Chapter 43
Bovine Paratuberculosis and Human Crohn's Disease—Is There a Zoonotic Linkage?

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Abstract *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is an acid-fast bacterium, which causes paratuberculosis, an infectious enteric disease of ruminants, also called Johne's disease (JD). Since the publication by Thomas Kennedy Dalziel in the year 1913 MAP has been discussed as probable causative agent of Morbus Crohn (syn. Crohn's Disease (CD)), an inflammatory disease of the human intestinal tract. Here we describe the history, etiology, diagnosis, clinical and epidemiological aspects of paratuberculosis and CD to elucidate the role of MAP in the pathogenesis of CD. The theory still remains open for controversial discussion and future studies are needed to find a final conclusion. At the moment, however, there is not enough evidence to convincingly demonstrate that MAP is the etiological agent for CD.

43.1 Introduction

Morbus Crohn (syn. Crohn's Disease (CD)) is an inflammatory disease that has the potential to involve any part of the human intestinal tract anywhere from the mouth to the anus. The disease is generally located at the terminal ileum and the proximal colon. A linkage between CD and *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is discussed since the publication by Thomas Kennedy Dalziel in the year 1913 (Dalziel 1989).

43.2 Johne's Disease/Paratuberculosis

Paratuberculosis, also called Johne's disease (JD) is an infectious disease of ruminants caused by MAP. Paratuberculosis can be found worldwide. Only Sweden and some states in Australia are proven to be free of this disease (http://www.cfsph.iastate.edu/Factsheets/pdfs/paratuberculosis.pdf).

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Primary susceptible species are cattle, sheep, goats, deers including wild ruminant species, and other ruminants like camels (Kennedy and Benedictus 2001; Tiwari et al. 2006). The host range of JD is wide. Infections of non-ruminants like wild rabbits, foxes, stoats (a weasel species), mandrills or macaques are described (Beard et al. 2001; McClure et al. 1987; Zwick et al. 2002). Calves inoculated with MAP from a free-living rabbit developed typical histological lesions consistent with JD, demonstrating that wild animals other than ruminants may also contribute to the spread of the disease. But the main source of infection for calves is the exposure to feces of infected mature cattle (Beard et al. 2001).

43.3 History

Since the middle of the nineteenth century both the clinical signs and pathological anatomy of paratuberculosis are known as chronic enteritis with marked transformation of the intestinal mucosa. Johne and Frothingham demonstrated acid-fast bacilli in altered tissues and described for the first time this disease as a singular case of tuberculosis in cattle (Johne and Frothingham 1895).

Due to the different biological properties of the infectious agent and due to the different pathological patterns Bang proposed in the year 1906 to separate the illness from tuberculosis. Since then bovine paratuberculosis is classified as a separate disease (Bang 1906).

In 1910/1912 Twort published the isolation and cultivation of the infectious agent for the first time. Finally, in 1933 the experimental infection of cattle with MAP succeeded. By that the Henle-Koch postulates were fulfilled. Since then it is proven that MAP is the monocausal agent of bovine paratuberculosis (Twort and Ingram 1912).

43.4 Etiology

43.4.1 Infectious Agent

MAP belongs to the family of *Mycobacteriaceae*, gram-positive bacteria, which comprise more than 100 species. They proliferate intracellularly and are characterized by their acid-stability and lipid-enriched cell wall. The subspecies MAP is clearly distinct from other pathogens of the family like *Mycobacterium tuberculosis*, the infectious agent of tuberculosis or *Mycobacterium leprae*, the infectious agent of leprosy. MAP is a mycobacterial subspecies with a slow replication rate and a generation time of over 20 h. Its cultural growth is mycobactin dependent (Rowe and Grant 2006). MAP is a subspecies member of the *M. avium* complex. While members of the *M. avium* complex typically cause disease in immunocom-

promised hosts, MAP has been elucidated as causative agent of JD in immunocompetent ruminants (Behr and Kapur 2008).

43.4.2 Pathogenesis of Johne's Disease

MAP can be transmitted directly, most commonly fecal-orally or orally by colostrum. Infections by the intrauterine route or semen are also reported (Uzoigwe et al. 2007).

