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***Mycobacterium avium* subspecies *paratuberculosis*: A possible causative agent in human morbidity and risk to public health safety**

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Abstract

Mycobacterium avium subspecies *paratuberculosis* is a bacterial parasite and the causative agent of paratuberculosis, a disease predominately found in cattle and sheep. Infection with this microorganism results in substantial farming economic losses and animal morbidity. The link between infection with this pathogen and human disease has been theorised for many years with Crohn's disease being one of many suspected resultant conditions. *Mycobacterium avium* may be spread from animal to human hosts by water and foodborne transmission routes, where the foodborne route of exposure represents a significant risk for susceptible populations, namely children and the immune-compromised. Following colonisation of the host, the parasitic organism evades the host immune system by use of molecular mimicry, displaying peptide sequences similar to that of the host cells causing a disruption of self-verses non self-recognition. Theoretically, this failure to recognise the invading organism as distinct from host cells may result in numerous autoimmune conditions. Here, the author presents current information assessing the link between numerous diseases states in humans such inflammatory bowel disease, Type 1 diabetes, rheumatoid arthritis, Hashimoto's thyroiditis, multiple sclerosis and autism following infection with *Mycobacterium avium paratuberculosis*. The possibility of zoonotic transmission of the organism and its significant risk to public health safety as a consequence is also discussed.

Keywords: Auto-immune, Human morbidity, *Mycobacterium paratuberculosis*, Public health, Zoonotic.

Introduction

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of paratuberculosis (paraTB) (Malvisi *et al.*, 2016) or Johne's disease (JD) in cattle, a chronic granulomatous gastroenteritis. MAP is an obligate intracellular parasite of animals (Grant, 2005); where it can only reproduce within a host cell (in this case a macrophage) of a susceptible species and is unable to reproduce outside of this cellular environment.

Johne and Frothingham first identified MAP as the bacterial pathogen responsible for chronic gastroenteritis of bovine species in 1895, since then its significance as a potential zoonotic pathogen has become increasingly evident. MAP can frequently be found in dairy cattle where it results in significant economic losses due to persistent contagious infection and animal morbidity (Khol *et al.*, 2017).

ParaTB is endemic worldwide occurring frequently in ruminants such as cattle, sheep, goats, and farmed deer. The disease has also been reported in non-ruminants such as wild rabbits, foxes and stoats (Whittington and Sergeant, 2001). Due to the disseminated nature of the pathogen, infected livestock regularly shed the organism in their faeces, milk, and colostrum, resulting in faecal-oral transmission which is considered the primary route of infection (Khol *et al.*, 2017).

At present there is no effective cure for JD and efforts to develop an effective vaccine for MAP have resulted in the commercialisation of Mycopar® which contains inactivated MAP and an adjuvant (Ganusov *et al.*, 2015). The vaccine however, has some side effects and its effectiveness remains unknown, with some studies reporting limited success (Harris and Barletta, 2001) while others report some protection against JD (Knust *et al.*, 2013). The use of vaccines has been a hugely effective tool for controlling the rate of other infectious diseases in humans and animals. An effective MAP vaccine would reduce clinical disease prevalence, improve animal welfare and reduce financial losses to the agricultural industry due to animals culled and poor weight gains.

The aim of this review is to linking the role of this bacterial species in human morbidity. Understanding the health implications of human infection with MAP is of paramount importance, as it is essential to determine its link to chronic debilitating conditions, namely autoimmune diseases such as inflammatory bowel disease (IBD) as either causation or merely correlation.

Routes of transmission of MAP

Typically the transmission of any pathogenic species to humans may be from one of three general routes, depending on the life cycle and environmental

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survivability of the organisms. MAP is predominately an intestinal parasite of numerous species which is capable of surviving for long periods of time in harsh conditions outside of its host environment. To date, published literature has focused on routes of transmission relating to waterborne (Whiley *et al.*, 2012), food (Roberston *et al.*, 2017) and zoonotic exposure (Wynne *et al.*, 2011).

Waterborne transmission

The extensive shedding of this microbial species by numerous and various animal hosts suggests that large quantities of viable and potentially infectious bacteria are present in the environment at any given time. Studies have shown that clinically and sub-clinically infected animals can shed MAP in variable numbers into the environment in their faeces, depending on the infected host, the pathogen strain and the manifestations of the disease (Rhodes *et al.*, 2014). Furthermore, the organism can survive for extended periods of time in agricultural slurry, run off and consequently in the wider environment where it exists as either a vegetative state or spheroplast (McNees *et al.*, 2015).

