The association of nontuberculous mycobacteria with immune-mediated chronic inflammatory and autoimmune diseases: a call for action

Karel Hruska\textsuperscript{a,b}, Arnost Cepica\textsuperscript{c}

\textsuperscript{a} Institute for Research and Education, Brno, Czech Republic, \textsuperscript{b} Veterinary Research Institute, Brno, Czech Republic
\textsuperscript{c} The Atlantic Veterinary College, The University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, C1B4X4

Corresponding author: Karel Hruska <karel.hruska@upvav.cz> or <hruska.publishing@gmail.com>

Feedback is welcomed

Running title: Non-tuberculous mycobacteria: A global public health threat

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\textsuperscript{1} Environmental Health Perspective: Submitted April 15, 2018, rejected May 2, 2018

The Editor-in-Chief: The EHP editorial team has completed a preliminary evaluation of your manuscript for overall scientific quality, relevance to environmental health science, originality, and potential to contribute new or important information to the field. Based on this evaluation we have decided not to consider it further for publication in EHP. We regret that, due to the number of manuscripts we receive, we are unable to provide more detailed feedback on your manuscript. We appreciate your interest in EHP and hope that we may be more helpful on another occasion.

\textsuperscript{2} Journal of Inflammation: Submitted June 4, 2018, rejected October 11, 2018

The Editor: Thank you for considering Journal of Inflammation. Peer review of your manuscript and my own assessment is now complete and, in the light of the reports, and my own assessment as Editor, I regret to inform you that your manuscript cannot be accepted for publication in Journal of Inflammation.

I wish you every success with your research and hope that you will consider us again in the future.

Reviewer #1: This is an interesting article but is highly speculative. Although there associations are described there is not enough evidence presented to prove causation. The article may be of more interest from an environmental health perspective rather than an inflammation journal.

KH note: "Our speculation" is based on tracking prestigious interdisciplinary journals for 25 years and carefully selection of 288 articles to be presented in this review. See for yourself the Boxes.
ABSTRACT

**Background:** In 2000 the European Commission issued its Report “Possible links between Crohn’s disease and paratuberculosis”, and in 2005 the American Academy of Microbiology issued its Colloquium Report “Microbial triggers of chronic human illness”. Nevertheless, the knowledge regarding this health problem continues to grow.

**Objectives:** Nontuberculous mycobacteria (NTM) are not only conventional opportunistic pathogens, but they are important sources of triggers of chronic immune-mediated and autoimmune disorders. We highlight the risk posed by NTM in water, food, and soil, primarily through the Web of Knowledge (Clarivate Analytics) references.

**Discussion:** NTM are associated with the so called “diseases of civilisation” (Type 1 diabetes, Crohn’s disease, multiple sclerosis, asthma, psoriasis, rheumatoid arthritis, and others). Considering the wide exposure of public to mycobacterial triggers from the milk of cows affected by *Mycobacterium avium* subsp. *paratuberculosis*, by drinking water from communal distribution systems, water from swimming pools, and contaminated surface waters, NTM present a global threat to public health. NTM are not being currently controlled for.

**Conclusion:** Alarming spread and subsequent public exposure to NTM has followed unmanaged spread of bovine paratuberculosis, as well as the fast pace urbanization, and westernization of life style of both developed and developing countries.

**Recommendations:** Minimization of infant exposure to immunomodulatory triggers of NTM should be the highest priority, primarily through promotion of breast feeding, monitoring of milk used in manufacturing of baby formula for *M. avium* subsp. *paratuberculosis*, as well as monitoring of water used for food preparation, bathing, and baby swimming, for the levels of live or dead NTM.

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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>CD</th>
<th>Crohn's disease</th>
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<tr>
<td>CE</td>
<td>cell equivalent</td>
</tr>
<tr>
<td>CFA</td>
<td>Complete Freund Adjuvant</td>
</tr>
<tr>
<td>cfu</td>
<td>colony forming units</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
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<td>HSP</td>
<td>heat shock protein</td>
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<tr>
<td>IBD</td>
<td>inflammatory bowel disease</td>
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<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
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<tr>
<td>MDP</td>
<td>muramyl dipeptid</td>
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<tr>
<td>MAP</td>
<td><em>Mycobacterium avium</em> subsp. <em>paratuberculosis</em></td>
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<tr>
<td>MB</td>
<td>mycobacteria</td>
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<td>MS</td>
<td>multiple sclerosis</td>
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<td>NMT</td>
<td>nontuberculous mycobacteria</td>
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<tr>
<td>PD</td>
<td>Parkinson disease</td>
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<td>T1D</td>
<td>type 1 diabetes mellitus</td>
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</table>
Motto:

All truth passes through three stages. First, it is ridiculed. Second, it is violently opposed. Third, it is accepted as self-evident. Arthur Schopenhauer (1788-1860)

Motto:

Don’t just publish your research—publicize it. Scientists simply can’t be silent, or else science truly will be silenced. Christopher Coons, a United States senator from Delaware (Science 2017, Vol 357 Issue 6350, p. 4321)

Mycobacteria – sophisticated and treacherous microorganisms with multifaceted pathogenesis

Mycobacteria have been found in 8,000-year-old human bones, and the diseases traditionally associated with these bacteria, tuberculosis and leprosy, have had a catastrophic impact on human health up to the 20th century. The history of mycobacterial involvement in delayed-type hypersensitivity goes back to 1882, when Robert Koch described the tuberculin reaction. In the 1940s, Landsteiner and Chase proved that the reaction was mediated by the cellular arm of the immune system. Both live and inactivated mycobacteria act as immunomodulators, and they do so after ingestion or inhalation. These activities are properties of the derivatives of the peptidoglycans of mycobacterial cell walls. The N-acetyl muramyl-dipeptide was designated as the smallest adjuvant molecule already in 1974 (Ellouz et al. 1974). N-glycolyl MDP, a constituent of mycobacteria, is more potent and efficacious at inducing innate responses and T cell-mediated immunity than N-acetyl MDP derived from most bacteria, (Coulombe et al. 2009; Behr and Divangahi 2015). In accordance with these properties of mycobacteria, in addition to being conventional pathogens, the importance of nontuberculous mycobacteria as immuno-modulatory environmental pollutants has emerged in last two decades (Falkinham 2003a, 2003b; Hruska and Pavlik 2014).

During the last decades, human exposure to nontuberculous bacteria has increased dramatically, and interestingly, an increase in the incidence of serious chronic inflammatory and autoimmune diseases has generally followed (Vatn 2012; Konkel 2016). The public is generally surrounded by, and exposed to mycobacteria, and human activities contribute to their environmental buildup (Falkinham 2009a; Falkinham 2010b). Exposure to mycobacteria has considerably increased as a consequence of the phenomena associated with urbanisation, e.g., use of municipal water distribution systems, increase of airborne dust, the use of indoor and outdoor recreational swimming pools and the practice of baby swimming. Increased exposure burden is caused by changes in the technologies used in cattle farming, by the move to global marketing of cattle infected with paratuberculosis and by global marketing of powdered infant formula produced from cow milk frequently containing high numbers of Mycobacterium avium subsp. paratuberculosis (MAP). It is this powdered infant formula that harbours an especially great potential to negatively affect public health in the long term (Hruska et al. 2005; Hruska et al. 2011). In addition, the widespread consumption of fast food prepared from infected ground beef can also be part of this mycobacterial assault on public health (Wickens et al. 2005). Inhalation of aerosol contaminated with mycobacteria in showers, whirlpools and indoor swimming pools represents yet another path of human exposure. A further increase in the mycobacterial burden can be expected from the application of hydroponic and aeroponic technologies in high-capacity growing systems.
The clear chronological association between the worldwide build-up of mycobacteria in the environment and the appearance of chronic inflammatory and autoimmune diseases has been well documented. The most plausible mechanism of mycobacterial pathogenesis of these disorders involves the immunomodulatory substances known to be released by both live and dead mycobacteria. Such immunomodulatory triggers have only been widely studied during the last 15 years, but it is already obvious that human technological and environmental developments are the driving force behind the sharp rise in immune-mediated chronic inflammatory and autoimmune diseases (Box 1). Nontuberculous mycobacteria have been so far considered saprophytes or at the most opportunistic pathogens (Box 2). Various nosological entities may manifest clinically in many patients in parallel (Box 3).

Environmental factors play a big role in introducing ambiguity to the classification of diseases, whether through pollutants, physical factors, socioeconomic status or other factors. Consequently, the same aetiological factor can initiate different pathways of pathogenesis due to host differences, which in turn induce different clinical manifestations of the disease, or even diseases considered to be entirely distinct nosological entities. Frequently reported co-morbidities, together with clustering of the affected subjects indicate the involvement of the same or similar trigger factor. While Alzheimer’s disease is not listed in Box 2, a frequent association of mycobacterioses and dementia has been noted (Mohamed 1990; Heckman et al. 2004; Broxmeyer 2005; Sethi et al. 2011; Sakakibara et al. 2014; Gendelman et al. 2017). Similarly, a recently published case report has described neutrophilic urticarial dermatosis concurrent with inflammatory bowel disease and systemic lupus erythematosus (Hou et al. 2017). However, interpretations of clinical observations of various chronic inflammatory and autoimmune disorders and their analyses often do not take into consideration the new and changing paradigms of mycobacterial immunopathogenetic capacity (Hruska and Pavlik 2014). Most authors, however, suspect the involvement of unknown environmental contributing factors in the causality of this class of disorders. Mycobacterial immunomodulatory triggers fulfil the criteria for the missing “environmental contributing factor” very well.

The well-documented relatively recent worldwide build-up of nontuberculous bacteria, and their marked capacity to trigger a variety of immunopathological processes, has, however, not yet fully permeated the awareness of medical practitioners, medical educators, various related research fields and that of the general public. Powerful evidence regarding the correlation between the significant environmental increase in mycobacteria from bovine infections with *M. paratuberculosis* and the increase in the incidence of Crohn’s disease has been well documented in Iceland and in the Czech Republic (Hruska and Pavlik 2014) Based on the knowledge of the historical events leading to the spread of *M. paratuberculosis*, it can be projected that the long-term environmental burden from these mycobacteria will almost certainly continue to increase worldwide.
Important characteristics of mycobacteria that lead to high rates of human exposure

Mycobacteria are perfectly equipped for survival in water and soil, due to their remarkable properties, like the ability to grow in nutritionally poor environments, resistance to higher temperatures and common disinfectants as well as their ability to replicate over an unusually large range of temperatures (Falkinham 2010b; Falkinham 2015). Mycobacteria grow well in the biofilms created within household plumbing (Esteban and Garcia-Coca 2018) and also within amoebas (Salah et al. 2009; Thomas et al. 2010; Ovrutsky et al. 2013; Samba-Louaka et al. 2018) in addition they can be found in host macrophages (Marino et al. 2017). Furthermore, their survival is aided by their abilities to form spores (Lamont et al. 2012) and to change the structure of their cell membranes (Beran et al. 2006). Mycobacteria easily enter into aerosol created during showering or by surf breaks. Mycobacteria are, however, also able to “jump out” of still waters, by first accumulating in water-air interfaces and then being propelled into the air with the release of air bubbles (Blanchard and Syzdek 1970). Mycobacteria survive in river water and also in sediments for many months and even years (Pickup et al. 2005; Whittington et al. 2005). Mycobacterial ecology is described in detail in a monograph (Kazda et al. 2009) and in many review papers (Pedley et al. 2004; Pickup et al. 2005; Pickup et al. 2006; Falkinham 2009a, 2009b; Hruska and Kaevska 2012).