85 % of the animals get infected in the first days of their life, 5 % during the first year and 10 % in utero. Commonly the infection starts in the first 30 days of life, later on an increasing age-resistance is acquired. Calves are mainly infected orally by fecally contaminated drugs or by colostrum, older animals by contaminated food, water or pastures. The incubation time is reported to range from 2 to 10 years (Chiodini et al. 1984).

The disease is characterized by a profuse and intractable diarrhea that ends up in severe weight loss and death.

The disease develops chronically and intermittently. Over a very long period infected animals show no signs of illness. Therefore, JD usually stays undetected for a long time. However, the spreading of bacteria starts before the onset of clinical symptoms.

Hence mainly animals in a late subclinical stage play an important role in the propagation of paratuberculosis. Whitlock and Buergelt described that one animal with clinical symptoms represents only the tip of an iceberg. Every cow with symptoms may indicate 15–25 more affected animals in the herd (Whitlock and Buergelt 1996).

Furthermore the intermittent spread is another cornerstone of the maintenance of MAP in the herd. While one clinically affected cow releases up to 10 million infectious particles per gram feces (Whitlock et al. 2005), the excretion dose of a subclinically diseased cow ranges between 10 and 100 infectious agents per gram.

The infection dose is reported to be 10,000 agents for the infection of one calf (Gerlach 2002).

43.4.3 Ability to Survive

The thick, waxy cell wall renders the organism highly resistant. The pathogen survives in soil up to 11 months. In running water a survival time up to 163 days is reported. In standing water or bovine feces the organism was detectable for at least 35 weeks (Lovell et al. 1944; Whittington et al. 2005; Whittington et al. 2004).

Beside the high environmental survival time of MAP also the high resistance to heat is significant. MAP in milk was stated to survive pasteurization (Grant 2003; Millar et al. 1996).

43.4.4 Time Line of Paratuberculosis

The course of disease can be divided into four phases of different immunology, clinical signs and pathomorphology (Whitlock and Buergelt 1996):

1. the silent early stage of infection
2. the subclinical stage
3. the clinical stage
4. the advanced clinical stage

1. The Silent Early Stage of Infection After oral ingestion MAP is taken up by M cells, mainly in the ileum, and transported into the Peyer's patches. There the organism is phagocytized by resident macrophages and persists in their phagosomes, thereby eliciting a cell mediated immune reaction. In this stage there is no detectable spread of the agent by feces.
The animal shows no signs of disease. When examining the tissue acid-fast bacilli can be seen histologically in intestinal lymph nodes or intestinal sections and MAP can be cultivated (Whitlock and Buergelt 1996).

2. The Subclinical Stage The intracellular proliferation of the pathogen in macrophages results ultimately in cell death and release of the agent into surrounding tissues and the gut lumen. By the liberation of MAP the humoral immune response is initiated. Acid-fast bacilli can be (intermittently) detected in the feces (Coussens 2001; Sweeney et al. 1992).
The animal still shows no signs of disease. Stages 1 and 2 correspond to the incubation time of the disease and can range between 2 to 10 years.
Pathomorphologically a moderate hypertrophy of the mucosa and an enlargement of the mesenterial lymph nodes is accompanied by infiltration of epitheloid and Langhans giant cells and foamy macrophages containing phagocytized acid-fast bacilli.

3./4. The Clinical Stage/Advanced Clinical Stage Initial clinical signs follow the subclinical stage. The first apparent sign is gradual weight loss. Congruent with the weight loss the manure consistency becomes more fluid. The clinical symptoms can be seen for months with intermittent times of amelioration. Later, clinically affected animals become increasingly lethargic, weak and emaciated. "Water-hose" or "pipe stream" diarrhea, hypoproteinemia and intermandibular edema (bottle jaw) characterize the advanced stage of the disease (Tiwari et al. 2006).
Morphological changes in JD include chronic inflammation involving all layers of the intestinal wall (transmural involvement), thickening of involved segments, with narrowing of the lumen, linear ulceration of the mucosa, and a submucosal edema with elevation of the surviving mucosa, producing a characteristic cobblestone appearance.

