in patients with Crohn's disease. Scand J Infect Dis Suppl, 19, 61–67.

Peyrin-Biroulet L, Lémann M. (2011). Review article: remission rates achievable by current therapies for inflammatory bowel disease. Aliment Pharmacol Ther, 33, 870–879.

Peyrin-Biroulet L, Neut C, Colombel JF. (2007). Antimycobacterial therapy in Crohn's disease: game over? Gastroenterology, 132, 2594–2598.

Pickup RW, Rhodes G, Arnott S, Sidi-Boumedine K, Bull TJ, Weightman A, Hurley M, Hermon-Taylor J. (2005). *Mycobacterium avium* subsp. *paratuberculosis* in the catchment area and water of the River Taff in South Wales, United Kingdom, and its potential relationship to clustering of Crohn's disease cases in the city of Cardiff. Appl Environ Microbiol, 71, 2130–2139.

Pinedo PJ, Buergelt CD, Donovan GA, Melendez P, Morel L, Wu R, Langaee TY, Rae DO. (2009). Association between CARD15/NOD2

gene polymorphisms and paratuberculosis infection in cattle. Vet Microbiol, 134, 346–352.

Prantera C, Kohn A, Mangiarotti R, Andreoli A, Luzi C. (1994). Antimycobacterial therapy in Crohn's disease: results of a controlled, double-blind trial with a multiple antibiotic regimen. Am J Gastroenterol, 89, 513–518.

Primm TP, Lucero CA, Falkinham JO 3rd. (2004). Health impacts of environmental mycobacteria. Clin Microbiol Rev, 17, 98–106.

Purdie AC, Plain KM, Begg DJ, de Silva K, Whittington RJ. (2011). Candidate gene and genome-wide association studies of *Mycobacterium avium* subsp. *paratuberculosis* infection in cattle and sheep: a review. Comp Immunol Microbiol Infect Dis, 34, 197–208.

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J; MetaHIT Consortium. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature, 464, 59–65.

Qual DA, Kaneene JB, Varty TJ, Miller R, Thoen CO. (2010). Lack of association between the occurrence of Crohn's disease and occupational exposure to dairy and beef cattle herds infected with *Mycobacterium avium* subspecies *paratuberculosis*. J Dairy Sci, 93, 2371–2376.

Quesniaux VF, Jacobs M, Allie N, Grivennikov S, Nedospasov SA, Garcia I, Olleros ML, Shebzukhov Y, Kuprash D, Vasseur V, Rose S, Court N, Vacher R, Ryffel B. (2010). TNF in host resistance to tuberculosis infection. Curr Dir Autoimmun, 11, 157–179.

Radomski N, Thibault VC, Karoui C, de Cruz K, Cochard T, Gutiérrez C, Supply P, Biet F, Boschiroli ML. (2010). Determination of genotypic diversity of *Mycobacterium avium* subspecies from human and animal origins by mycobacterial interspersed repetitive-unit-variable-number tandem-repeat and IS1311 restriction fragment length polymorphism typing methods. J Clin Microbiol, 48, 1026–1034.

Raizman EA, Wells SJ, Godden SM, Bey RF, Oakes MJ, Bentley DC, Olsen KE. (2004). The distribution of *Mycobacterium avium* ssp. *paratuberculosis* in the environment surrounding Minnesota dairy farms. J Dairy Sci, 87, 2959–2966.

Rankin JD. (1955). An attempt to prevent the establishment of *Mycobacterium johnei* in calves by means of isoniazid alone and in combination with streptomycin. Vet Rec, 66, 550–551.

Rath T, Roderfeld M, Blöcher S, Rhode A, Basler T, Akineden Ö, Abdulmawjood A, Halwe JM, Goethe R, Bülte M, Roeb E. (2011). Presence of intestinal *Mycobacterium avium* subspecies *paratuberculosis* (MAP) DNA is not associated with altered MMP expression in ulcerative colitis. BMC Gastroenterol, 11, 34.