Pickup *et al.* (2005) reported a 32.3% detection rate of MAP in freshwaters receiving runoff from farming and domestic locations in South Wales. Waterborne transmission is therefore an inevitability following contamination of surface waters including lakes and rivers (Waddell *et al.*, 2016) with a potential for drinking water contamination as a result. MAP DNA was detected in over 80% of domestic water samples in Ohio USA (Sechi and Dow, 2015). Studies have shown that MAP is highly resistant to standard water disinfection methods (Naser *et al.*, 2014). Norton *et al.* (2004) reported up to 103 colony forming units (CFU) of planktonic MAC per 100 ml of potable water, with biofilm samples taken straight from water distribution pipes containing MAC counts up to 106 CFU/100 ml (Schulze-Robbecke *et al.*, 1992). Biofilm or sessile communities are notoriously difficult to eradicate and are extremely resistant to chemical disinfection techniques (Garvey *et al.*, 2015). Biofilm organisms in water piping networks can pass in to domestic water and be aerosolised by the tap outlets (Whiley *et al.*, 2012) where there is a potential for human exposure as reported by Zhou (2007). MAP was detected by qPCR (by amplifying the *IS900* gene) in approximately 90% of both water and biofilm samples from 31 cold water faucets in southwestern Ohio (Gill *et al.*, 2011). Additionally, studies by George *et al.* (1980) have demonstrated that MAC can survive and proliferate in temperatures ranging from 15 to 45°C and salinities from 0 to 2% sodium chloride (NaCl). Nonetheless, the link between water contamination, survival of the organism in treated potable water supplies and human infection has not been extensively investigated.

Foodborne transmission

The excretion of MAP in both milk and faeces by dairy cows with clinical and subclinical JD (Grant *et al.*, 2015) results in foodborne transmission. It has also been suggested that meat from old dairy cows used for human consumption may be an additional source of infection as it can become concentrated in tissues remote from the intestinal tract and lymph nodes (Gill *et al.*, 2011).

A review by Waddell *et al.* (2016) summarises several studies which demonstrate MAP detection on meat from animals showing clinical JD and/or positive for *M. paratuberculosis* by ELISA, PCR or culture (Waddell *et al.*, 2016). A study by Mutharia *et al.* (2010) reports a 1000 cfu/g recovery of MAP from 7 of 15 liver, mesenteric and ileocaecal lymph node samples, with smaller numbers isolated from 5 of 15 kidney, superficial inguinal and prescapular lymph node samples (Mutharia *et al.*, 2010). Research has shown that pasteurisation, heating milk to 72°C for 15 seconds, is not effective for inactivating MAP (Grant *et al.*, 2015).

Wynne *et al.*, 2011 reports on a study of 567 pasteurised milk samples from the UK where 11.8% were MAP positive by PCR analysis and that MAP could be cultured from 1.8% of samples (Wynne *et al.*, 2011). Additionally, Botsaris *et al.* (2016) reports the presence of MAP in powdered infant formula using phage amplification coupled with a polymerase chain reaction (PCR) assay, which suggests that freeze drying may be insufficient to remove the pathogen especially in situations of bacterial clumping. A recent study reported testing infant formula for MAP in 65 samples from 18 countries, with greater than 40% testing positive for viable MAP (Grant *et al.*, 2015). The presence of MAP in a dairy herd is controlled by culling of the herd with subclinical infection and improving calf management (Dore *et al.*, 2012) as transmission via the colostrum is likely. However, the culture of MAP from the milk sourced from asymptomatic cows (Naser *et al.*, 2014) indicates that the spread of MAP is not effectively controlled by these methods.

Additionally, the isolation of MAP from samples containing a mixed variety of microbial species which may outgrow or inhibit it (Roberston *et al.*, 2017) leads to issues when attempting to culture viable numbers. In order to efficiently control the presence of the pathogen in food supplies, better detection methods must be established.

Recent studies have focused on the use of PCR methods with varied success rates due to the presence on PCR inhibitory substances in the food itself (Acharya *et al.*, 2017). Additionally, PCR methods detect DNA of target species and are not a direct indication of viability, in order to determine viable numbers present culture methods must be employed.