According to Clarke (Clarke 1997), the histopathology of JD ranges from the more common pluribacillary or lepromatous form to the less common paucibacillary or

paucimicrobial tuberculoid form comparable with leprosy in humans. Due to the histopathological features of CD which closely resemble those found in animals with the paucibacillary form of JD, it has been suggested that the two diseases may share the same etiology (Grant 2003, Collins et al. 2000; Greenstein 2003; Moss et al. 1992).

43.5 Diagnosis of Bovine Paratuberculosis

For microbiological diagnosis of MAP direct and indirect methods are distinguished. While the first detect the agent or parts of the agent itself, the latter are based on specific immune responses that occur after contact with the pathogen (exposure, infection, immunization).

43.5.1 Direct Detection of MAP in Feces or Organs

The gold standard is the cultural detection of the pathogen. It is considered as 100 % specific, but its sensitivity is not higher than 35 %. For improving sensitivity the culture can be combined with PCR. The main disadvantage of MAP culture is the slow growth of the pathogen with an incubation period of up to 18 weeks in primary culture (Whan et al. 2005).

However, a positive result is proof for the presence of living reproductive MAP in the animal.

The fecal culture is able to detect most animals in advanced stages of the disease, but identifies only a few animals in early stages of infection. It will detect infected animals 6 months or more before they develop clinical signs, and during the clinical stage its sensitivity approaches 100 %.

Furthermore, the intermittent shedding of bacteria contributes also to the relative low sensitivity cited above.

In case of massive shedding MAP can be detected directly in the feces by light microscopy after Ziehl-Neelsen staining. PCR assays using IS*900*, *f57* or other target sequences are used for the direct detection of MAP. Since 2012 a commercial real-time PCR assay is authorized in Germany for the detection of MAP in feces (Life Technologies S.A.S.; Carlsbad, CA, USA).

43.5.2 Indirect Detection of MAP in Serum or Milk

The serological tests commonly used for detection of a humoral response to paratuberculosis in cattle are complement fixation (CF), enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion (AGID).

At present the ELISA is the most sensitive and specific test for serum antibodies to *M. avium* ssp. *paratuberculosis* in cattle. According to the OIE manual (http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/) the ELISA detects in total about 30–40 % cattle identified as infected by fecal culture.

In Germany several different commercial ELISA assays are licensed for the detection of paratuberculosis specific antibodies (http://www.fli.bund.de/en/startseite/institutes/institute-of-molecular-pathogenesis/reference-laboratories/nrl-for-paratuberculosis.html). The sensitivity of these methods, however, is also very limited. As cited above: for an early diagnosis of paratuberculosis in young animals all detection methods are not effective enough. Because of the limited sensitivity antibody tests are useful to determine the health state in a herd, but are not an adequate tool for determining the disease in individual animals.

There are two tests for detection of a cell-mediated immunity: the gamma interferon release assay for blood samples and the skin test for delayed-type hypersensitivity. According to the OIE manual both have limited value in the field at present and further research is needed with respect to the interpretation criteria.

In summary, at present there are various diagnostic methods suitable for the detection of animals with JD in a progressive stage. But in spite of intense research they still have considerable sensitivity problems. A reliable diagnosis of the early stages of the disease is still missing. The fact that MAP is shed intermittently in feces and milk lead to post mortem screening attempts like testing of lymph nodes in the slaughterhouse (Munster et al. 2011), as mesenteric lymph nodes are generally accepted to be the main locus of MAP colonization.

Nevertheless, according to the guidelines for handling of paratuberculosis in ruminant populations (Federal Gazette No. 28 from 10.02.2005, p. 2165) there is currently no approval for *intra vitam* tests that are appropriate for a comprehensive over-all monitoring in Germany.

After all, the time consuming culture is still presumed to be the most significant tool to identify MAP.

43.5.3 Therapy and Vaccination

It is not possible to cure MAP infected, clinically ill animals. Antibiotics, although showing growth inhibition *in vitro* do not lead to permanent treatment success in animals. A short-term alleviation of clinical symptoms by use of various antibiotics or antihistamines has been described in the literature. The MAP excretion, however, could not be prevented.