Ren Z, Turton J, Borody T, Pang G, Clancy R. (2008). Selective Th2 pattern of cytokine secretion in *Mycobacterium avium* subsp. *paratuberculosis* infected Crohn's disease. J Gastroenterol Hepatol, 23, 310–314.

Ricanek P, Lothe SM, Szpinda I, Jorde AT, Brackmann S, Perminow G, Jørgensen KK, Rydning A, Vatn MH, Tønjum T; IBSEN II study group. (2010). Paucity of mycobacteria in mucosal bowel biopsies from adults and children with early inflammatory bowel disease. J Crohns Colitis, 4, 561–566.

Roffey DM, Wai EK, Bishop P, Kwon BK, Dagenais S. (2010). Causal assessment of occupational sitting and low back pain: results of a systematic review. Spine J, 10, 252–261.

Roiz MP, Palenque E, Guerrero C, Garcia MJ. (1995). Use of restriction fragment length polymorphism as a genetic marker for typing *Mycobacterium avium* strains. J Clin Microbiol, 33, 1389–1391.

Romero C, Hamdi A, Valentine JF, Naser SA. (2005). Evaluation of surgical tissue from patients with Crohn's disease for the presence of *Mycobacterium avium* subspecies *paratuberculosis* DNA by in situ hybridization and nested polymerase chain reaction. Inflamm Bowel Dis, 11, 116–125.

Rosu V, Ahmed N, Paccagnini D, Gerlach G, Fadda G, Hasnain SE, Zanetti S, Sechi LA. (2009). Specific immunoassays confirm association of *Mycobacterium avium* subsp. *paratuberculosis* with type-1 but not type-2 diabetes mellitus. PLoS ONE, 4, e4386.

Rothman KJ, Greenland S. (2005). Causation and causal inference in epidemiology. Am J Public Health, 95 Suppl 1, S144–S150.

Rowbotham DS, Howdle PD, Trejdosiewicz LK. (1995). Peripheral cell-mediated immune response to mycobacterial antigens in inflammatory bowel disease. Clin Exp Immunol, 102, 456–461.

Rowbotham DS, Mapstone NP, Trejdosiewicz LK, Howdle PD, Quirke P. (1995). *Mycobacterium paratuberculosis* DNA not detected in Crohn's disease tissue by fluorescent polymerase chain reaction. Gut, 37, 660–667.

Roy E, Lowrie DB, Jolles SR. (2007). Current strategies in TB immunotherapy. Curr Mol Med, 7, 373–386.

Ruiz-Larrañaga O, Garrido JM, Iriondo M, Manzano C, Molina E, Koets AP, Rutten VP, Juste RA, Estonba A. (2010a). Genetic association between bovine NOD2 polymorphisms and infection by *Mycobacterium avium* subsp. *paratuberculosis* in Holstein-Friesian cattle. Anim Genet, 41, 652–655.

Ruiz-Larrañaga O, Garrido JM, Manzano C, Iriondo M, Molina E, Gil A, Koets AP, Rutten VP, Juste RA, Estonba A. (2010b). Identification of single nucleotide polymorphisms in the bovine solute carrier family 11 member 1 (SLC11A1) gene and their association with infection by *Mycobacterium avium* subspecies *paratuberculosis*. J Dairy Sci, 93, 1713–1721.

Ruiz-Larrañaga O, Garrido JM, Iriondo M, Manzano C, Molina E, Montes I, Vazquez P, Koets AP, Rutten VP, Juste RA, Estonba A. (2010c). SP110 as a novel susceptibility gene for *Mycobacterium avium* subspecies *paratuberculosis* infection in cattle. J Dairy Sci, 93, 5950–5958.

Rutgeerts P, Geboes K, Vantrappen G, Van Isveldt J, Peeters M, Penninckx F, Hiele M. (1992). Rifabutin and ethambutol do not help recurrent Crohn's disease in the neoterminal ileum. J Clin Gastroenterol, 15, 24–28.