Thus, nontuberculous bacteria not only act as conventional pathogens in people and animals causing local inflammation or granulomas, but perhaps more importantly also release immunomodulatory substances, triggers of chronic inflammatory and autoimmune diseases (Box 4).

The immunomodulators are capable of triggering chronic inflammatory and autoimmune disorders, often dubbed as “diseases of civilisation”, the aetiology of which, however, remains widely debated among researchers. The tissues affected, and the clinical symptoms exhibited, often vary widely, and clinical symptoms often manifest only with a delay of several years (Carbone et al. 2005; Hermon-Taylor 2009; Hruska and Pavlik 2014). Considerable evidence has been accumulated in recent years regarding the aetiological participation of nontuberculous mycobacteria in several serious diseases like Crohn's disease, Type 1 diabetes, multiple sclerosis, asthma, psoriasis, etc. The degree of clinical manifestation of these chronic inflammatory diseases is variable, as it likely depends on the number of mycobacterial cells entering the host, the route of exposure, previous priming, the length of action in the host, the host age, genetic factors, general immune competence as well as on other factors. Most publications address the relationship between paratuberculosis and Crohn's disease (Chiodini et al. 2012). However, the etiology and pathogenesis of chronic inflammatory diseases do not meet the classical paradigm of infectious diseases and zoonoses and their clarification will require challenging research and interdisciplinary collaboration.

Human exposure may occur orally with food or water, or by inhalation in dusty environments or during showering or swimming in indoors pools or heavily contaminated rivers or lakes. Generally, the exposed individuals are unaware of the danger, because there are no immediate symptoms of acute
disease. After a period of latency, any subsequent symptoms of chronic inflammatory or autoimmune disorders that may develop are difficult to associate with the past exposure, regardless of whether the exposure was short and substantial, or cumulative over an extended period of time. The most vulnerable group for exposure to mycobacterial triggers of chronic inflammatory and autoimmune disorders are undoubtedly new-borns and infants with immature immune systems. This mainly occurs in those children who are not breastfed, and whose dried formula or the water used for the preparation of such formula, may contain high numbers of mycobacteria.

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) and other mycobacterial species have been linked to several immune-mediated chronic inflammatory and autoimmune diseases. Presumably, mycobacterial constituents act as triggers of these diseases in conjunction with certain host genetic factors or functionally altered leukocytes. This results in interference in certain metabolic pathways, which then leads to pathological processes in respective target tissues. It is important to keep in mind that this aetiologi cal function can be accomplished even by exposure to dead mycobacteria. In the absence of trials involving long-term or even lifetime follow-up of humans deliberately exposed to mycobacteria, which would be clearly ethically impermissible, the best experimental evidence for the immunopathogenic activity of *Mycobacterium avium* subsp. *paratuberculosis* in humans with respect to Crohn's disease comes from Golan et al. (2009). This report significantly extended the previous evidence of MAP detection in breast milk, blood and intestinal tissues of patients with Crohn's disease by determining that in the human small intestine xenograft model, MAP actively invades epithelial goblet cells and induces tissue damage and inflammation.

**Non-tuberculous mycobacteria are widely present in the environment**

The sources of human exposure to nontuberculous mycobacteria are water (Box 6A reviews, Box 6B references), aerosols, food (Box 7) and soil (Rhodes et al. 2013; Rhodes et al. 2014).

So far, attention has been primarily directed towards speciation of isolates obtained by sample cultivation in liquid or solid media. However, current methods of reproducible determination of the total count of living and dead mycobacteria are not suitable for routine screening. Moreover, in the course of establishing the counts of mycobacteria in water and food according to the colony forming units, the number of the bacterial cells is significantly underestimated.

Taken together, the above observations demonstrate that any finding of mycobacteria in water or food has to be acknowledged as a risk, irrespective of the viability or the number of cells present.
Non-tuberculous mycobacteria have generally only been considered to be opportunistic pathogens of humans. In addition, pasteurisation of milk and heat treatment of meat during food preparation have been considered sufficient measures for devitalisation of these bacteria. However, there are serious problems with this rationale, as many studies have shown that approximately 2% of samples of pasteurised retail milk contained cultivable live bacteria. More importantly, even the bacteria that are killed by milk pasteurisation or heat treatment of meat are not rendered harmless, as they can still act as triggers of immunologically mediated chronic inflammatory and autoimmune disorders in people.

Dairy products and water are currently not tested for mycobacteria, even though they do pose a great risk. Paratuberculosis in cattle is currently generally not considered to be very important, because in infected animals it may be subclinical for years, and it generally does not cause mass mortality. Calves get infected from their mothers or from the contaminated environment, and the disease appears often only after two or three years. diarrhoea is the main clinical symptom, but initially it does not cause concern for the farmers. Loss of body condition and cachexia develop only after months or years. During the course of the infection cows shed large numbers of MAP in milk and faeces (up to $10^4$ per millilitre of milk and $10^8$ per gram of faeces). One infected cow daily sheds up to $4 \times 10^{13}$ MAP cells in faeces and millions in milk. Beef can also contain mycobacteria (up to $10^4$ per gram). The problem is compounded by the fact that paratuberculosis in cattle has undergone huge global spread during the last decades due to the very active trade of cows. The bacteria cannot be detected during the latent phases of the infection, and serological detection of carriers is not reliable. Therefore, movement of animals among farms is tolerated, and international trade is not regulated with respect to the spread of paratuberculosis. Currently, in many countries, up to 90% of farms are affected. Paratuberculosis affects not only cattle, but also buffaloes, camels, sheep and goats, and the milk and meat of these animals also poses a risk to the public (Chaubey et al. 2017).

It also needs to be pointed out that the above-mentioned immunological triggers can be released not only from *Mycobacterium avium* subsp. *paratuberculosis*, but also from other non-tuberculous mycobacteria commonly present in the environment. The incidence of lung disease is related with the occurrence of factors contributing to exposition of population with NTM (Honda et al. 2018). Up to 10,000 mycobacteria can be present in 1 ml of tap water and up to 8 million in one package of powdered infant milk (Hruska et al. 2011). The health of infants may also be endangered if such water is used for formula, food preparation or bathing. Once again, mycobacteria are not subject to control; thus, exposure of formula-fed babies can may occur not only through dried milk powder, but also from the water used to prepare the formula and later in the life from vegetable-based meals.

Interactions between environmental factors and mucosal tissues in the early neonatal period and infancy may be critical in directing and controlling the expression of disease-specific responses in later life (Ogra and Welliver 2008). Powdered infant formula has been the subject of intensive research.
Non-tuberculous mycobacteria: A global public health threat

since 1991, undoubtedly due to the huge increase in production and globally marketing of this food product which saves the lives of new-borns and infants who cannot be breastfed. Formulas are made from milk with unknown numbers of live and dead MAP, unless the manufacturer controls for this bacterial contaminant. There are no regulatory measures to keep MAP under control. This situation has the potential to negatively affect the health of infants in the long term, worldwide. The danger for the health of children consuming such milk, the main constituents of infant formulas, stems from the presence of triggers of several serious chronic diseases. The benefits of breast colostrum and milk in protection against infectious agents have been well-studied and are indisputable. However, bottle-fed children are not protected from the triggers, as indicated by their higher incidence of chronic inflammatory and autoimmune disorders. This evidence is highly suggestive of an aetiological association between formula and this class of diseases.

Importantly, the level of human exposure has been dramatically increasing in the last decades due to the global spread of paratuberculosis in cattle, small ruminants, buffaloes and camels. High levels of mycobacteria are present at certain stages of bovine paratuberculosis in milk and meat of clinically healthy animals. Such products are still marketed without control of mycobacteria contamination, although their impact on public health is obvious. The production of mycobacteria-free or mycobacteria-low infant powdered milk should be regarded as a priority. Human exposure to nontuberculous mycobacteria has been intensified not only through the globalisation of foodstuffs and animal trade, but also through the increasing density of urban inhabitation accompanied by the municipal distribution of water and by global climate change. Further increases in global warming and urbanisation are expected (United Nations 2018; Papalexiou et al. 2018).

Mycobacteria and the so-called “diseases of civilisation”

In the developed parts of the world there has been a steady and simultaneous increase of allergies, Crohn’s disease and ulcerative colitis, type 1 diabetes and multiple sclerosis (Rook et al. 2004). Both the world-wide trend for human migration from rural to urban areas and the development of new technologies in the dairy cattle industry have had a negative impact on human health worldwide, due to the alarming rise in the incidence of non-tuberculous mycobacteria. The accumulating evidence from the published research data regarding the threat to public health of these bacteria, formerly considered innocuous, has been ignored for more than 50 years. The preponderance of the evidence strongly suggests that these ubiquitous mycobacteria are the triggers responsible for the alarming global rise in the incidence of chronic immune-mediated inflammatory and autoimmune diseases. In this respect, Type 1 diabetes, Crohn’s disease, multiple sclerosis, asthma, psoriasis, rheumatoid arthritis and others, have been subject to extensive studies. In spite of the fact that the triggers for the development of these chronic inflammatory and autoimmune diseases are bacterial, the aetiology, pathogenesis, diagnosis and therapy of these conditions are vastly different from common infectious diseases. The pathogenic activity of mycobacteria is indirect, and is mediated through an impairment
of a variety of elements of innate and adaptive immunity. Conversely, confirmation of the presence of mycobacteria, whether by culture or through the presence of their DNA, may not be associated with any disease, or disease may become clinically manifested only after several years.