In Germany no vaccine is currently approved. However, in principle vaccinations are possible. The first vaccine was developed in 1926. Many authors describe the use of vaccines in terms of advantages and disadvantages.

The excretion of the pathogen and thus the spread of infection can be reduced by vaccination. Available vaccines, based on whole killed or live-attenuated bacteria

will not protect against infection and disease and the use of serological methods for animal disease control in vaccinated herds is no longer suitable. Furthermore interference with the tuberculin skin test used for the control of bovine tuberculosis is described (Bastida and Juste 2011; Juste 2012; Patton 2011; Rosseels and Huygen 2008).

43.6 Animal Disease Control Measures

43.6.1 Economic Importance of the Disease

The economic impacts on dairy and beef industry as a result of decreased milk production, increased susceptibility to other disease, progressive weight loss and veterinary costs are considerable.

Calculations from The Netherlands came to the conclusion that the damage for a farm with 100 dairy cows over a period of 20 years may cost 6800 € per year. In farms with clinically diseased animals the damage is estimated to be about 900 € per animal showing clinical symptoms (von Schloss 2000).

43.6.2 Control Measures in Other Countries

Since 1970 control measures are implemented in The Netherlands, but according to the OIE (http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasetimelines), The Netherlands are not yet free of paratuberculosis infection.

Only Sweden is free of paratuberculosis, because the most restrictive measures were applied. Stocks with animals tested positive in cultural and/or serological tests were completely culled.

Rehabilitation programs were performed amongst others in the USA, the Czech Republic, Italy and some regions of Germany (voluntary programs). This demonstrates that the fight against paratuberculosis is a very complex and a long lasting approach (Köhler et al. 2003). Over 10 years may be necessary to achieve a JD-free status in a herd.

In Germany different sanitary programs are published for example the "Ratgeber Paratuberkulose" of the Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health) (http://www.bmelv.de/SharedDocs/Downloads/Landwirtschaft/Tier/Tiergesundheit/Tierseuchen/Paratuberkulose_Ratgeber.html) or hygiene measures of the German Farmers' Federation (DBV) (http://media.repro-mayr.de/61/101361.pdf).

In essence, they are all based on a strict hygiene management emphasizing on a paratuberculosis free young stock and the elimination of MAP positive animals.

43.7 MAP in Milk and Cheese

43.7.1 MAP in Milk

In principle there are two possibilities for MAP to get into raw milk: directly by the mammary gland (only in case of clinically infected animals leading to a suspected bacterial load of about two to eight bacteria per 50 ml milk), and indirectly through fecal contamination. Compared to the secretory route the fecal contamination is much more important, because significant amounts of MAP can enter into the milk from infected animals including also the subclinical infected population.

In the dairy industry different heating methods are used. The most important is the high-temperature, short-time (HTST) pasteurized milk process (72–75 °C, 15–30 s), with a market share of approximately 40 % (pasteurization) and the ultra-high temperature (UHT) process (≥ 135°C, ≥ 1 s), with a market share of about 60 % (UHT milk) (Protocol of the German Federal Institute for Risk Assessment, 2001: http://www.bfr.bund.de/cm/343/protokollmcpara.pdf). Dairy products are mainly produced on the bases of HTST milk.

To investigate the thermal resistance of MAP in milk, numerous experimental investigations were performed. After the UHT process, no surviving MAP were found in milk.

In HTST treated milk, experimentally infected with 10^2–10^3 colony forming units (CFU)/ml of milk, viable MAP could be detected. However, there was a reduction of microbial count to five orders of magnitude. This corresponds to a reduction of 99.999 %. After experimental contamination of milk with lower bacterial counts (10 CFU/ml) no viable pathogen could be detected (Publication of the Bavarian Health and Food Safety Agency, 2005: http://www.lgl.bayern.de/tiergesundheit/tierkrankheiten/bakterielle_pilzinfektionen/paratuberkulose/doc/paratuberkulose_ag.pdf).