Zoonotic transmission

At present the MAP species is not recognised as a zoonotic pathogen (Whiley *et al.*, 2012), however the economic losses and animal health issues which occur due to JD coupled with the familiarity of the disease to IBD means that the possibility of zoonotic transmission must not be overlooked. Waddell *et al.* (2008) concluded that although the evidence supporting the zoonotic transmission of MAP is not strong it cannot be ignored. More recently the publication of Dore *et al.* (2012) concluded that direct contact with animal faeces is the most likely route of zoonotic transmission of MAP with calves faeces, the housing environment, colostrum, milk (Dore *et al.*, 2012) and grazing (McNees *et al.*, 2015) all listed as potential risk factors. A major concern for the zoonotic transmission of MAP is that MAP-infected animals, such as cattle, remain asymptomatic in a lengthy subclinical phase (Naser *et al.*, 2014) where they may potentially transmit the parasite to humans via contact with excreted faeces and/or milk (Sweeney, 1996).

MAP and human morbidity

The relationship between human morbidity and infection with *Mycobacterium avium paratuberculosis* is one of significant importance as more and more researchers try to understand and establish a causative agent behind what are currently classified as autoimmune diseases in humans. Epitope mimicry is the term used to describe the mechanism by which an infectious agent has epitopes which are so similar to the self-antigenic determinants of the host (Bitti *et al.*, 2012), that they result in cross reactivity of the immune system, host cells and the infective agent. This cross reactivity may then result in disease states which are classified as autoimmune conditions. Furthermore, it is generally accepted that autoimmune disorders are a result of environmental triggers in genetically susceptible individuals (Miller, 2011). MAP is one such organism having developed similar epitopes to human host cells as a means of evading the innate immune response (Dow, 2012) and may be an environmental trigger associated with the following conditions.

Johne's Disease and IBD

As previously stated JD in a chronic, contagious, granulomatous, inflammatory disease (Souriau *et al.*, 2017) affecting numerous animal species resulting from an infection of the gastrointestinal tract (GIT) with *Mycobacterium paratuberculosis*. Colonisation of the GIT with MAP leads to manifestations of the disease affecting the integrity of the intestinal wall causing oedematous (Tiwari *et al.*, 2006) and weight loss which can ultimately lead to death of the animal. Histologically, infection is evident as the extensive formation of submucosal granulomas and a thickened intestinal wall (Arsenault *et al.*, 2014) resulting in cachexia due to an inability to absorb nutrients.

Infection with MAP has an incubation period which can last from 2 to 6 years (Malvisi *et al.*, 2016) where no signs of infection are seen, progression from this subclinical form to the symptomatic clinical form results in the disease state JD. After infection has occurred, mycobacteria remain hidden in the intestine or associated lymph nodes, predominately in macrophages and Peyer's patches (Tiwari *et al.*, 2006). Once infected intestinal macrophages have lysed, mycobacteria distribute to different sites such as the uterus, the mammary gland or the immune foetus (Mercier *et al.*, 2016).

Post MAP subclinical infection, a cellular immune response is activated causing an influx of mononuclear inflammatory cells. With progression to the clinical stage the humoral immune response is initiated leading a production of MAP-specific antibodies (Ganusov *et al.*, 2015) which can be detected via ELISA specific antibody testing. In human patients with IBD such as ulcerative colitis (UC) and Crohn's disease (CD), the appearance of the gut wall is similar to that seen in animals with JD (Gill *et al.*, 2011).

Furthermore, Crohn's disease is also a chronic, granulomatous inflammatory disease which primarily affects the terminal ileum and colon (Thia *et al.*, 2010) resulting in intestinal lesions, diarrhoea, chronic pain and malnutrition. As with bovine species, the intestinal lesions manifest from continual stimulation of the mucosal and systemic immune systems that prolong the inflammatory cascade (Rubery, 2002).

The cellular response may increase the permeability of the intestinal wall and the entry of MAP into the systemic circulatory systems of the host; studies have described culturing MAP from 50% of patients with CD (Naser *et al.*, 2004). Furthermore, reports have shown the presence of MAP in breast milk samples from Crohn's patients (Grant *et al.*, 2015) suggesting a possible route of transmission in familial cases of IBD. Genetic similarities between patients with IBD and cattle with JD have been described, for example the NOD2 mutation was found to be more frequent in both ruminant and human cases of infection (Kuenstner *et al.*, 2015).