Ryan P, Bennett MW, Aarons S, Lee G, Collins JK, O'Sullivan GC, O'Connell J, Shanahan F. (2002). PCR detection of *Mycobacterium paratuberculosis* in Crohn's disease granulomas isolated by laser capture microdissection. Gut, 51, 665–670.

Salonen A, de Vos WM, Palva A. (2010). Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives. Microbiology (Reading, Engl), 156, 3205–3215.

Sanderson JD, Moss MT, Tizard ML, Hermon-Taylor J. (1992). *Mycobacterium paratuberculosis* DNA in Crohn's disease tissue. Gut, 33, 890–896.

Sasikala M, Reddy DN, Pratap N, Sharma SK, Balkumar PR, Sekaran A, Banerjee R, Reddy DB. (2009). Absence of *Mycobacterium avium* ss *paratuberculosis*–specific IS900 sequence in intestinal biopsy tissues of Indian patients with Crohn's disease. Indian J Gastroenterol, 28, 169–174.

Scanu AM, Bull TJ, Cannas S, Sanderson JD, Sechi LA, Dettori G, Zanetti S, Hermon-Taylor J. (2007). *Mycobacterium avium* subspecies *paratuberculosis* infection in cases of irritable bowel syndrome and comparison with Crohn's disease and Johne's disease: common neural and immune pathogenicities. J Clin Microbiol, 45, 3883–3890.

Schirbel A, Fiocchi C. (2010). Inflammatory bowel disease: Established and evolving considerations on its etiopathogenesis and therapy. J Dig Dis, 11, 266–276.

Schuller JL, Piket-van Ulsen J, Veeken IV, Michel MF, Stolz E. (1979). Antibodies against Chlamydia of lymphogranuloma-venereum type in Crohn's disease. Lancet, 1, 19–20.

Schulzke JD, Ploeger S, Amasheh M, Fromm A, Zeissig S, Troeger H, Richter J, Bojarski C, Schumann M, Fromm M. (2009). Epithelial tight junctions in intestinal inflammation. Ann N Y Acad Sci, 1165, 294–300.

Schurr E, Gros P. (2009). A common genetic fingerprint in leprosy and Crohn's disease? N Engl J Med, 361, 2666–2668.

Schwartz D, Shafran I, Romero C, Piromalli C, Biggerstaff J, Naser N, Chamberlin W, Naser SA. (2000). Use of short-term culture for identification of *Mycobacterium avium* subsp. *paratuberculosis* in tissue from Crohn's disease patients. Clin Microbiol Infect, 6, 303–307.

Scollard DM, Joyce MP, Gillis TP. (2006). Development of leprosy and type 1 leprosy reactions after treatment with infliximab: a report of 2 cases. Clin Infect Dis, 43, e19–e22.

Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL. (2006). The continuing challenges of leprosy. Clin Microbiol Rev, 19, 338–381.

Sechi LA, Mura M, Tanda E, Lissia A, Fadda G, Zanetti S. (2004). *Mycobacterium avium* sub. *paratuberculosis* in tissue samples of Crohn's disease patients. New Microbiol, 27, 75–77.

Sechi LA, Mura M, Tanda F, Lissia A, Solinas A, Fadda G, Zanetti S, Manuela M, Francesco T, Amelia L, Antonello S, Giovanni F, Stefania Z. (2001). Identification of *Mycobacterium avium* subsp. *paratuberculosis* in biopsy specimens from patients with Crohn's disease identified by in situ hybridization. J Clin Microbiol, 39, 4514–4517.

Sechi LA, Scanu AM, Molicotti P, Cannas S, Mura M, Dettori G, Fadda G, Zanetti S. (2005). Detection and Isolation of *Mycobacterium avium* subspecies *paratuberculosis* from intestinal mucosal biopsies of patients with and without Crohn's disease in Sardinia. Am J Gastroenterol, 100, 1529–1536.

Sechi LA, Gazouli M, Ikonomopoulos J, Lukas JC, Scanu AM, Ahmed N, Fadda G, Zanetti S. (2005). *Mycobacterium avium* subsp. *paratuberculosis*, genetic susceptibility to Crohn's disease, and Sardinians: the way ahead. J Clin Microbiol, 43, 5275–5277.