For interpretation of these data it is important to consider which methodology of thermal inactivation was applied. In terms: was the experiment conducted under commercial-type pasteurizers with continuous turbulent flow for heat distribution comparable to the ones used in the dairy industry? This has been neglected in many studies and could be the cause of conflicting results (Chiodini and Hermon-Taylor 1993; Grant et al. 1996; Meylan et al. 1996; Robertson et al. 2012).

However, experiments performed in the Max Rubner Institute (Federal Research Institute of Nutrition and Food) in Germany between 2006 and 2010 demonstrated that the common pasteurization process leads to a profound reduction of MAP in milk. Due to the fact that MAP can only replicate in the host and that the dilution process starts immediately after its entry into the food chain, experts state that if at all only minimal amounts of viable MAP may reach the consumer by milk.

Furthermore there is no scientific evidence that CD is driven by the uptake of MAP by food (Study of Germany's national Public Health Institute (RKI), 2003: http://www.bfr.bund.de/cm/343/morbus_crohn_und_mycobacterium_avium_ssp_paratuberculosis_literaturstudie.pdf; Research report of the Federal Ministry of Food,

Agriculture and Consumer Protection: (https://openagrar.bmelv-forschung.de/servlets/MCRFileNodeServlet/Document_derivate_00000093/FoRep_2_2000_14-17_hammer.pdf).

43.7.2 MAP in Cheese

Only limited data are available on the occurrence of MAP in raw milk cheeses: experimental production of cheese from artificially contaminated raw milk showed that MAP are also greatly reduced during the ripening process (Donaghy et al. 2004; Spahr and Schafroth 2001; Sung and Collins 2000).

43.7.3 MAP in Retail Milk

To address the occurrence of MAP in HTST-treated milk from the market field, studies in the US (Ellingson et al. 2005), Great Britain (GB) (Food Standards Agency UK: http://www.foodstandards.gov.uk/multimedia/pdfs/milksurvey.pdf;) (Grant 2003) and Ireland (O'Reilly et al. 2004) were conducted. MAP in low numbers and frequency has been demonstrated in the US and UK: In UK 1.8 % of 567 tested packages contained MAP, in the US 2.8 % of 702 packs. In an Irish study, which was published in September 2004, no viable MAP was detected in any of the 357 examined milk cartons. In the Irish study 56 % of the investigated manufacturing firms treated the milk at least at 75 °C for 25 s. Poor stable hygiene is discussed as main reason for positive milk, which may explain regional differences in the studies.

43.8 Crohn's Disease

CD is a chronic relapsing inflammatory bowel disease (IBD) affecting the human gastrointestinal tract with preference for the terminal ileum and colon, but possible involvement of all its other parts (Baumgart and Sandborn 2012). All age groups and both genders can be affected, with the main peak for disease onset between the ages of 17–40 years (Thia et al. 2010). Patients are often febrile and suffer from painful abdominal cramps and chronic diarrhea; their stool is bloody or mucous. The Montreal classification was established to categorize the different phenotypic behavior of CD, with the majority of patients being affected by the non-stricturing non-penetrating phenotype, the remainder by the more aggressive stricturing or penetrating phenotypes which are characterized by gut stenoses or fistulas, respectively (Satsangi et al. 2006). The etiology of the disease is unknown, but genome wide association studies have identified an enormous amount of susceptibility loci (Franke et al. 2010; Jostins et al. 2012). Besides a genetic susceptibility to the disease, environmental factors also play a very important role in its development. A

whole lot of such lifestyle factors have been found to be associated with CD, like e.g., a reduction in women breastfeeding, air pollution, tobacco use, increased hygiene conditions, or the consumption of Western diet. Interestingly, CD is frequently triggered or exacerbated after an infectious gastroenteritis (Garcia Rodriguez et al. 2006). In an animal model, a virus infection was able to induce a CD-like phenotype in genetically susceptible individuals (Cadwell et al. 2010). It has been tempting to speculate from the first discovery of CD up to now, that a pathogen might be the etiological agent for development of CD.