Studies report that this gene is directly involved with recognition of bacterial cell wall peptidoglycans, activating the nuclear factor immune pathway (Girardin *et al.*, 2003) and in the direct killing of intracellular organisms (Grant, 2005). The identification of this genetic link in both diseases strongly suggests a common causative agent, however one cannot ignore the possibility that the presence of this mutation in Crohn's patients merely leads to an increased risk of MAP infection.

Zamani *et al.* (2017) concluded that there was a lack of detectable MAP by both ELISA and PCR in non-IBD control individuals (Zamani *et al.*, 2017) compared to

IBD patients. Additionally, Jeyanathan *et al.* (2007) reports the visualisation of MAP in 60% of CD and 40 % of UC patient tissue samples using oil immersion microscopy. A study examining ileocolonic mucosal biopsies from CD patients for MAP DNA using PCR specific primers showed that 92% of samples were positive for the presence of MAP compared to the control which had 26% (Bull *et al.*, 2003).

In Iceland, JD incidence increased from 0% to 30% over an 18-year period after introduction of MAP-infected sheep into the local sheep population in 1938 (Fridriksdottir *et al.*, 2000). Research has correlated this with a study reporting increased IBD incidence in Iceland occurring over a combined period of 40 years from 1950 (Gitlin *et al.*, 2012).

Arguments against MAP as a causative agent of IBD relate to the lack of culturable isolates from IBD patients, matching the requirements of Koch's postulates. Studies have shown however, that it is extremely difficult to grow MAP from human samples where MAP exists with a modified cell wall making it resistant to standard identity staining techniques (Sechi *et al.*, 2005) couple with specific growth requirements such as certain levels of iron which it sources from its hosts (Naser *et al.*, 2014). Additionally, JD does not display some of the manifestations of CD such as the segmental localization and features such as fistula, fissures, bleeding, stenosis, adhesions, perforation and the formation of abscesses (Liverani *et al.*, 2014).

In order to prove without a doubt that MAP causes IBD, live organisms must be isolated and cultured from a patient and shown to be able to infect and cause morbidity in a second host. The difficulty relating to this stems from culture techniques and the ethical implications of host infection. However, research by Van Kruiningen *et al.* (1986) achieved this when they isolated and cultured MAP from a CD patient which subsequently then caused terminal ileitis in goats 5 months post infection.

Resistance to treatment

As a member of the *M. avium* complex MAP is typically hard to treat with standard antibiotics (McNees *et al.*, 2015). Additionally, when infecting humans MAP is present without a cell wall, meaning that treatment with antibiotic drugs targeting the bacterial cell wall is unlikely to be effective for this intracellular parasite (Alcedo *et al.*, 2016). Therefore, therapeutics which target peptidoglycan biosynthesis (e.g. penicillin's, cephalosporin's and vancomycin), which is unique to bacteria, are ineffective against *Mycobacterium* (Dubuisson *et al.*, 2010). Furthermore, the use of such antibiotics may also lead to complications by inhibiting the gut micro-flora and contributing to multidrug resistant bacteria (Naser *et al.*, 2004). Other factors such as an extremely slow growth rate and an inactive dormant stage outside of the

host also make MAP difficult to treat with antimicrobials. For the treatment of mycobacterial infections a combination of antimicrobial therapeutics are used to improve efficacy and to limit the development of drug resistance. Typically, the antimicrobial combinations used are macrolide protein synthesis inhibitors such as clarithromycin and azithromycin combined with ethambutol affecting cell metabolism and a rifamycin which inhibits RNA synthesis. At present, there is no long term *in vitro* infection model for testing the efficacy of antimicrobials against MAP infections.

Antibiotic treatment of IBD

The successful treatment of CD patients with antibiotic therapy and subsequent remission of the disease in these patients further supports the role of MAP in IBD. Table (1) summarises the drug therapy used, studied and implemented in the treatment of IBD.