Sechi LA, Gazouli M, Sieswerda LE, Molicotti P, Ahmed N, Ikonomopoulos J, Scanu AM, Paccagnini D, Zanetti S. (2006). Relationship between Crohn's disease, infection with *Mycobacterium avium* subspecies *paratuberculosis* and SLC11A1 gene polymorphisms in Sardinian patients. World J Gastroenterol, 12, 7161–7164.

Selby W, Pavli P, Crotty B, Florin T. (2007). Author's reply. Gastroenterol, 133, 1745–1746.

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; Antibiotics in Crohn's Disease Study Group. (2007). Two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Crohn's disease. Gastroenterology, 132, 2313–2319.

Seldenrijk CA, Drexhage HA, Meuwissen SG, Meijer CJ. (1990). T-cellular immune reactions (in macrophage inhibition factor assay) against *Mycobacterium paratuberculosis, Mycobacterium kansasii, Mycobacterium tuberculosis, Mycobacterium avium* in patients with chronic inflammatory bowel disease. Gut, 31, 529–535.

Semret M, Turenne CY, Behr MA. (2006). Insertion sequence IS900 revisited. J Clin Microbiol, 44, 1081–1083.

Shaffer JL, Hughes S, Linaker BD, Baker RD, Turnberg LA. (1984). Controlled trial of rifampicin and ethambutol in Crohn's disease. Gut, 25, 203–205.

Shafran I, Kugler L, El-Zaatari FA, Naser SA, Sandoval J. (2002). Open clinical trial of rifabutin and clarithromycin therapy in Crohn's disease. Dig Liver Dis, 34, 22–28.

Shaoul R, Karban A, Weiss B, Reif S, Wasserman D, Pacht A, Eliakim R, Wardi J, Shirin H, Wine E, Leshinsky-Silver E, Levine A. (2004). NOD2/CARD15 mutations and presence of granulomas in pediatric and adult Crohn's disease. Inflamm Bowel Dis, 10, 709–714.

Shin AR, Kim HJ, Cho SN, Collins MT, Manning EJ, Naser SA, Shin SJ. (2010). Identification of seroreactive proteins in the culture filtrate antigen of *Mycobacterium avium* ssp. *paratuberculosis* human isolates to sera from Crohn's disease patients. FEMS Immunol Med Microbiol, 58, 128–137.

Sidoti F, Banche G, Astegiano S, Allizond V, Cuffini AM, Bergallo M. (2011). Validation and standardization of IS900 and F57 real-time quantitative PCR assays for the specific detection and quantification of *Mycobacterium avium* subsp. *paratuberculosis*. Can J Microbiol, 57, 347–354.

Sigurdardóttir OG, Bakke-McKellep AM, Djønne B, Evensen O. (2005). *Mycobacterium avium* subsp. *paratuberculosis* enters the small intestinal mucosa of goat kids in areas with and without Peyer's patches as demonstrated with the everted sleeve method. Comp Immunol Microbiol Infect Dis, 28, 223–230.

Singh PK, Singh SV, Singh AV, Sohal JS. (2008). Evaluation of four methods of DNA recovery from *Mycobacterium avium* subspecies *paratuberculosis* present in intestine tissue of goats and comparative sensitivity of IS900 PCR with respect to culture for diagnosis of Johne's disease. Indian J Exp Biol, 46, 579–582.

Sisto M, Cucci L, D'Amore M, Dow TC, Mitolo V, Lisi S. (2010). Proposing a relationship between *Mycobacterium avium* subspecies *paratuberculosis* infection and Hashimoto's thyroiditis. Scand J Infect Dis, 42, 787–790.

Smego RA, Ahmed N. (2003). A systematic review of the adjunctive use of systemic corticosteroids for pulmonary tuberculosis. Int J Tuberc Lung Dis, 7, 208–213.