43.8.1 Theories and Attempted Methods to Elucidate the Role of MAP in CD

As early as 1913 Thomas Kennedy Dalziel suggested that the histological characters of CD and JD are so similar as to justify the proposition that they might be the same, even though, as he also stated, the absence of the acid-fast bacillus would suggest a clear distinction (Dalziel 1989). Since then, the hypothesis of MAP as etiological agent for CD has been tried to be verified by multiple approaches, like immunohistochemistry, attempts to cultivate the bacterium, experiments to transmit CD to animals, by serological tests, molecular methods, and treatment programs of CD with antimycobacterial antibiotics.

43.8.2 Microbiological Approach

It is amazing that up to now no final conclusion about the role of MAP in CD could be drawn. The reason for this most probably lies in the elusive behavior of MAP in the human body. Histological immunostaining of resected granuloma tissue of CD patients against MAP antigen could up to now only confirm the primary statement of Dalziel that the acid-fast bacillus is absent, even though it has been performed repeatedly by several work groups (Van Kruiningen 2011). Cultural growth of MAP requires extremely long culture times, special selective cultural media and experienced lab personnel (Turenne et al. 2007). Additionally, because of the difficult handling procedures laboratory cross-contamination in mycobacterial laboratories is not rare, which should always be regarded when interpreting mycobacterial culture results (Van Kruiningen 2011). This might in some cases explain the discrepancies between different studies, where in a few smaller studies MAP could be detected in very small patient collectives, while it was undetectable in major study groups. MAP was detected in breast milk samples of two patients with CD, but not in five controls (Naser et al. 2000a), in another study it could be cultivated from four out of ten biopsies of children with early onset CD, but not in two ulcerative colitis or four non-IBD patients (Kirkwood et al. 2009). The group of Naser also reported the detection of MAP from blood samples of CD patients; however, there was also growth of MAP in samples from ulcerative colitis and healthy patients (Naser et al.

2004). Conversely, in a major culturing attempt on IBD samples from 191 patients, including 79 CD patients, from US and Denmark, not one of 3985 cultures had been positive (Collins et al. 2000).

43.8.3 Epidemiological Approach

If MAP infection would really be an etiological agent for CD, one would imagine that cattle farmers who are exposed to animals with JD are more often afflicted by CD than farmers with healthy animals. However, studies from the US and UK could not show a higher prevalence of CD in farmers handling JD animals (Jones et al. 2006; Qual et al. 2010).

43.8.4 Infection Experiments

Also all inoculation experiments of animals with triturated intestinal material from CD patients were up to now unsuccessful in induction of a JD-like infection, even though also susceptible animals, like goats or rabbits, were infected (Van Kruiningen 2011).

Because of these above-mentioned problems in detecting MAP in the tissue, but the striking similarity of the pathology of CD with JD, other hypotheses concerning the role of MAP in CD have arisen. A plausible explanation for the limited detectability of MAP could be that the pathogen is ingested at a young age and stimulates the immune system to create a chronic intestinal inflammatory disease but cannot consistently be found in the mucosa years after its ingestion (Bernstein et al. 2004). According to this hypothesis, the pathogen itself might only be present in very small numbers in the human body or might already have completely disappeared, but its former or residual presence would still be detectable by MAP-specific antibody measurements or nucleic acid tests (NAT).

43.8.5 Serological Approach

Therefore many studies compared antibody titers against MAP in serum of CD patients and controls. In this context different capture antigens in the assays with expectably different specificity for MAP antibodies were used (Van Kruiningen 2011). The results gained by these antibody measurements were often inconclusive; in some cases, there were significantly higher antibody responses for CD than for control patients (Collins et al. 2000; Naser et al. 2000b), in other studies no significant differences could be found (Bernstein et al. 2004; Cho et al. 1986; Kobayashi et al. 1988). This even sometimes occurred when using the identical test in different countries (Collins et al. 2000). As atypical mycobacteria comprise a huge group of

different species, many of them existing ubiquitously in the environment, e.g. in tap water or in the soil, and with probably also a considerable amount of still undiscovered species, it is tempting to speculate that cross-reactivity of MAP-"specific" antibodies towards antigens of different atypical mycobacteria is highly probable (Osterstock et al. 2007). Therefore, it is very difficult to interpret the meaning of positive serological test results regarding their specificity for previous or present MAP colonization, infection or immunity.