Type 1 diabetes

Type 1 diabetes (T1D) is an autoimmune disease characterised by destruction of the insulin producing cells of the islets of Langerhans by misguided T cells, specifically autoimmunity towards heat shock protein (HSP) 60, insulinoma - associated protein-2 (IA-2) and glutamic acid decarboxylase (GAD) present on cells within the pancreas (Rani *et al.*, 2010). Although genetic factors are known to be involved in the pathogenesis of T1D there is also evidence suggesting a role of environmental factors such as viruses in the incidence of disease (Naser *et al.*, 2014). Additionally, studies are looking at the role of MAP in cases of T1D, where there is increasing evidence of shared genetic susceptibility between T1D and MAP infections (Rani *et al.*, 2010).

Genetic similarities (Table 2) which have been found relate to the SLC11A1 gene (formerly the NRAMP1 gene) which codes for a membrane protein of monocytes and macrophages (Paccagnini *et al.*, 2009). Mutations in this gene lead to malfunction of the protein and a lack of phagocytosis of the pathogen. Consequently infectious agents can avoid the host immune response and proliferative *in vivo*. Research studies have isolated MAP DNA from the blood of 63% of Sardinian T1D patients, but only 16% of healthy controls (Bitti *et al.*, 2012). Furthermore, the MAP envelope protein was detected in the blood of 47% Sardinian T1D patients, but in only 8% of type 2 diabetes (T2D) patients and 13% of non-diabetic controls (Rosu *et al.*, 2009). GAD65 is expressed in human pancreatic islets cells and has similar amino acid sequences to mycobacterial HSP 65, cross reactivity between both molecules occurs causing destruction of the islet cells. Additionally, HSPs are produced by both human and bacteria cells in response to environmental stresses, where they provide a protective function aiding in cell survival (Dow and Sechi, 2011). These

HSPs share sequence similarities and therefore can result in a cross reactivity between the human and microbial cells where the immune system fails to differentiate between the two. This cross reactivity and epitope homology between bacterial and human HSPs and GAD65 and bacterial HSP 65 can ultimately lead to destruction of the human cells (Dow and Sechi, 2011) causing issues with insulin production and finally, diabetes in the host. Furthermore, studies have reported that up to 70 percent of T1D patients have antibodies to GAD 65, compared to 4 percent of healthy individuals (Rani *et al.*, 2010). More recently autoantibodies against the islets have been supported by detection of antibodies against the β -cell antigen zinc transporter 8 (ZnT8) on cell membranes which displays similarities to a MAP protein (MAP3865c) (Masala *et al.*, 2015).

Rheumatoid arthritis and Hashimoto's thyroiditis

Rheumatoid arthritis (RA) is an autoimmune condition affecting the peripheral joints in a symmetrical fashion, resulting in chronic inflammation, joint erosion and pain (Peters *et al.*, 2007). Recent studies have identified a role of the SLC11A1 gene in different autoimmune diseases such as RA, juvenile rheumatoid arthritis and multiple sclerosis (MS) (Paccagnini *et al.*, 2009). Mycobacterial HSP65 has also been implicated in human mycobacterial infection, where it is estimated that up to 40% of the T-cell response is directed against this single protein. Immune responses to mycobacterial HSP65 have been identified in autoimmune disease such as RA and Hashimoto's thyroiditis (Dow, 2012). Yang *et al.* (2000) reports on the role of NRAMP1 gene polymorphisms in patient susceptibility to RA (Yang *et al.*, 2000). Hashimoto's thyroiditis (HT), chronic lymphocytic thyroiditis or autoimmune thyroiditis results from immune cells attacking the thyroid gland. Studies have reported MAP as an agent in HT based on the same molecular mimicry principle as MAP and T1D (D'Amore *et al.*, 2010) specifically cross reactivity with ZnT8. While ZnT8 is predominantly expressed in pancreatic islet cells, it is also expressed in the follicular and para-follicular epithelial cells of the thyroid gland (Sechi and Dow, 2015).

Multiple Sclerosis and Autism

Multiple sclerosis (MS) is a neurodegenerative disorder affecting the central nervous system (CNS) and is classified as an autoimmune disease resulting from genetic and environmental influences. The disease manifests as a loss of the protective myelin sheaths surrounding the axons of neurons within the CNS, leading to inflammatory demyelination due to specific CD4 T cells which attack the myelin sheaths (Cossu *et al.*, 2017). Sardinia in Italy has the highest incidence of autoimmune MS, with MAP being detected in 42% of MS patients, but only in 6.3% of healthy controls in one study (Cossu *et al.*, 2011).