Sobhani I, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, Langella P, Corthier G, Tran Van Nhieu J, Furet JP. (2011). Microbial dysbiosis in colorectal cancer (CRC) patients. PLoS ONE, 6, e16393.

Sohal JS, Singh SV, Singh AV, Singh PK. (2010). Strain diversity within *Mycobacterium avium* subspecies *paratuberculosis*—a review. Indian J Exp Biol, 48, 7–16.

St-Jean G, Jernigan AD. (1991). Treatment of *Mycobacterium paratuberculosis* infection in ruminants. Vet Clin North Am Food Anim Pract, 7, 793–804.

Stainsby KJ, Lowes JR, Allan RN, Ibbotson JP. (1993). Antibodies to *Mycobacterium paratuberculosis* and nine species of environmental mycobacteria in Crohn's disease and control subjects. Gut, 34, 371–374.

Steingart KR, Henry M, Laal S, Hopewell PC, Ramsay A, Menzies D, Cunningham J, Weldingh K, Pai M. (2007). Commercial serological antibody detection tests for the diagnosis of pulmonary tuberculosis: a systematic review. PLoS Med, 4, e202.

Stephan R, Schumacher S, Tasara T, Grant IR. (2007). Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Swiss raw milk cheeses collected at the retail level. J Dairy Sci, 90, 3590–3595.

Stevenson K. (2010). Diagnosis of Johne's Disease: Current limitations and prospects. Cattle Pract, 18, 104–109.

Stewart LC, Day AS, Pearson J, Barclay ML, Gearry RB, Roberts RL, Bentley RW. (2010). SLC11A1 polymorphisms in inflammatory bowel disease and *Mycobacterium avium* subspecies *paratuberculosis* status. World J Gastroenterol, 16, 5727–5731.

Stinear TP, Seemann T, Harrison PF, Jenkin GA, Davies JK, Johnson PD, Abdellah Z, Arrowsmith C, Chillingworth T, Churcher C, Clarke K, Cronin A, Davis P, Goodhead I, Holroyd N, Jagels K, Lord A, Moule S, Mungall K, Norbertczak H, Quail MA, Rabbinowitsch E, Walker D, White B, Whitehead S, Small PL, Brosch R, Ramakrishnan L, Fischbach MA, Parkhill J, Cole ST. (2008). Insights from the complete genome sequence of *Mycobacterium marinum* on the evolution of *Mycobacterium tuberculosis*. Genome Res, 18, 729–741.

Subramanian S, Campbell BJ, Rhodes JM. (2006). Bacteria in the pathogenesis of inflammatory bowel disease. Curr Opin Infect Dis, 19, 475–484.

Suenaga K, Yokoyama Y, Okazaki K, Yamamoto Y. (1995). Mycobacteria in the intestine of Japanese patients with inflammatory bowel disease. Am J Gastroenterol, 90, 76–80.

Suenaga K, Yokoyama Y, Nishimori I, Sano S, Morita M, Okazaki K, Onishi S. (1999). Serum antibodies to *Mycobacterium paratuberculosis* in patients with Crohn's disease. Dig Dis Sci, 44, 1202–1207.

Swift GL, Srivastava ED, Stone R, Pullan RD, Newcombe RG, Rhodes J, Wilkinson S, Rhodes P, Roberts G, Lawrie BW. (1994). Controlled trial of anti-tuberculous chemotherapy for two years in Crohn's disease. Gut, 35, 363–368.

Tabaqchali S, O'Donoghue DP, Bettelheim KA. (1978). *Escherichia coli* antibodies in patients with inflammatory bowel disease. Gut, 19, 108–113.

Taddei S, Robbi C, Cesena C, Rossi I, Schiano E, Arrigoni N, Vicenzoni G, Cavirani S. (2004). Detection of *Mycobacterium avium* subsp. *paratuberculosis* in bovine fecal samples: comparison of three polymerase chain reaction-based diagnostic tests with a conventional culture method. J Vet Diagn Invest, 16, 503–508.