The impact of civilisation factors, also called “westernisation of lifestyle”, on human exposure to mycobacteria can be summarised as follows:

- More people are dependent on municipal water supplies
- Environment and foodstuffs are important sources of mycobacteria due to the paratuberculosis pandemic in animals
- Pre-term born babies are mostly fed by formula, and more of these infants survive this critical period of life
- More people practice jogging and swimming in polluted environments
- Even some current technological progress like cattle slurry spraying (Burch et al. 2017; Seltenrich 2017), biogas plants (Mazzone et al. 2018) and the use of air blowers for cleaning of sidewalks and public spaces (Liu et al. 2015) contribute to human exposure to mycobacteria via the respiratory route

Conclusions

The published data present convincing evidence of the following:

- Nontuberculous mycobacteria are opportunistic pathogens, particularly for people with compromised immunity (infants, seniors, transplant recipients, persons infected with HIV and other immunosuppressive infections and non-infectious immunosuppressive conditions like certain cancers)
- Mycobacteria release immuno-modulatory triggers, causing chronic inflammatory and autoimmune disorders
- Mycobacteria enter the hosts orally (food, water), by inhalation, via damaged skin, by contaminated catheters and endoscopic instruments, during tooth extraction or loss, during percutaneous procedures under ultrasound examinations, during ultrasound examinations in the vicinity of injury or surgical incision or during tattooing, etc.
- *M. avium* subsp. *paratuberculosis* can be shed in high numbers in the milk of cows with subclinical paratuberculosis, and they have also been found in dairy products and beef
- Nontuberculous mycobacteria are rather commonly detected in drinking water, rivers, swimming pools, municipal water distribution systems and even in bottled mineral and table waters
- Mycobacteria survive for a long time not only in the environment, but also in some disinfection solutions, in gels used for ultrasound examination or in tattoo ink

Starting in the 1960s, publications on nontuberculous mycobacteria have appeared in the Web of Science database (Clarivate Analytics) with increasing frequency. Among the most active authors are J. Kazda (50 publications, 1964–2005), J. O. Falkinham (118 publications, 1976–2017) and I. Pavlik (177 publications, 1994–2017). The number of annual publications retrieved with the keyword “paratuberculosis” ranged between one and 16 between 1945 and 1989, but it increased to more than 200 publications annually in the 2006 to 2017 period. Search results for “Crohn” retrieved less than ten papers per year from 1945 to 1960 and less than 1,000 from 1961 to 1995. However, there were 3,000 to 4,000 publications annually from 2011 to 2017 (Hruska 2016, updated by KH 2018).

The impact of mycobacteria on human chronic inflammatory diseases can only be studied indirectly, as experimental studies are ethically unacceptable. Nevertheless, many rigorous, well-executed studies are available, and based on empirical data many hypotheses have been proposed.
Unfortunately, the interpretation of these studies is often done without full awareness of the complementary and compounding nature of these results, just because they come from seemingly unrelated disciplines. Urbanisation, hygiene status, microbial exposures and pollution have been implicated as potential environmental global risk factors for Crohn's disease or ulcerative colitis (Ng et al. 2013), the global diseases in the 21st century (Ng et al. 2018). As far as we know, the association of human chronic inflammatory and autoimmune diseases and civilization factors, mediated through exposure to mycobacteria has not yet been opened.

The well-documented connection between chronic inflammatory and autoimmune diseases on one side, and mycobacterial triggers on the other, offers in our view a plausible interpretation of the current known facts. (Box 8). The aim of this commentary is to bring the attention of the reader to the global risk posed by mycobacteria. In particular, we call for focus on baby formula, in which triggers released from the mycobacteria present can significantly influence the development of the baby's immune responsiveness. As documented in papers cited in this commentary, in spite of the accumulating evidence in this regard for over 100 years, the association with chronic inflammatory and autoimmune disorders has become buried in squabbles over proposed competing hypotheses, and, regrettably, it remains woefully underestimated.

Ample evidence for the important role of mycobacterial triggers associated with chronic inflammatory diseases, particularly in the cell-mediated arm of the response, comes from the analysis of many publications regarding Complete Freund Adjuvant (CFA), intensively used in research since the late 1950s. It is the mycobacterial component that makes the discussion of CFA relevant for the purpose of this report. In addition to its action as a powerful immune adjuvant, there has been a dawning realisation that CFA also causes serious side effects by acting on the same pathways of the immune response. As a result, CFA has been banned for use in human immunisation (Gupta et al. 1993) because of these serious and debilitating side effects (Claassen and Boersma 1992). The unacceptable negative effects on the recipients of the CFA were unambiguously linked to the inactivated mycobacterial content, which prompted the search for mycobacterial subunits that would retain the potent immunostimulatory activity of the bacteria but lack the undesirable toxic side effects. The minimal structure needed for the immunomodulatory effect of CFA was identified as N-acetyl muramyl-lalanyl-D-isoglutamine (Ellouz et al. 1974). However, even this structure was still too toxic for humans (Gupta et al. 1993). Synthetic N-glycolyl MDP, the constituent of mycobacteria, is more potent and efficacious at inducing innate responses and T cell-mediated immunity than N-acetyl MDP derived from most bacteria,(Coulombe et al. 2009; Behr and Divangahi 2015) Some mechanisms exerted by immunomodulators are listed in Box 5. This gives cause for alarm, especially as oral and respiratory exposure of humans and particularly children to mycobacteria is rapidly increasing but still unacknowledged as a very probable serious health risk.

Immunomodulatory triggers may impact either leukocyte activities or target tissues, or they may interfere with host metabolic pathways. The net effect of the triggers is undoubtedly influenced by the quantity of mycobacteria and the length of exposure, by whether previous sensitisation has taken
place, by the stage of the host’s immune development and host genetic factors. Epigenetic factors have also been found to play a role (Tang and Ho 2007). The fact that triggers participate in the aetiology of diseases with diverse symptomatologies can be explained by their variable natures, the route of exposure, the affected metabolic pathways and the state of target host tissues. By far the most information in this respect has been obtained on triggers present in the mycobacterial cell wall. Consequently, the global rise in the incidence of bovine paratuberculosis is closely paralleled by a dramatic increase in the prevalence of chronic inflammatory and autoimmune diseases, often dubbed “diseases of civilisation”. However, the already established critical role of mycobacteria in the causation of chronic inflammatory and autoimmune disorders is still underestimated by some.

In conclusion, there are large amount of research data strongly suggesting that mycobacteria in food and water are dangerous, and that it does not matter if they are consumed live or dead. However, clinical disease is not a regular outcome of all exposures, as it presumably depends on the quantity of mycobacteria consumed, the stage of life and frequency of exposure, prior sensitisations and on the genetic predisposition of the affected person. This makes the causative association of mycobacteria with inflammatory and autoimmune conditions clinically less obvious.
The first step in correcting the problem must be official statements by health and food hygiene authorities that human exposure to mycobacterial triggers poses a definite risk with respect to immune-mediated chronic inflammatory and autoimmune diseases. In the European Union, such an official document should be requested by the European Commission from the European Food Safety Authority as the Scientific Opinion of EFSA and should be followed by European Commission Regulation. Similar legal procedures should proceed globally to manage the global threat of powdered infant milk; such initiatives would gradually decrease the risk posed by exposure.

Various approaches to lowering the risk associated with human exposure to mycobacterial triggers of chronic inflammatory and autoimmune diseases are summarised in Box 9. In our view, only the requirement for “MAP-free” powdered infant milk requires strict regulations. Other commodities would not require introduction of regulations, but voluntary testing may be suggested to manufacturers of dairy products, ground beef, fruits and vegetables and bottled drinking water, as a means of product quality promotion. Public swimming pool water, water of spa facilities, hospitals and dental clinics should be tested at the expense of the owners of businesses. In all cases where the products or water are designated “Mycobacteria-free” or “Mycobacteria-low” (lower than 500 cells equivalent) there should be regulations in place enabling monitoring of the declared status, associated with enforcement of sanctions for false labelling and deception of consumers.

The public has to have access to general information and to answers to the important questions. The responsibility for public awareness of the importance of mycobacteria as participants in the causation of chronic diseases lies firstly with the public health authorities, family physicians and serious investigative media but also with the communities affected by these disorders.

Mycobacteria should be subject to compulsory food hygienic inspection of powdered infant formulas. Self-imposed voluntary inspection would be highly desirable for milk, ice cream, drinking water (both tap and bottled) and beef from cows slaughtered at ages higher than 30 months. The expenditures needed should be easily recoverable if such products were labelled “MAP-free” or “Mycobacteria-low”. This would, undoubtedly, motivate cattle farmers to cull their mycobacteria-shedding animals. Continuous monitoring of MAP in milk samples collected before transfer from farm refrigeration tanks to transport tanks would enable early detection of increases in MAP shedding. Testing of pooled samples would make early identification of a new shedder possible, and the immediate elimination of the shedders should facilitate production of “MAP-free” or “MAP-low” milk within a reasonable period of time.

Furthermore, it would be prudent to monitor the mycobacterial content of water used in hospitals, in hydrotherapy and in wellness facilities. Swimming pools, especially those where baby swimming is practiced, should be encouraged to monitor mycobacterial content in the water and air. Furthermore,
commercial services regarding mycobacterial contamination should be available to households interested in such testing. This service depends on the development of a simple sampling procedure, methods of postal delivery of samples to the testing laboratories and by financially viable testing. All results should be available for evaluation and publication. It is hoped that the data accumulated by such testing will then facilitate more research regarding the association of mycobacteria with various chronic inflammatory and autoimmune disorders. Furthermore, it is hoped that the results of such testing will also provide the needed incentive for the manufacture and distribution of affordable microbial filters.

Women suffering with Crohn’s disease shed mycobacteria in milk (Naser et al. 2000). Therefore, breast milk of mothers with Crohn’s disease should be tested for mycobacteria throughout the nursing period, and interruption of breastfeeding in cases of shedding would be a prudent in order to prevent the exposure of babies and to safeguard the development of proper immune responsiveness.

Agencies supporting research should make it a priority to encourage the screening of the mycobacterial contamination of milk, water, food items and the establishment of a single national database for all laboratories. The certified methods of monitoring must be simple, aimed at detecting MAP in milk and dairy products and Mycobacterium spp. in other commodities and validated to reliably determine when an arbitrary count limit, e.g., 500 or 1,000 cell equivalents per gram or millilitre, is exceeded.

While it is not possible to completely eliminate human exposure to mycobacteria, this does not mean that the substantial body of important research findings pointing to the potential of nontuberculous mycobacteria in inducing chronic inflammatory and autoimmune diseases should continue to be ignored. A basic measure that can, and should be taken now, is a risk awareness campaign, so that people can avoid unnecessary exposure to large numbers of mycobacteria, which after ingestion or inhalation release triggers of disease affecting millions of people. It is undeniable that the increasing numbers of patients affected with chronic inflammatory and autoimmune disorders could be reduced if a global public campaign to raise awareness of this connection were conducted. Individuals could then take their own measures to minimise their risk of developing these disorders. Consequently, they could influence the development of proper public health policies by lobbying health experts and lawmakers.