An increase in MS has been reported in Iceland from the 1950s as seen with the increase in IBD following the introduction of infected sheep. Remarkably, this geographically secluded island was paratuberculosis free until 1938 (Cossu *et al.*, 2013) suggesting exposure to MAP a possible environmental factor leading to the MS outbreak. The studies of Frau *et al.* (2016) hypothesize that both genetic and MAP have an independent role in conferring MS risk. Whilst Cusco *et al.*, 2017 concluded that the role of mycobacteria in the initiation and progression of MS could be a population-specific occurrence, reliant on different genetic and non-genetic factors (Cusco *et al.*, 2017). Autism is a neurodevelopmental disorder which manifests in the early stages of childhood, typically identified in affected individuals by three years of age. This condition is predominately recognised by impaired communication, social interaction and behavioural issues (Singh, 2001). Autism spectrum disorders (ASD) are a collection of developmental neurobehavioral conditions that include autistic disorder, Asperger's disorder, and pervasive developmental disorder (Dow, 2012). ASD is predominately a heritable disorder with estimates as high as 80-90% (Mahajan and Mostofsky, 2015). Epidemiologic studies suggest that some cases of autism can be attributed to environmental factors such as toxic exposures, teratogens, perinatal insults, and prenatal infections such as rubella and cytomegalovirus (Muhle *et al.*, 2004). The link between autoimmune dysfunction and neurodevelopmental diseases such as ASD has been hypothesised based on immunological abnormalities found in autistic patients, but remains unproven (Jung *et al.*, 2011). Specifically, autoimmunity to brain tissue antigens has been recognised in autistic patients with the most common being the CNS myelin-derived myelin basic protein (MBP) (Dow, 2012) and neuron-axon filament proteins (NAFP). Nerve cell myelination is one of the most important postnatal developmental events (Stiles and Jernigan, 2010); therefore, it can be assumed that autoimmunity targeting this process will have huge impact on affected individuals. Studies report an altered blood brain barrier in ASD patient resulting from neurological inflammation, immune dysregulation and increased cytokines in the brain (Samsam *et al.*, 2014). The research of Mameli *et al.* (2014) reports cross reactivity between MBP and MAP epitopes due to molecular mimicry in MS patients. To date no such studies have been reported on ASD patients, but represents an interesting hypothesis where MAP and MBP cross reactivity may play a role in developing autoimmune ASD. Strength is given to the theory that autism is an autoimmune condition by the fact that many autistic patients respond positively to treatment with immune modulating drugs (Singh, 2001).

Table 1. Summary of drug therapy and combinations used for the treatment of IBD.

Drug type	Target effect	Disease condition	Effective	Reference	
Antimicrobials	Penicillin's, Cephalosporin's Vancomycin	peptidoglycan biosynthesis – cell wall inhibition	IBD	No	Alcedo <i>et al.</i> , 2016 Dubuisson <i>et al.</i> , 2010
	Clarithromycin Azithromycin	macrolide protein synthesis inhibitors	IBD	Yes	
	Ethambutol	cell metabolism	IBD	Yes	
	Rifamycin	inhibits RNA synthesis	IBD	Yes	
Antimicrobials	Ciprofloxacin Fluoroquinolone	inhibition of DNA gyrase	CD	Varied results	Lal and Steinhart, 2006
Anti-parasitic	Metronidazole	inhibits nucleic acid synthesis	CD	47% remission rate in patients	Prantera <i>et al.</i> , 1996
Steroid	Methylprednisolone	Anti-inflammatory	CD	68% remission of patients	Prantera <i>et al.</i> , 1996
Steroid and antimicrobial combination	Clarithromycin Rifabutin, Clofazimine and 16-week course of corticosteroids	Antimicrobial Anti-inflammatory	CD	Yes	Selby <i>et al.</i> , 2007
Antimicrobial	Clarithromycin, Rifabutin, Ciprofloxacin, UVBI therapy and Clofazimine	Antimicrobial	CD	Yes	Kuenstner <i>et al.</i> , 2015

Table 2. Outlining genetic and epitope similarities between MAP and host cells and their relationship autoimmune conditions.