Talhari C, Ferreira LC, Araújo JR, Talhari AC, Talhari S. (2008). Immune reconstitution inflammatory syndrome or upgrading type 1 reaction? Report of two AIDS patients presenting a shifting from borderline lepromatous leprosy to borderline tuberculoid leprosy. Lepr Rev, 79, 429–435.

Tanaka K, Wilks M, Coates PJ, Farthing MJ, Walker-Smith JA, Tabaqchali S. (1991). *Mycobacterium paratuberculosis* and Crohn's disease. Gut, 32, 43–45.

Tasara T, Hoelzle LE, Stephan R. (2005). Development and evaluation of a *Mycobacterium avium* subspecies *paratuberculosis* (MAP) specific multiplex PCR assay. Int J Food Microbiol, 104, 279–287.

Thamer M, Ray NF, Henderson SC, Rinehart CS, Sherman CR, Ferguson JH. (1998). Influence of the NIH Consensus Conference on *Helicobacter pylori* on physician prescribing among a Medicaid population. Med Care, 36, 646–660.

Thayer WR. (1992). The use of antimycobacterial agents in Crohn's disease. J Clin Gastroenterol, 15, 5–7.

Thayer WR Jr, Coutu JA, Chiodini RJ, Van Kruiningen HJ, Merkal RS. (1984). Possible role of mycobacteria in inflammatory bowel disease. II. Mycobacterial antibodies in Crohn's disease. Dig Dis Sci, 29, 1080–1085.

Thia KT, Sandborn WJ, Harmsen WS, Zinsmeister AR, Loftus EV Jr. (2010). Risk factors associated with progression to intestinal complications of Crohn's disease in a population-based cohort. Gastroenterology, 139, 1147–1155.

Thiam S, LeFevre AM, Hane F, Ndiaye A, Ba F, Fielding KL, Ndir M, Lienhardt C. (2007). Effectiveness of a strategy to improve adherence to tuberculosis treatment in a resource-poor setting: a cluster randomized controlled trial. JAMA, 297, 380–386.

Thomas Dow C. (2008). Cows, Crohn's and more: is *Mycobacterium paratuberculosis* a superantigen? Med Hypotheses, 71, 858–861.

Thomas GA, Swift GL, Green JT, Newcombe RG, Braniff-Mathews C, Rhodes J, Wilkinson S, Strohmeyer G, Kreuzpainter G. (1998). Controlled trial of antituberculous chemotherapy in Crohn's disease: a five year follow up study. Gut, 42, 497–500.

Thorel MF, Krichevsky M, Lévy-Frébault VV. (1990). Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. nov., *Mycobacterium avium* subsp. *paratuberculosis* subsp. nov., and *Mycobacterium avium* subsp. *silvaticum* subsp. nov. Int J Syst Bacteriol, 40, 254–260.

Thornton GF. (1970). The role of corticosteroids in the management of tuberculous infections. In: Johnson JEI, ed., Rational Therapy and Control of Tuberculosis. A Symposium. Gainesville: University of Florida Press.

Toracchio S, El-Zimaity HM, Urmacher C, Katz S, Graham DY. (2008). *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease granulomas. Scand J Gastroenterol, 43, 1108–1111.

Tuci A, Tonon F, Castellani L, Sartini A, Roda G, Marocchi M, Caponi A, Munarini A, Rosati G, Ugolini G, Fuccio L, Scagliarini M, Bazzoli F, Belluzzi A. (2011). Fecal detection of *Mycobacterium avium* paratuberculosis using the IS900 DNA sequence in Crohn's disease and ulcerative colitis patients and healthy subjects. Dig Dis Sci, 56, 2957–2962.

Turenne CY, Wallace R Jr, Behr MA. (2007). *Mycobacterium avium* in the postgenomic era. Clin Microbiol Rev, 20, 205–229.

Turenne CY, Collins DM, Alexander DC, Behr MA. (2008). *Mycobacterium avium* subsp. *paratuberculosis* and *M. avium* subsp. avium are independently evolved pathogenic clones of a much broader group of *M. avium* organisms. J Bacteriol, 190, 2479–2487.

Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med, 1, 6ra14.

Uematsu S, Fujimoto K. (2010). The innate immune system in the intestine. Microbiol Immunol, 54, 645–657.

USDA. (2008). Johne's disease on U.S. dairies, 1991–2007. Fort Collins, CO: USDA, APHIS, Veterinary Services, Center for Epidemiology and Animal Health.

USDA, National Advisory Committee on Microbiological Criteria for Foods. (2010). Assessment of food as a source of exposure to *Mycobacterium avium* subspecies *paratuberculosis* (MAP). J Food Prot, 73, 1357–1397.

Ustianowski AP, Lawn SD, Lockwood DN. (2006). Interactions between HIV infection and leprosy: a paradox. Lancet Infect Dis, 6, 350–360.

Uzoigwe JC, Khaitsa ML, Gibbs PS. (2007). Epidemiological evidence for *Mycobacterium avium* subspecies paratuberculosis as a cause of Crohn's disease. Epidemiol Infect, 135, 1057–1068.

Van Kruiningen HJ. (1999). Lack of support for a common etiology in Johne's disease of animals and Crohn's disease in humans. Inflamm Bowel Dis, 5, 183–191.

Van Kruiningen HJ, Chiodini RJ, Thayer WR, Coutu JA, Merkal RS, Runnels PL. (1986). Experimental disease in infant goats induced by a *Mycobacterium* isolated from a patient with Crohn's disease. A preliminary report. Dig Dis Sci, 31, 1351–1360.

van Vliet MJ, Harmsen HJ, de Bont ES, Tissing WJ. (2010). The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. PLoS Pathog, 6, e1000879.

Vary PH, Andersen PR, Green E, Hermon-Taylor J, McFadden JJ. (1990). Use of highly specific DNA probes and the polymerase chain reaction to detect *Mycobacterium paratuberculosis* in Johne's disease. J Clin Microbiol, 28, 933–937.

Vatn MH. (2009). Natural history and complications of IBD. Curr Gastroenterol Rep, 11, 481–487.

Verma RK, Jain A. (2007). Antibodies to mycobacterial antigens for diagnosis of tuberculosis. FEMS Immunol Med Microbiol, 51, 453–461.

Verna AE, Garcia-Pariente C, Muñoz M, Moreno O, García-Marin JF, Romano MI, Paolicchi F, Pérez V. (2007). Variation in the immuno-pathological responses of lambs after experimental infection with different strains of *Mycobacterium avium* subsp. *paratuberculosis*. Zoonoses Public Health, 54, 243–252.

Verschoor CP, Pant SD, You Q, Schenkel FS, Kelton DF, Karrow NA. (2010). Polymorphisms in the gene encoding bovine interleukin-10 receptor alpha are associated with *Mycobacterium avium* ssp. *paratuberculosis* infection status. BMC Genet, 11, 23.

Viazis N, Vlachogiannakos J, Georgiou O, Rodias M, Georgiadis D, Papastamopoulos V, Baraboutis IG, Karamanolis DG, Skoutelis A. (2010). Course of inflammatory bowel disease in patients infected with human immunodeficiency virus. Inflamm Bowel Dis, 16, 507–511.

Wagner J, Sim W, Bishop RF, Catto-Smith AG, Cameron DJ, Kirkwood CD. (2011). *Mycobacterium avium* subspecies *paratuberculosis* in children with early-onset Crohn's disease: a longitudinal follow-up study. Inflamm Bowel Dis, 17, 1825–1826.

Wall S, Kunze ZM, Saboor S, Soufleri I, Seechurn P, Chiodini R, McFadden JJ. (1993). Identification of spheroplast-like agents isolated from tissues of patients with Crohn's disease and control tissues by polymerase chain reaction. J Clin Microbiol, 31, 1241–1245.

Walmsley RS, Ibbotson JP, Chahal H, Allan RN. (1996). Antibodies against *Mycobacterium paratuberculosis* in Crohn's disease. QJM, 89, 217–221.