A call for Action (Box 10) will be published on the internet. Relevant servers can share this activity, which could start a massive information campaign. Supporters are welcome.
Box 1

Non-tuberculous mycobacteria are suspected triggers of immune mediated chronic inflammatory and autoimmune diseases (selected references). See also Box 3.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMYOTROPHIC LATERAL SCLEROSIS</td>
<td>Pierce 2018b</td>
<td>Outdoor sports players may develop amyotrophic lateral sclerosis after multiple oral, nasal or subcutaneous doses of MAP present in the dirt, dust and grass of their playing fields</td>
</tr>
<tr>
<td>ANENCEPHALY</td>
<td>Pierce 2019</td>
<td>The inhalation of aerosolized MAP-contaminated manure by women in the first four weeks of pregnancy may be responsible for the development of anencephaly in the fetus</td>
</tr>
<tr>
<td>ASThma</td>
<td>Arrieta et al. 2015</td>
<td>Infants at risk of asthma exhibited transient gut microbial dysbiosis during the first 100 days of life</td>
</tr>
<tr>
<td></td>
<td>Cepeda et al. 2017</td>
<td>Intake of fastfood was positively associated with a higher prevalence of wheeze in adolescents</td>
</tr>
<tr>
<td>ATOPIC DISEASES</td>
<td>van Odijk et al. 2003</td>
<td>Breastfeeding seems to protect from the development of atopic disease *)</td>
</tr>
<tr>
<td>AUTISM</td>
<td>Dow 2011</td>
<td>MAP; through molecular mimicry to its heat shock protein HSP65, triggers autism by stimulating antibodies that cross react with myelin basic protein</td>
</tr>
<tr>
<td>CROHN’S DISEASE</td>
<td>Bergstrand and Hellers 1983</td>
<td>Possible association with no or very short periods of breast-feeding *)</td>
</tr>
<tr>
<td></td>
<td>Rigas et al. 1993</td>
<td>Negative association of breast-feeding with Crohn’s disease *)</td>
</tr>
<tr>
<td></td>
<td>Naser et al. 2000</td>
<td>MAP role in CD pathogenesis supported</td>
</tr>
<tr>
<td></td>
<td>Karlinger et al. 2000</td>
<td>A great number of bacterial and viral factors has been suspected of being infectious factors in IBD, mostly in CD</td>
</tr>
<tr>
<td></td>
<td>Hanson et al. 2001</td>
<td>Breastfeeding decreases the risk of several chronic inflammatory diseases *)</td>
</tr>
<tr>
<td></td>
<td>Shanahan 2002</td>
<td>Crohn’s disease is a disorder mediated by T lymphocytes which arises in genetically susceptible individuals as a result of a breakdown in the regulatory constraints on mucosal immune responses to enteric bacteria</td>
</tr>
<tr>
<td></td>
<td>Rook et al. 2004</td>
<td>The simultaneous increase in diseases of immunodysregulation is at least partly attributable to malfunction of regulatory T cells in a subset of the population living in the modern environment; mycobacteria, helminths and lactobacilli, are recognised by the innate immune system as harmless, and as adjuvants for Treg induction</td>
</tr>
<tr>
<td></td>
<td>Klement et al 2004</td>
<td>Breastfeeding is associated with lower risks of Crohn disease *)</td>
</tr>
<tr>
<td></td>
<td>Shanahan and O’Mahony 2005</td>
<td>The heterogeneity of Crohn’s disease suggests that it would be unwise to dismiss an infectious contribution to the pathogenesis in a subset of patients. The most enduring infectious candidate has been Mycobacterium paratuberculosis</td>
</tr>
<tr>
<td></td>
<td>Hruska et al. 2005, 2011</td>
<td>Infant dried milk can be a source of mycobacterial triggers</td>
</tr>
<tr>
<td></td>
<td>Sonntag et al. 2007</td>
<td>Exposure to bacterial antigens and other environmental factors in combination with a genetic susceptibility was implicated in the etiology of inflammatory bowel disease; preterm birth and other perinatal circumstances were associated with the development of IBD</td>
</tr>
<tr>
<td></td>
<td>Pierce 2009a</td>
<td>Cluster of Crohn's disease possibly linked to fully treated drinking water</td>
</tr>
<tr>
<td></td>
<td>Pierce 2009b</td>
<td>MAP might directly infect endothelial cells and adipocytes and cause them to proliferate, causing focal obstruction within already existing vessels (including granuloma formation), the development of new vessels (neangiogenesis and lymphangiogenesis), and the &quot;creeping fat&quot; of the mesentry that is unique in human pathology to Crohn’s disease but also occurs in bovine Johne's disease</td>
</tr>
<tr>
<td></td>
<td>Golan et al. 2009</td>
<td>MAP can specifically colonize the normal human small intestine and can elicit inflammation and severe mucosal damage</td>
</tr>
<tr>
<td></td>
<td>Hermon-Taylor 2009</td>
<td>Intracellular infection with the primary pathogen causes an immune dysregulation and a specific chronic enteric neuropathy with loss of mucosal integrity</td>
</tr>
<tr>
<td></td>
<td>Economou et al. 2009</td>
<td>Industrialized status and affluence are the common denominators</td>
</tr>
<tr>
<td></td>
<td>Chiodini et al. 2012</td>
<td>A great deal of information has been accumulated that clearly establishes an association between M. paratuberculosis and Crohn's disease: a review</td>
</tr>
<tr>
<td></td>
<td>Naser et al. 2014</td>
<td>The majority of the studies definitively support the role of MAP in at least 30%-50% of CD patients</td>
</tr>
<tr>
<td></td>
<td>Agrawal et al. 2014</td>
<td>A possible transmission of MAP from animal-derived products to humans</td>
</tr>
<tr>
<td></td>
<td>Zamani et al. 2017</td>
<td>Nine individuals with ulcerative colitis or Crohn disease grew up or lived in Plains, Montana, a 1,200-person community adjacent to the Clark Fork River near herds of free ranging Rocky Mountain bighorn sheep</td>
</tr>
<tr>
<td>HYPERSENSITIVE PNEUMONITIS, HOT TUB LUNG</td>
<td>Fjallbrant et al. 2013</td>
<td>Diffuse granulomatous lung disease caused by inhalation of water aerosol containing non-tuberculous mycobacteria</td>
</tr>
<tr>
<td></td>
<td>Vergez et al. 2017</td>
<td>A type of hypersensitivity pneumonitis caused by inhalational exposure to the Mycobacterium avium complex</td>
</tr>
</tbody>
</table>

*) Some evidence supports a contribution of MAP to the development of Crohn’s disease.
Box 1 Hypersensitive pneumonitis, cont.

Miller et al. 2018  
Pathogenesis is attributed to a combination of immune complex-mediated and delayed hypersensitivity reactions to the inciting agent, mostly mycobacteria

Wassilew et al. 2016  
NTM isolation and pulmonary disease are reported to rise in frequency

Gebert et al. 2018  
The regions in the United States where NTM lung infections are most common were the same regions where pathogenic mycobacteria were most prevalent in showerheads, highlighting the important role of showerheads in the transmission of NTM infections.

James et al. 2018  
A close spatial relationship between the abundance of a mycobacterium-like organism, most probably M. avium, and a localised outbreak of metal working fluid associated hypersensitivity pneumonitis

Saussereau et al. 2019  
Hot tub lung is a hypersensitivity pneumonitis (HP) due to exposure to inhaled non-tuberculous mycobacteria, the most frequent being Mycobacterium avium complex. HP may be enhanced by chronic exposure to multiple microorganisms.

INFERTILITY ...... CONGENITAL MALFORMATIONS

Laurence et al. 1968a,b  
It was concluded that there is a polygenically inherited predisposition to produce offspring with central nervous system malformations in certain populations, interacting with environmental trigger mechanisms.

Carter et. al. 1968  
The findings are compatible with the hypothesis that the genetic predisposition to spina bifida and anencephaly is polygenic, and that additional intrauterine environmental factors also play a part in causation. The risk of recurrence in this region of Britain would not necessarily apply to other regions with a lower incidence at birth of neural tube malformations.

Elzaatari et al.1995  
In humans, the high intensity of antibody reactions against PTB65K heat shock protein of some sera from Crohn’s disease patients compared with that from noninflammatory bowel disease patients showed a positive correlation with mycobacterial diseases.

Fisher et al. 1995  
GD10 rat embryos were exposed in utero to a heat treatment previously demonstrated to produce skeletal malformations; maternal core temperature was raised and maintained at 42-42.4 degrees C for 5 min. In addition, explanted GD10 embryos were cultured in vitro and exposed to temperatures of 42-42.5 degrees C for 15 min. Results demonstrated that the expression of 70- and 90-kD proteins was transcriptionally regulated. The 70-kD protein was identified, using Western blot analysis, as a eukaryotic inducible stress protein (hsp72), and the presence of this protein was detected between 2 and 27 hr post-treatment.

Fisher et al. 1996  
Heat-induced alterations in proteins comprising intermediate filaments occur concomitantly with the induction of stress proteins and precede aberrant somite morphology.

Wali et al. 1996  
Mycobacterium fortuiitum infection of the endometrium - a rare cause of infertility

Buckiova et al. 1998  
Heat-exposed chick embryos exhibited the heat shock response, with protein expression reaching a maximum 4-6 h following heat treatment. Malformed embryos showed an intense heat shock response for a further 6 h. The levels of induced heat shock proteins were similar in the affected neural tube and in the heart, where neither induced cell death nor malformations were observed.

Dominitz et al. 2002  
Maternal IBD is associated with increased odds of preterm delivery, low birth weight, smallness for gestational age and reporting of congenital malformations.

Felkner et al. 2003  
One or more episodes of periconceptional diarrhea were associated with increased risk of neural tube defects-affected pregnancies compared to no episodes of diarrhea.

Ha et al.2003  
Reverse transcriptase-polymerase chain reaction (RT-PCR) showed the increased expression of sHSP and nestin mRNA in the cerebral arteriovenous malformation (AVM) specimens. Embryonic reversion of the mature cytoskeleton to nestin and the increased expression of sHSP in response to cerebral injury are associated with increased wall tension caused by dilating AVM vessels and with the hemodynamic stress that surrounds AVMs.

Child et al. 2006  
The finding of increased maternal anti-Hsp70 in patients who later gave birth to babies with a birth defect suggests a previous stressful event may have contributed to the pathogenesis. The results suggest that there is a relationship between hsp60 and hsp70 levels and their respective antibodies in rats and alterations in maternal reproductive performance and impaired fetal development and growth in pregnancies associated with diabetes.

Early foetal loss in cows was positively correlated with seroconversion to MAP.

Garcia-Ispierto and Lopez-Gatus 2016  
Successful implantation is dependent on the appropriate decidualization of endometrial stromal cells for the establishment of pregnancy in women. Mycobacterial heat shock protein 65 is involved in pathogenesis of the genital tuberculosis (GTB). In presence of HSP65, significant reduction in the decidual phenotype of endometrial stromal cells and prolatin expression is suggestive of impairment in decidualization.