Gene affected	Function	Disease state	Reference
NOD2 mutation	Recognition of bacterial cell wall peptidoglycans, activating the nuclear factor immune pathway, in the direct killing of intracellular organisms	<i>Johne's Disease</i> <i>Inflammatory Bowel Disease</i>	Kuenstner <i>et al.</i> , 2015 Zamani <i>et al.</i> , 2017 Girardin <i>et al.</i> , 2003
SLC11A1 gene	Codes for a membrane protein of monocytes and macrophages, glutamic acid decarboxylase (GAD), heat shock protein (HSP) 60 HSP60	<i>Type 1 diabetes</i> Epitope homology between MAP and human heat shock proteins and pancreatic GAD may trigger anti-GAD antibodies that secondarily destroy the pancreas <i>RA and Hashimoto's thyroiditis</i> Cross reactivity with mycobacterial HS65	Paccagnini <i>et al.</i> , 2009 Dow and Sechi, 2011 Dow, 2012 Yang <i>et al.</i> , 2000
β -cell antigen zinc transporter 8 (ZnT8)	Zinc transporter present on cell membranes of the pancreas which displays similarities to a MAP protein MAP3865c Present on follicular and para-follicular epithelial cells of the thyroid gland	<i>Type 1 diabetes</i> Cross reactivity with MAP3865c <i>Hashimoto's thyroiditis</i> Cross reactivity with MAP3865c	Masala <i>et al.</i> , 2011 Sechi and Dow, 2015 D'Amore <i>et al.</i> , 2010
CNS myelin-derived myelin basic protein (MBP)	Nerve cell myelination	<i>Multiple sclerosis</i> <i>Autism</i>	Dow, 2012 Cossu <i>et al.</i> , 2011 Mameli <i>et al.</i> , 2014
Neuron-axon filament proteins (NAFP)	Component of the neuronal cytoskeleton	Molecular mimicry between MBP, NAFP and MAP epitopes	Cusco <i>et al.</i> , 2017 Singh, 2001
<i>IS900</i> gene	Encodes for p43 insertion sequence	Detection in patients presenting with disease states. Detection in contaminated water samples	Gill <i>et al.</i> , 2011

Control measures

Given the severity of JD in cattle and the severity of the conditions observed in humans it is essential to implement control measures to prevent the environmental spread of this pathogen and to protect public health safety. Control measures to prevent zoonotic outbreaks relate to vigilant monitoring of food products throughout the food production line. Food production must adhere strictly to Good Manufacturing Practice (GMP) and follow the hazard analysis critical control point (HACCP) guide documents. Effective suitable disinfection procedures must be in place from “farm to fork” to prevent contamination of the food chain. The “farm to fork” food safety approach was initiated by the European Food Safety Authority (EFSA) and involves risk assessment, risk management and risk communication measures which are undertaken by all EU member states to protect public health safety. At a farm level proper sewage disposal and control of agricultural runoff is essential including the use of faecal matter as a fertilizer. At an individual level, adequate hand washing and sanitation is also critical. Following the implementation of such measures, epidemiology studies could give an indication of the success rate in reducing the incidence of JD in cattle.

Conclusion

At present there is a lack of definitive evidence regarding the contribution of MAP to autoimmune diseases such as IBD, diabetes and rheumatoid arthritis from a causation or correlation aspect. Establishing the undisputable link and risk of MAP transfer from animals to humans must therefore be considered an important precautionary action. Determining the zoonotic transfer of this pathogen will allow for a better assessment of safety measures in terms of zoonotic morbidity in humans which will contribute to a better level of public health safety. The economic cost of infected animals particularly in the dairy industry and medical costs of human cases of morbidity is hugely significant, coupled with animal and patient quality of life means that this issue cannot be disregarded until a definitive answer to the question can be found. The presence of epitope homology between human antigens and MAP proteins may initiate cross reactivity and a failure of self and non-self-recognition resulting in autoimmunity. Molecular mimicry which is based on structural similarities between shared amino acid sequences on pathogens and host cells may provide an explanation to pathogen induced autoimmunity. The manifestations of this cross reactivity might then result in one or more of the many autoimmune conditions discussed depending on location, target organs or specifically target cells. Therefore, even though no definite evidence exists, the mycobacterial hypothesis cannot be completely ruled out; MAP may be a

plausible infectious agent linked to the inflammatory process of IBD and other autoimmune disorders (Hermon-Taylor, 2002).

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Conflict of interest

The authors declare that there is no conflict of interests.

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