Wen Z, Fiocchi C. (2004). Inflammatory bowel disease: autoimmune or immune-mediated pathogenesis? Clin Dev Immunol, 11, 195–204.

Whan L, Ball HJ, Grant IR, Rowe MT. (2005). Occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in untreated water in Northern Ireland. Appl Environ Microbiol, 71, 7107–7112.

Whitlock RH, Wells SJ, Sweeney RW, Van Tiem J. (2000). ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. Vet Microbiol, 77, 387–398.

Whittington RJ, Marshall DJ, Nicholls PJ, Marsh IB, Reddacliff LA. (2004). Survival and dormancy of *Mycobacterium avium* subsp. *paratuberculosis* in the environment. Appl Environ Microbiol, 70, 2989–3004.

Whittington RJ, Marsh IB, Saunders V, Grant IR, Juste R, Sevilla IA, Manning EJ, Whitlock RH. (2011). Culture phenotypes of genomically and geographically diverse *Mycobacterium avium* subsp. *paratuberculosis* isolates from different hosts. J Clin Microbiol, 49, 1822–1830.

Windsor PA, Whittington RJ. (2010). Evidence for age susceptibility of cattle to Johne's disease. Vet J, 184, 37–44.

Windsor PA, Bush R, Links I, Eppleston J. (2005). Injury caused by self-inoculation with a vaccine of a Freund's complete adjuvant nature (Gudair) used for control of ovine paratuberculosis. Aust Vet J, 83, 216–220.

Winthrop KL, Chang E, Yamashita S, Iademarco MF, LoBue PA. (2009). Nontuberculous mycobacteria infections and anti-tumor necrosis factor-alpha therapy. Emerging Infect Dis, 15, 1556–1561.

Wynne JW, Seemann T, Bulach DM, Coutts SA, Talaat AM, Michalski WP. (2010). Resequencing the *Mycobacterium avium* subsp. *paratuberculosis* K10 genome: improved annotation and revised genome sequence. J Bacteriol, 192, 6319–6320.

Yim JJ, Selvaraj P. (2010). Genetic susceptibility in tuberculosis. Respirology, 15, 241–256.

Zhang FR, Huang W, Chen SM, Sun LD, Liu H, Li Y, Cui Y, Yan XX, Yang HT, Yang RD, Chu TS, Zhang C, Zhang L, Han JW, Yu GQ, Quan C, Yu YX, Zhang Z, Shi BQ, Zhang LH, Cheng H, Wang CY, Lin Y, Zheng HF, Fu XA, Zuo XB, Wang Q, Long H, Sun YP, Cheng YL, Tian HQ, Zhou FS, Liu HX, Lu WS, He SM, Du WL, Shen M, Jin QY, Wang Y, Low HQ, Erwin T, Yang NH, Li JY, Zhao X, Jiao YL, Mao LG, Yin G, Jiang ZX, Wang XD, Yu JP, Hu ZH, Gong CH, Liu YQ, Liu RY, Wang DM, Wei D, Liu JX, Cao WK, Cao HZ, Li YP, Yan WG, Wei SY, Wang KJ, Hibberd ML, Yang S, Zhang XJ, Liu JJ. (2009). Genomewide association study of leprosy. N Engl J Med, 361, 2609–2618.

Zhang H, Massey D, Tremelling M, Parkes M. (2008). Genetics of inflammatory bowel disease: clues to pathogenesis. Br Med Bull, 87, 17–30.

Zhang MZ, Zhang S. (2011). An efficient DNA extraction method for polymerase chain reaction-based detection of *Mycobacterium avium* subspecies *paratuberculosis* in bovine fecal samples. J Vet Diagn Invest, 23, 41–48.

Zwick LS, Walsh TF, Barbiers R, Collins MT, Kinsel MJ, Murnane RD. (2002). Paratuberculosis in a mandrill (Papio sphinx). J Vet Diagn Invest, 14, 326–328.