Subramani et al. 2017  
The findings provide valuable new insights into Cerebral Cavernous Malformations pathogenesis and novel options for the development of preventive and therapeutic strategies

Antognelli et al. 2018  
The inhalation of aerosolized MAP-contaminated manure by women in the first four weeks of pregnancy, and intrauterine transmission to the embryo, may be responsible for the development of anencephaly in the foetus

Pierce 2019  

MULTIPLE SCLEROSIS, NEUROMYELITIS OPTICA

Pisacane et al. 1994  
Patients with multiple sclerosis were less likely than controls to have been breast fed for a prolonged period of time

Hanson et al. 2001  
Protective effects of breastfeeding decreases the risk of several chronic inflammatory diseases *)
## Box 1 Multiple sclerosis, Neuromyelitis optica, cont.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dow 2012</td>
<td>Molecular mimicry is the proposed etiopathology by which MAP stimulates autoantibodies associated with autoimmune (type 1) diabetes, autoimmune (Hashimoto's) thyroiditis, and multiple sclerosis</td>
</tr>
<tr>
<td>Cossu et al. 2016</td>
<td>MAP could act as a risk factor or a triggering agent of MS in some Japanese patients with a genetic susceptibility to the mycobacterium.</td>
</tr>
<tr>
<td>Cossu et al. 2017a</td>
<td>Our study provided further evidence for a possible association between MAP and MS.</td>
</tr>
<tr>
<td>Slavin et al. 2018</td>
<td>High level of antibodies against MAP virulence factors protein tyrosine phosphatase A (PtpA) and protein kinase G (PknG) in multiple sclerosis and neuromyelitis optica spectrum disorder patients obviously exposed with MAP</td>
</tr>
<tr>
<td>Cossu et al. 2017b</td>
<td>We provide a detailed review of the mycobacteria that have been linked to MS over the last three decades, with a focus on Mycobacterium bovis bacille Calmette-Guerin vaccine for human and oral exposure to Mycobacterium avium subsp. paratuberculosis.</td>
</tr>
<tr>
<td>Bo et al. 2018b</td>
<td>The sera of 34 neuromyelitis optica spectrum disorder patients showed elevated levels of antibodies against MAP and human epitope MBP85-98 compared to healthy controls</td>
</tr>
<tr>
<td>Yokoyama et al. 2018</td>
<td>Serum and CSF samples from 46 patients with multiple sclerosis, 42 patients with neuromyelitis optica spectrum disorder, and 29 age-matched and sex-matched control subjects were screened retrospectively for the presence of antibodies against MAP pentapeptide (MAP_5p), MAP_2694(295-303), and myelin basic protein (85-98) peptides. The results highlight the involvement of MAP in MS etiopathogenesis</td>
</tr>
<tr>
<td>Cossu et al. 2019</td>
<td>The adjuvant effect of MAP on experimental autoimmune encephalomyelitis development was demonstrated</td>
</tr>
</tbody>
</table>

### PARKINSON'S DISEASE

- **Dow 2014**
  - This article proposes that genetic defects associated with PD also result in a permissive environment for MAP infection—ineffective xenophagy. It postulates that beginning as an enteric infection, MAP--via the vagus nerve--initiates a pathologic process that results in a targeted neuroinvasion of the CNS

### RHEUMATOID ARTHRITIS

- **Hanson et al. 2001**
  - Protective effects of breastfeeding decreases the risk of several chronic inflammatory diseases

- **Bo et al. 2018a**
  - Prevalence of Abs against at least one of the assessed epitopes reached 72% in rheumatoid arthritis (RA) patients and 15% among healthy controls. Interferon regulatory factor 5 is a potential autoimmune target of RA. Epstein-Barr virus and MAP infections may be involved in the pathogenesis of rheumatoid arthritis, igniting a secondary immune response that cross-reacts against rheumatoid arthritis self-peptides.

- **Sharp et al. 2018**
  - Genetic polymorphisms may play vital role in T-cell regulation, susceptibility to mycobacteria and ultimately response to treatment of rheumatoid arthritis patients.

### SARCOIDOSES

- **Deake and Newman 2006**
  - Molecular analysis for and humoral immunity to mycobacterial antigens from sarcoidosis patients have renewed interest in a potential role of mycobacteria in sarcoidosis

- **Spagnolo I al. 2008**
  - Mycobacteria, may be causative in some sarcoidosis cases

- **Schouten et al. 2018**
  - The study included 615 cases and 1334 referents in Alberta, Canada, 1999-2005. The consumption of farm milk appeared to be consistenly associated with sarcoidosis.

### THYROIDITIS

- **Dow 2012**
  - MAP HSP65 acts as a trigger of autoimmune diseases

- **Gupta et al. 2017**
  - IS900 PCR: 28/76 (36.8%) positive patients of thyroiditis

### TYPE 1 DIABETES

- **Gale 2002**
  - The incidence of childhood type 1 diabetes increased worldwide in the closing decades of the 20th century. Childhood type 1 diabetes was rare but well recognized before the introduction of insulin. Low incidence and prevalence rates were recorded in several countries over the period 1920-1950. The overall pattern since then is one of linear increase. Steep rises in the age-group under 5 years have been recorded recently. The disease process underlying type 1 diabetes has changed over time and continues to evolve. Understanding why and how this produced the pandemic of childhood diabetes would be an important step toward reversing it. (KH note: The incidence copies the onset of civilisation factors involved in NTM exposition of the population).

- **Schack-Nielsen et al. 2005**
  - Breastfeeding reduced incidence of Type-1 diabetes *)

- **Dow 2006**
  - Genetic susceptibilities, epitope homologies and epidemiologic studies are presented that support MAP as a causative agent of T1DM in the genetically at risk

- **Dow 2012**
  - Molecular mimicry is the proposed etiopathology by which MAP stimulates autoantibodies associated with autoimmune (type 1) diabetes, autoimmune (Hashimoto's) thyroiditis, and multiple sclerosis

- **Lamb et al. 2015**
  - Cow's milk intake may increase risk of islet autoimmunity and progression to T1D

- **Lund-Blix et al. 2015**
  - Breast-feeding for 12 months predicts a lower risk of type 1 diabetes *)

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* KH note: The incidence copies the onset of civilisation factors involved in NTM exposition of the population.
**Box 1 Type 1 diabetes, cont.**

<table>
<thead>
<tr>
<th>Author</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virtanen 2016</td>
<td>Cow’s milk consumption is associated with an increased risk of type 1 diabetes</td>
</tr>
<tr>
<td>Niegowska et L. 2017</td>
<td>A significantly elevated positivity for MAP/proinsulin was detected among patients, with the highest prevalence in the 32-41 year-old T1D-like LADA subgroup, supporting our hypothesis of a possible MAP contribution in the development of autoimmunity</td>
</tr>
<tr>
<td>Songini et al. 2017</td>
<td>Recent studies have linked MAP to T1D in the Sardinian population having the second highest incidence of T1D in the world</td>
</tr>
</tbody>
</table>

*) in other words: formula feeding has an opposite effect, obviously due to the mycobacterial triggers (Hruska and Pavlik 2014)
### Disorders associated with ingestion or inhalation of nontuberculous mycobacteria

#### SKIN AND SOFT TISSUES INFECTIONS
- Sugita et al. 2000: M. avium in bath tub heating unit, familial cluster (father and two children)
- Atkins and Gottlieb 2014: A review

#### CERVICAL LYMPHADENITIS IN CHILDREN
- Hermon-Taylor et al. 1998: M. paratuberculosis cervical lymphadenitis, followed by terminal ileitis similar to Crohn's disease
- van Bremen et al. 2014: Atypical mycobacteriosis
- Miqueleiz-Zapatero et al. 2018: M. lentiflavum should be considered an important emergent pathogen cause of cervical lymphadenitis in the paediatric population

#### OSTEOMYELITIS
- Bi et al. 2015: Severe consequences and a poor prognosis

#### PULMONARY DISEASES
- Rose et al. 1998: Endemic granulomatous pneumonitis "Lifeguard lung" (M. abscessus)
- Johnson and Odell 2014: Pulmonary infections are most commonly due to M. avium complex (MAC), M. kansasii, and M. abscessus
- Prevots and Marras 2015: Pulmonary Infection with NTM: a review
- Kwon and Koh 2016: Two types of NTM lung diseases reported; fibrocavitary and nodular bronchiectatic forms
- Mougari et al. 2016: M. abscessus is responsible for lung diseases and healthcare-associated extrapulmonary infections
- Yeung et al. 2016: Respiratory diseases and increased mortality associated with pulmonary NTM infection
- Dietz et al. 2017: Pulmonary disease caused by NTM is steadily increasing worldwide
- Larson et al. 2017: NTM disease is becoming more prevalent
- Martiniano et al. 2017: Pulmonary NTM infections has increased in recent decades (pleurisy, pneumothorax)
- Naito et al. 2018: A close spatial relationship between the abundance of a mycobacterium-like organism, most probably M. avium, and a localised outbreak of metal working fluid associated hypersensitivity pneumonitis
- M. abscessus is responsible for lung diseases and healthcare-associated extrapulmonary infections

#### GENERAL
- Prevots and Marras 2015: Host factors important for a worldwide increase in the prevalence of human nontuberculous mycobacterial infections include thoracic skeletal abnormalities, rheumatoid arthritis, and use of immunomodulatory drugs. Clustering of disease within families suggests a heritable genetic predisposition to disease susceptibility. Warm, humid environments with high atmospheric vapor pressure contribute to population risk. (KH note: ... or a household related source of exposition to NTM)

#### Diseases associated with use of mycobacterially contaminated medical tools

#### REVIEW ARTICLE
- Sood and Parrish 2017: Mycobacterial outbreaks in healthcare settings have been underrecognized.

#### BRONCHOSCOPY RISK
- Falkinham 2010a: Water and biofilm samples collected from the bronchoscopy preparation laboratory yielded M. avium, M. intracellulare, M. malmoense and M. gordonae

#### CATHETERS
- Montero et al. 2016: Venticuloperitoneal shunt placement complicated with M. abscessus
- Blair et al. 2017: M. fortuitum empyema associated with an infected pleural catheter

#### COSMETIC MEDICINE
- Singh et al. 2016: Seeking cosmetic surgery in the developing world (medical tourism)
- Hammond et al. 2017: M. chelonae infection after abdominal liposuction and gluteal fat injection