43.8.6 Molecular Biological Approach

Comparably, an enormous amount of studies has been published with focus on MAP-DNA detection in intestinal tissue, granulomas, or peripheral blood mononuclear cells (PBMC). In most studies, the main target gene was the IS element IS*900*, which has been postulated to be specific for MAP. Most of these studies were based on classical PCR or nested PCR on biopsies, buffy coat of blood or PBMC (Kirkwood et al. 2009; Naser et al. 2004; Autschbach et al. 2005; Bernstein et al. 2003; Bull et al. 2003; Juste et al. 2009; Suenaga et al. 1995), one study used laser-microdissected tissue (Ryan et al. 2002), another performed in-situ labeling on paraffin-embedded tissues (Hulten et al. 2001). Also—similar to the serological analyses- there were many conflicting results between these studies, with some studies showing a significantly higher presence of IS*900* DNA in CD samples and others showing no difference to controls. However, a meta-analysis of NAT-based techniques detected an association between MAP and CD (Abubakar et al. 2008). Given that the IS*900* element would be exclusively present in MAP and therefore indeed highly specific, then one possible explanation might be a variable sensitivity of the applied NAT in the study-specific analyzed tissues. Alternatively, as NAT are extremely sensitive methods, sample contamination could be a major issue in some of these studies. Even the water used during endoscopy for taking the biopsies could be contaminated with mycobacteria (Van Kruiningen 2011). Moreover, it becomes increasingly clear that IS*900* is indeed also present in other mycobacteria species; it could be detected by NAT in mycobacterial isolates related to *M. cookii*, *M. scrofulaceum*, and the *M. avium-intracellulare* complex (Van Kruiningen 2011; Cousins et al. 1999; Englund et al. 2002; Motiwala et al. 2004), which questions the specificity of the IS*900*-PCR method and heightens the contamination risk.

43.8.7 Therapeutical Approach

As MAP obviously is an easily detectable pathogen in cattle with JD, but very elusive and highly debated in humans, other researchers tried to demonstrate the implication of MAP or mycobacteria in CD indirectly by studying the effect of antimycobacterial antibiotic therapy on CD patients. By this approach the ultimate proof for the role of MAP in CD can never be achieved, as the application of antibiotics can

influence many factors other than MAP, e.g., by their antiphlogistic effects on the immune system or maybe by elimination of other unknown causative bacteria, or even sometimes by their curative effect on an existing but unrecognized gut tuberculosis. Even more problematic for this approach is the actual possibility that MAP disease might not even be curable by antibiotics, as it is the case with its hypothetical animal counterpart JD. Besides different uncontrolled clinical studies, a five-year follow-up study and a randomized prospective, parallel, placebo-controlled, double-blind study have tried to find a benefit of antimycobacterial therapy for CD patients (Borody et al. 2002; Selby et al. 2007; Thomas et al. 1998) with variable success. Selby's study had the statistically best set-up and found no evidence for a beneficial effect over a time period of 2 years; however, the study was criticized for low dosage and suboptimal application of the antibiotics.

43.9 Summary

In conclusion, the scientific community is still divided into supporters and critics for a possible role of MAP in CD. While the supporters bring forward the detection of MAP in blood, intestine and even milk samples of human CD patients, the opponents still miss the final convincing evidence that the presence of MAP in the human body can really initiate CD. One of the main arguments of critics still stands fast, namely that in all of recorded medical and veterinary medical history there are no published accounts of the transmission of JD to humans (Van Kruiningen et al. 2011). Novel data from a meta-analysis of CD genome-wide association scans detected considerable overlap between susceptibility loci for IBD and mycobacterial infection (Jostins et al. 2012), which could indeed mean that mycobacteria are involved in the development of IBD or that the associated changes in these susceptibility loci necessary for mycobacterial control somehow auto-induce autoimmune processes with subsequent pathogen-free granuloma formation. Therefore, the theory still remains open for controversial discussion and future studies will hopefully lead to a final conclusion. At the moment, however, there is not enough evidence to convincingly demonstrate that MAP is a possible etiological agent for CD.

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