#### HEATER-COOLER UNIT USED IN CARDIOSURGERY
- Gotting et al. 2016: M. chimaera detected in the water tanks of the HCU's and in exhaust air
- Garvey et al. 2016, 2017: UK, Birmingham, M.chimaearain water from heater-cooler units of cardiopulmonary bypass equipment, 3000 cfu/l
- Schreiber and Sax 2017: M. chimaera infections following cardiac surgery
- Stewartson et al. 2017: M. chimaera infection should be considered after surgery with cardiopulmonary bypass; disseminated infection or sternal wound infection
- Walker et al. 2017: Many HCU's are contaminated with M. chimaera and complex biofilms

#### RESPIRATORY INFECTION
- Towle et al. 2016: Contaminated nebulizers
**TATOOING**

<table>
<thead>
<tr>
<th>Study</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kennedy et al. 2012</td>
<td>M. chelonae associated with skin and soft-tissue infections</td>
</tr>
<tr>
<td>Falsey et al. 2013</td>
<td>Regulation and use of tattoo inks should be considered</td>
</tr>
<tr>
<td>Conaglen et al. 2013</td>
<td>MB isolated from 71/142 (50%) cases, M. chelonae identified in 48/71 (67.6%) isolates</td>
</tr>
<tr>
<td>Sergeant et al. 2013</td>
<td>M. chelonae cultured from the ink</td>
</tr>
<tr>
<td>Simunovic and Shinohara 2014</td>
<td>INTM infections happen in tattoos with increasing frequency through contaminated ink or water or water used to dilute inks</td>
</tr>
</tbody>
</table>

**ULTRASOUND TRANSMISSION GEL**

<table>
<thead>
<tr>
<th>Study</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng et al. 2016</td>
<td>M. massiliense infections associated with ultrasound transmission gel, one death in a neonate</td>
</tr>
</tbody>
</table>
Box 3
Reported co-morbidity of immune-mediated diseases and familiar clusters likely impacted by mycobacteria, namely *M. avium subsp. paratuberculosis* (selected references)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tlaskalova-Hogenova et al 2004</td>
<td>Involvement of commensal microflora and its components with strong immunoactivating properties (e.g. LPS, peptidoglycans, superantigens, bacterial DNA, Hsp) in etiopathogenetic mechanism of various complex, multifactorial and multigenic diseases, including inflammatory bowel diseases, periodontal disease, rheumatoid arthritis, atherosclerosis, allergy, multiorgan failure, colon cancer has been recently suggested.</td>
</tr>
<tr>
<td>Bernstein et al. 2005</td>
<td>The finding of asthma as the most common comorbidity increased in Crohn's disease patients compared with the general population is novel.</td>
</tr>
<tr>
<td>Green et al. 2006</td>
<td>Incidences of Crohn's disease and ulcerative colitis were observed to be highest among non-Aboriginal persons, persons of high socioeconomic status, persons with the lowest rates of enteric infection, and persons with the highest rates of multiple sclerosis.</td>
</tr>
<tr>
<td>Banwell et al. 2008</td>
<td>Children with inflammatory demyelination, CNS injury, and T1D exhibited heightened T-cell reactivities to self-antigens, and these responses were not strictly limited to the disease target organs.</td>
</tr>
<tr>
<td>Spagnolo et al. 2008</td>
<td>Both individual predisposition to develop pulmonary granulomatous diseases, specifically sarcoidosis, Blau syndrome, and systemic vasculitides and mycobacteria may be causative in some cases.</td>
</tr>
<tr>
<td>Dow 2014</td>
<td>Genetic studies reveal an association between Parkinson's disease (PD), leprosy and Crohn's disease.</td>
</tr>
<tr>
<td>Brassard et al. 2015</td>
<td>The incidence of Crohn's disease in asthma and chronic obstructive pulmonary disease patients was 27% and 55% higher than in the general population of Quebec.</td>
</tr>
<tr>
<td>Liao et al. 2016</td>
<td>Prior tuberculosis history, hypertension, diabetes mellitus, interstitial lung disease, chronic obstructive pulmonary disease and exposure to oral corticosteroids in a dose-dependent manner were associated with a significantly increased risk of NTM disease in RA patients.</td>
</tr>
<tr>
<td>Sechi and Dow 2015</td>
<td>Incriminating for MAP as a zoonotic agent is the increasing number of diseases with which MAP has been related: Blau syndrome, type 1 diabetes, Hashimoto thyroiditis, and multiple sclerosis.</td>
</tr>
<tr>
<td>Gendelman et al. 2017</td>
<td>Systemic lupus erythematosus is significantly associated with dementia.</td>
</tr>
<tr>
<td>Lee et al. 2017</td>
<td>We describe a rare case of multiple autoimmune syndrome presenting with psoriasis, vitiligo, and Crohn's disease, and suggest that tumor necrosis factor-alpha may be associated with the pathogenesis of all three conditions.</td>
</tr>
<tr>
<td>Yavne et al. 2017</td>
<td>Giant cell arteritis patients have a higher proportion of hypothyroidism in comparison with matched controls.</td>
</tr>
<tr>
<td>Nedelkopoulou et al. 2018</td>
<td>Inflammatory bowel disease and autoimmune liver disease are closely associated, the former often dictating progression of the latter.</td>
</tr>
<tr>
<td>Yavne et al. 2018</td>
<td>Giant cell arteritis patients had a significantly increased proportion of both Crohn's disease and ulcerative colitis in comparison with controls.</td>
</tr>
<tr>
<td>Iwata et al. 2018</td>
<td>Nontuberculous mycobacterial pulmonary disease is occasionally associated with rheumatoid arthritis.</td>
</tr>
<tr>
<td>Pierce 2018a</td>
<td>MAP's invasion of intestinal goblet cells may result in the initial pathologic lesion of idiopathic inflammatory bowel disease and sporadic colorectal cancer.</td>
</tr>
</tbody>
</table>
Box 4
Some triggers and conditions associated with the immune mediated diseases (selected references and their titles)

<table>
<thead>
<tr>
<th>Trigger</th>
<th>Reference</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>MURAMYLDIPEPTIDE AND COMPONENTS OF THE CELL WALL</td>
<td>Lederer 1971</td>
<td>The mycobacterial cell wall</td>
</tr>
<tr>
<td></td>
<td>Lederer et al. 1975</td>
<td>Cell walls of mycobacteria and related organisms; chemistry and immunostimulant properties</td>
</tr>
<tr>
<td></td>
<td>Kawabata et al. 1994</td>
<td>Guinea-pigs prepared with various bacteria and their components to induce a necrotic reaction provoked with muramyl dipeptide</td>
</tr>
<tr>
<td></td>
<td>Hugot et al. 2003a</td>
<td>Crohn's disease: the cold chain hypothesis</td>
</tr>
<tr>
<td></td>
<td>Girardin et al. 2003a</td>
<td>Nod1 detects a unique muramopeptide from gram-negative bacterial peptidoglycan</td>
</tr>
<tr>
<td></td>
<td>Kobayashi et al. 2005</td>
<td>Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract</td>
</tr>
<tr>
<td></td>
<td>van Heel et al. 2005a</td>
<td>Synergistic enhancement of Toll-like receptor responses by NOD1 activation</td>
</tr>
<tr>
<td></td>
<td>van Heel et al. 2005b</td>
<td>Synergy between TLR9 and NOD2 innate immune responses is lost in genetic Crohn's disease</td>
</tr>
<tr>
<td></td>
<td>Traub et al. 2006</td>
<td>Muramyl dipeptide and toll-like receptor sensitivity in NOD2-associated Crohn's disease</td>
</tr>
<tr>
<td></td>
<td>Marina-Garcia et al. 2009</td>
<td>Clathrin- and dynamin-dependent endocytic pathway regulates muramyl dipeptide internalization and NOD2 activation</td>
</tr>
<tr>
<td>MYCOBACTERIAL CELL-WALL LIPID MONOMYCOLOYL GLYCEROL</td>
<td>Martin-Bertelsen et al. 2016</td>
<td>Nano-self-assemblies based on synthetic analogues of mycobacterial monomycoloyl glycerol and DDA: Supramolecular structure and adjuvant efficacy</td>
</tr>
<tr>
<td>HEAT-SHOCK PROTEINS, MOLECULAR MIMICRY</td>
<td>Rajaiah and Moudgil 2009</td>
<td>Heat-shock proteins can promote as well as regulate autoimmunity</td>
</tr>
<tr>
<td></td>
<td>Dow 2011</td>
<td>Mycobacterium paratuberculosis and autism: is this a trigger?</td>
</tr>
<tr>
<td></td>
<td>Cossu et al. 2016</td>
<td>Humoral response against host-mimetic homologous epitopes of Mycobacterium avium subspecies paratuberculosis in Japanese multiple sclerosis patients</td>
</tr>
<tr>
<td></td>
<td>Bo et al. 2018b</td>
<td>Mycobacterium avium subspecies paratuberculosis and myelin basic protein specific epitopes are highly recognized by sera from patients with Neuromyelitis optica spectrum disorder</td>
</tr>
<tr>
<td>PROINFLAMMATORY CYTOKINES AND OTHER PROINFLAMMATORY MOLECULES</td>
<td>Hugot et al. 2003</td>
<td>Lessons to be learned from the NOD2 gene in Crohn's disease</td>
</tr>
<tr>
<td></td>
<td>Muller and Lamprecht 2008</td>
<td>In Crohn's disease IL-17 as well as IL-17 plus IFN-gamma producing CD4(+) T-cells are detected in peripheral blood and inflamed intestinal mucosa</td>
</tr>
<tr>
<td></td>
<td>Moudgil and Choubey 2011</td>
<td>Cytokines in autoimmunity: Role in induction, regulation, and treatment</td>
</tr>
<tr>
<td>PARTICIPATION OF GENE POLYMORPHISM</td>
<td>Hugot et al. 2003</td>
<td>Lessons to be learned from the NOD2 gene in Crohn's disease</td>
</tr>
<tr>
<td></td>
<td>Girardin et al. 2003b</td>
<td>Lessons from Nod2 studies: towards a link between Crohn's disease and bacterial sensing</td>
</tr>
<tr>
<td></td>
<td>Hugot et al. 2007</td>
<td>Prevalence of CARD15/NOD2 mutations in Caucasian healthy people</td>
</tr>
<tr>
<td></td>
<td>Loddo and Romano 2015</td>
<td>Inflammatory bowel disease: genetics, epigenetics, and pathogenesis</td>
</tr>
<tr>
<td></td>
<td>Chauhan et al. 2016</td>
<td>Mechanism of action of the tuberculosis and Crohn disease risk factor IRGM in autophagy</td>
</tr>
<tr>
<td></td>
<td>Li et al. 2016</td>
<td>Variants in TRIM22 that affect NOD2 signaling are associated with very-early-onset inflammatory bowel disease</td>
</tr>
<tr>
<td>GENERAL</td>
<td>Feehan and Gilroy 2019</td>
<td>Resolution of inflammation is driven by a complex set of mediators that regulate cellular events required to clear inflammatory cells from sites of infection or injury to restore tissue function. Recent studies suggest that resolution is not the end of innate mediated immune responses to infection/injury. There is further immunological activity occurring after the resolution cascade is complete that alters the immune physiology of tissues, redefining what was once termed estorative homeostasis as adapted homeostasis.</td>
</tr>
</tbody>
</table>
Box 5

**Actions of the triggers** (selected references and their titles)

<table>
<thead>
<tr>
<th>Trigger Description</th>
<th>Reference</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muropeptides trigger intracellular signaling cascades, leading to altered gene expression and activation of the immune response muramyldipeptide/components of the cell wall</td>
<td>Traub et al. 2006</td>
<td>MDP and other muropeptides - direct and synergistic effects on the immune system</td>
</tr>
<tr>
<td>Innate immune activation can trigger experimental spondyloarthritides in HLA-B27/Hu beta 2m transgenic rats</td>
<td>van Tok et al. 2017</td>
<td></td>
</tr>
<tr>
<td>Activation of CARD15 by components of the bacterial wall and further activation of NFkappaB, a proinflammatory molecule</td>
<td>Hugot et al. 2003b</td>
<td>Lessons to be learned from the NOD2 gene in Crohn's disease</td>
</tr>
<tr>
<td>Peptidoglycan-derived muramyl dipeptide (MDP) activates innate immunity via the host sensor NOD2.</td>
<td>Girardin et al. 2003a</td>
<td>NOD1 detects a unique muropeptide from gram-negative bacterial peptidoglycan</td>
</tr>
<tr>
<td>Lessons from Nod2 studies: towards a link between Crohn's disease and bacterial sensing</td>
<td>Girardin et al. 2003b</td>
<td></td>
</tr>
<tr>
<td>Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide</td>
<td>Coulombe et al. 2009</td>
<td></td>
</tr>
<tr>
<td>Crohn's disease and related inflammatory diseases: from many single hypotheses to one &quot;superhypothesis&quot;</td>
<td>Hruska and Pavlik 2014</td>
<td></td>
</tr>
<tr>
<td>Human NOD2 recognizes structurally unique muramyl dipeptides from Mycobacterium leprae</td>
<td>Schenk et al. 2016</td>
<td></td>
</tr>
<tr>
<td>Nod2: The intestinal gate keeper</td>
<td>Al Nabhanzi Z. et al. 2017</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium avium ssp paratuberculosis (MAP) can be reliably detected and cultured from peripheral blood of 100% of patients with recent onset Crohn's disease</td>
<td>Thiebaut et al. 2016</td>
<td></td>
</tr>
<tr>
<td>Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide</td>
<td>Hruska and Pavlik 2014</td>
<td></td>
</tr>
<tr>
<td>Crohn's disease-specific mutated NOD2/CARD15 causes an impaired epithelial barrier</td>
<td>Begue et al. 2006</td>
<td>Microbial induction of CARD15 expression in intestinal epithelial cells via toll-like receptor 5 triggers an antibacterial response loop</td>
</tr>
<tr>
<td>Failure of response to the penetration of bacteria and other bowel contents through the intestinal mucosal barrier</td>
<td>Marks and Segal 2008</td>
<td>Innate immunity in inflammatory bowel disease: a disease hypothesis</td>
</tr>
<tr>
<td>Muramyl dipeptide induced little TNF alpha or interleukin 1 beta, but strong interleukin-8 secretion</td>
<td>van Heel et al. 2005</td>
<td>Muramyl dipeptide and toll-like receptor sensitivity in NOD2-associated Crohn's disease</td>
</tr>
<tr>
<td>Immune dysregulation and a specific chronic enteric neuropathy with loss of mucosal integrity</td>
<td>Hermon-Taylor 2009</td>
<td>Mycobacterium avium subspecies paratuberculosis, Crohn's disease and the Doomsday scenario</td>
</tr>
<tr>
<td>The crosstalk between autophagy and inflammation</td>
<td>Netea-Maier et al. 2016</td>
<td>Modulation of inflammation by autophagy: Consequences for human disease</td>
</tr>
<tr>
<td>Innate immunity and pathogenesis</td>
<td>Marks and Segal 2008</td>
<td>Innate immunity in inflammatory bowel disease: a disease hypothesis</td>
</tr>
<tr>
<td>Muramyl dipeptide and toll-like receptor sensitivity in NOD2-associated Crohn's disease</td>
<td>van Lierop et al. 2009</td>
<td>Role of the innate immune system in the pathogenesis of inflammatory bowel disease</td>
</tr>
<tr>
<td>Crohn's disease: how innate immune deficiency may result in chronic inflammation</td>
<td>Jung and Hugot 2009</td>
<td>Inflammatory bowel diseases: the genetic revolution</td>
</tr>
<tr>
<td>Crohn's disease: how innate immune deficiency may result in chronic inflammation</td>
<td>Vinh and Behr 2013</td>
<td>Crohn's as an immune deficiency: from apparent paradox to evolving paradigm</td>
</tr>
<tr>
<td>Crohn's disease: how innate immune deficiency may result in chronic inflammation</td>
<td>Lalande and Behr 2013</td>
<td></td>
</tr>
<tr>
<td>Gamma delta T cells have both innate and adaptive characteristics and functions</td>
<td>Shanahan 2002</td>
<td>Crohn's disease</td>
</tr>
<tr>
<td>The contribution of y delta T cells to the pathogenesis of EAE and MS</td>
<td>Blink and Miller 2009</td>
<td></td>
</tr>
<tr>
<td>Mycobacteria in Crohn's disease: how innate immune deficiency may result in chronic inflammation</td>
<td>Lalande and Behr 2010</td>
<td></td>
</tr>
<tr>
<td>Molecular mimicry is the proposed etiopathology by which MAP stimulates autoantibodies associated with several chronic diseases</td>
<td>Dow 2012</td>
<td>M. paratuberculosis heat shock protein 65 and human diseases: Bridging infection and autoimmunity</td>
</tr>
<tr>
<td>Naser et al. 2013</td>
<td>Exploring the role of Mycobacterium avium subspecies paratuberculosis in the pathogenesis of type 1 diabetes mellitus: a pilot study</td>
<td></td>
</tr>
<tr>
<td>Shoda et al. 2016</td>
<td>Immune responses to mycobacterial heat shock protein 70 accompany self-reactivity to human BIP in rheumatoid arthritis</td>
<td></td>
</tr>
</tbody>
</table>

Cont.
Box 5 cont.

**MAP epitopes as antigens stimulating beta-cell autoimmunity (T1DM)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niegowska et al. 2016a</td>
<td>Seroreactivity against specific L5P antigen from Mycobacterium avium subsp paratuberculosis in children at risk for T1D</td>
</tr>
<tr>
<td>Niegowska et al. 2016b</td>
<td>Type 1 diabetes at-risk children highly recognize Mycobacterium avium subspecies paratuberculosis epitopes homologous to human Znt8 and proinsulin</td>
</tr>
<tr>
<td>Niegowska et al. 2016c</td>
<td>Recognition of ZnT8, proinsulin, and homologous MAP peptides in Sardinian children at risk of T1D precedes detection of classical islet antibodies</td>
</tr>
<tr>
<td>Niegowska et al. 2017</td>
<td>Increased seroreactivity to proinsulin and homologous mycobacterial peptides in latent autoimmune diabetes in adults</td>
</tr>
</tbody>
</table>

**Induction of Th1 and Th17 responses**

<table>
<thead>
<tr>
<th>Study</th>
<th>Summary</th>
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<tbody>
<tr>
<td>Martin-Bertelsen et al. 2016</td>
<td>Nano-self-assemblies based on synthetic analogues of mycobacterial monomycoloyl glycerol and DDA: Supramolecular structure and adjuvant efficacy</td>
</tr>
<tr>
<td>Review</td>
<td>Title</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Collins et al. 1984</td>
<td>Mycobacteria in water</td>
</tr>
<tr>
<td>Kazda 2000</td>
<td>Pathogenic mycobacteria in water - A guide to public health consequences, monitoring and management</td>
</tr>
<tr>
<td>Pedley et al. 2004</td>
<td>The Ecology of Mycobacteria</td>
</tr>
<tr>
<td>Kazdra et al. 2009</td>
<td>Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment</td>
</tr>
<tr>
<td>Falkingham 2009a</td>
<td>The biology of environmental mycobacteria</td>
</tr>
<tr>
<td>Falkingham 2009b</td>
<td>Environmental disease: environmental alteration and infectious disease</td>
</tr>
<tr>
<td>Kazdra et al. 2011</td>
<td>Mycobacteria in water, soil, plants and air: a review</td>
</tr>
<tr>
<td>Hruska and Kaevaska 2012</td>
<td>Epidemiology and ecology of opportunistic premise plumbing pathogens</td>
</tr>
<tr>
<td>Falkingham 2015a</td>
<td>Common features of opportunistic premise plumbing pathogens</td>
</tr>
<tr>
<td>Falkingham et al. 2015b</td>
<td>Mycobacterium avium ssp. paratuberculosis detection in animals, food, water and other sources or vehicles of human exposure: A scoping review of the existing evidence</td>
</tr>
<tr>
<td>Proctor and Hammes 2015</td>
<td>Drinking water microbiology - from measurement to management</td>
</tr>
<tr>
<td>Waddell et al. 2016a</td>
<td>Mycobacterium avium ssp. paratuberculosis: Global opinion survey of topic specialists</td>
</tr>
<tr>
<td>Waddell et al. 2016b</td>
<td>The potential public health impact of Mycobacterium avium ssp. paratuberculosis: Global opinion survey of topic specialists</td>
</tr>
<tr>
<td>Li et al. 2017</td>
<td>A systematic review of waterborne infections from nontuberculous mycobacteria in health care facility water systems</td>
</tr>
<tr>
<td>Esteban and Garcia-Coca 2018</td>
<td>Mycobacterium Biofilms</td>
</tr>
<tr>
<td>Haig et al. 2018</td>
<td>A High-Throughput Approach for Identification of Nontuberculous Mycobacteria in Drinking Water Reveals Relationship between Water Age and Mycobacterium avium</td>
</tr>
<tr>
<td>Hamilton et al. 2018</td>
<td>Assessment of Water Quality in Roof-Harvested Rainwater Barrels in Greater Philadelphia</td>
</tr>
<tr>
<td>Hamilton and Falkingham 2018</td>
<td>Aerosolization of Mycobacterium avium and Mycobacterium abscessus from a household ultrasonic humidifier</td>
</tr>
<tr>
<td>Proctor et al. 2018</td>
<td>Biofilms in shower hoses</td>
</tr>
</tbody>
</table>
### DRINKING AND TAP WATER

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location, frequency and maximal numbers of NTB found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fischeder et al. 1991</td>
<td>Germany, in 82% of samples, 4.5 x 10(5) cfu/l</td>
</tr>
<tr>
<td>Peters et al. 1995</td>
<td>Berlin (D), in 42% of samples, 70% of sites</td>
</tr>
<tr>
<td>Kubalek and Mysak 1996</td>
<td>Olomouc (CZ), M. gordonae in 20% of samples</td>
</tr>
<tr>
<td>Covert et al. 1999</td>
<td>USA, MB in 35% water and in 54% of ice samples</td>
</tr>
<tr>
<td>Aronson et al. 1999</td>
<td>USA, Los Angeles, MB recovered from 12/13 (92%), 45/55 (82%) homes, 31/31 (100%) commercial buildings and 15/15 (100%) of hospitals</td>
</tr>
<tr>
<td>Lalande et al. 2001</td>
<td>reconditioned fountain in a hospital</td>
</tr>
<tr>
<td>Falkinham et al. 2001</td>
<td>USA, MB cultured from 15% from 528 samples, up to 700,000 cfu/l</td>
</tr>
<tr>
<td>Chang et al. 2002</td>
<td>Taiwan, MB (PCR) in 10/49 samples</td>
</tr>
<tr>
<td>Le Dantec et al. 2002</td>
<td>France, MB cultured from 104/144 (72%) of samples, &gt;600 cfu/l</td>
</tr>
<tr>
<td>Torvinen et al. 2004</td>
<td>Finland, MB cultured from water 80%, 140 cfu/l, from deposits 3.9 x 10(5) cfu/g</td>
</tr>
<tr>
<td>Santos et al. 2005</td>
<td>Portugal, 92.5% samples from the Lisbon water distribution system</td>
</tr>
<tr>
<td>Hillborn et al. 2006</td>
<td>USA, point-of-use sites in public or commercial buildings are persistently colonized</td>
</tr>
<tr>
<td>Shin et al. 2007</td>
<td>Korea, 50/150 samples of tapwater from hospitals</td>
</tr>
<tr>
<td>Feazel et al. 2009</td>
<td>100-fold above background water contents</td>
</tr>
<tr>
<td>Hussein et al. 2009</td>
<td>MB cultured from 21/49 (43%) cold and 32/44 (73%) warm water samples</td>
</tr>
<tr>
<td>Beumer et al. 2010</td>
<td>USA, MAP DNA in 88% of drinking water samples, &lt;500 target copies per liter</td>
</tr>
<tr>
<td>Briancesco et al. 2010</td>
<td>Italy, MAP cultured from 62% of water samples, 300 cfu/l</td>
</tr>
<tr>
<td>Falkinham 2011</td>
<td>USA, MB in 109/394 (28%) samples</td>
</tr>
<tr>
<td>Marshall et al. 2011</td>
<td>Australia, M. lentiflavum cultured from 13/206 (6.3%) drinking water sites</td>
</tr>
<tr>
<td>Fernandez-Rendon et al. 2012</td>
<td>Czech Rep., MAP DNA in 76.7% of samples (reservoir and household sediments</td>
</tr>
<tr>
<td>Klanciova et al. 2013</td>
<td>Thomson et al. 2013 Australia, Brisbane, MB cultured from 76/189 (40.2%) sites of municipal water distribution system in summer and from 160/195 (82%) sites in winter</td>
</tr>
<tr>
<td>Rhodes et al. 2014</td>
<td>UK, MB DNA detected in 28/30 (93%) samples from showers, 10(10) CE/l</td>
</tr>
<tr>
<td>Makovcova et al. 2014</td>
<td>Czech Rep., MB cultured from 94/396 (23.7%) of water samples</td>
</tr>
<tr>
<td>Azadi et al. 2016</td>
<td>Iran, MB isolated from 71/148 (48%) samples of hospital water samples</td>
</tr>
<tr>
<td>Hamilton et al. 2016</td>
<td>MB by qPCR in 78% of 134 roof-harvested rainwater tank samples water</td>
</tr>
<tr>
<td>Lecuona et al. 2016</td>
<td>Canary Island, MB (PCR) in 47.4% of 135 household potable water samples</td>
</tr>
<tr>
<td>Kaevksa et al. 2016</td>
<td>Czech Rep., MB in wastewater treatment plant effluent, 3.8 x 10(4) CE/ml</td>
</tr>
<tr>
<td>King et al. 2016</td>
<td>USA, MB detected in 36% of treated water samples</td>
</tr>
<tr>
<td>Moat et al. 2016</td>
<td>MB in biofilms of domestic shower hoses</td>
</tr>
<tr>
<td>Roguet et al. 2016</td>
<td>France, MB in water from 2 lakes, more in surface microlayer than in water column</td>
</tr>
<tr>
<td>Amha et al. 2017</td>
<td>diversity of the genus Mycobacterium was screened in treated municipal wastewater by pyrosequencing</td>
</tr>
<tr>
<td>Mohajeri et al. 2017</td>
<td>Iran, Kermanshah, MB cultured and typed in 35/110 (32%) of drinking water samples</td>
</tr>
<tr>
<td>Guspiel et al. 2017</td>
<td>New children's hospital: 6 months after plumbing system was flushed and disinfected, an increased incidence of rapidly growing mycobacteria was detected in clinical cultures</td>
</tr>
<tr>
<td>Espeschit et al. 2018</td>
<td>Brazil, MAP was identified by culture and/or polymerase chain reaction in water samples for animal (50%) and human (30%) consumption from ten dairy cow farms</td>
</tr>
<tr>
<td>Lande et al. 2019</td>
<td>USA, Philadelphia: M. avium was recovered from 81.1% households and from 90.5% M. avium patient households</td>
</tr>
<tr>
<td>Richards et al. 2018</td>
<td>USA, Montana, Groundwater and municipal drinking water systems on the Crow Reservation in Montana can harbor potential bacterial pathogens (mycobacteria, Legionella, and Helicobacter</td>
</tr>
<tr>
<td>Proctor et al. 2018</td>
<td>Shower hoses offer an excellent bacterial growth environment in close proximity to a critical end-user exposure route within building drinking water plumbing.</td>
</tr>
</tbody>
</table>

### BOTTLED TABLE AND MINERAL WATER

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location, frequency and maximal numbers of NTB found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papapetropoulos et al. 1997</td>
<td>Greece, MB in 23/150 (15.6%) of bottled table water (&gt;1000 cfu/l in 4%)</td>
</tr>
<tr>
<td>Tatchou-Nyamsi-Konig et al. 2009</td>
<td>growth of MB on a PET bottle wall in an oligotrophic environment</td>
</tr>
<tr>
<td>Totaro et al. 2018</td>
<td>Literature data assert a potential risk from microorganisms colonizing wellsprings and mineral water bottling plants. NTM qPCR units were detected in 18% of samples (from 1 x 10(2) to 1 x 10(5) qPCR units/L). The presence of free living amoebae increases the importance of microbiological risk assessment in natural mineral waters despite the absence of cultivable bacteria</td>
</tr>
<tr>
<td>Falcone-Dias et al. 2015</td>
<td>Brazil, Sao Paulo, MB cultured from bottled mineral water</td>
</tr>
</tbody>
</table>

### SWIMMING POOLS

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location, frequency and maximal numbers of NTB found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ilivainen et al. 1999</td>
<td>Finland, MB in 5 of 7 (71%)</td>
</tr>
</tbody>
</table>

### RIVER AND LAKE WATER AND SEDIMENTS

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location, frequency and maximal numbers of NTB found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pickup et al. 2005</td>
<td>UK, River Taff, MAP IS900 in 31/96 (32.3%) samples collected for one year</td>
</tr>
<tr>
<td>Whan et al. 2005</td>
<td>Northern Ireland, MAP in 15/90 (8%) of untreated water samples</td>
</tr>
<tr>
<td>Pickup et al. 2006</td>
<td>UK, River Tiwi, MAP IS900 in 48/70 (68.8%) samples collected for nine months</td>
</tr>
<tr>
<td>Rhodes et al. 2014</td>
<td>UK, Cardiff, MAP DNA detected in 1/5 aerosol samples above the river Taff</td>
</tr>
<tr>
<td>Klanciova et al. 2014</td>
<td>Czech Rep., MB in pond sediments and plants</td>
</tr>
<tr>
<td>Cui et al. 2017</td>
<td>China, Beijing, M. avium one of the most abundant species in urban recreational water</td>
</tr>
</tbody>
</table>
Box 6B cont.

**FISH TANK WATER AND BIOFILMS**
Yanong et al. 2010

<table>
<thead>
<tr>
<th>CE</th>
<th>cell equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>cfu</td>
<td>colony forming units</td>
</tr>
<tr>
<td>MAP</td>
<td><em>M. avium</em> subsp.<em>paratuberculosis</em></td>
</tr>
<tr>
<td>MB</td>
<td>mycobacteria</td>
</tr>
</tbody>
</table>

high numbers of colonies recovered from filters and swabs, 21,000 cfu/filter
### Box 7

**Mycobacteria in foodstuffs** (selected references)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description and Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argueta et al. 2000</td>
<td>USA, MB cultured from 25/121 (20.6%) samples of food collected from supermarkets</td>
</tr>
<tr>
<td>Corti and Stephan 2002</td>
<td>Switzerland, MAP IS900 in 1384 (19.7%) of bulk-tank milk samples</td>
</tr>
<tr>
<td>Hruska et al. 2005</td>
<td>IS900, the specific fragments for <em>Mycobacterium avium</em> subsp. paratuberculosis (MAP) have been detected using PCR in 25 samples (49.0%) and fragment f57 by real time PCR in 18 samples (35.3%).</td>
</tr>
<tr>
<td>Ayele et al. 2005</td>
<td>Czech Rep., MAP cultured from 4/244 (1.6%) of commercially pasteurized cow's milk, and from 13/66 (19.7%) raw milk from subclinically infected dairy cows</td>
</tr>
<tr>
<td>Hruska et al. 2011</td>
<td>MAP in concentrations from 48 to 32 500 cells per gram of powdered infant milk were found in 18 out of 51 investigated samples (35%) in this study. More than 10 000 cells per gram were present in four samples (7.8%).</td>
</tr>
<tr>
<td>Gill et al. 2011</td>
<td>General data: Infected animals can shed Map in feces and milk, and the organism can become disseminated in tissues remote from the gut and its associated lymph nodes</td>
</tr>
<tr>
<td>Collins 2011</td>
<td>USA, food manufacturing practices fail to reliably kill MAP, actions to limit human exposure to MAP needed</td>
</tr>
<tr>
<td>Lorencova et al. 2013</td>
<td>Czech Rep., MB DNA in 14/23 (60.9%) of retailed and in 21/23 (91.3%) frozen fresh water fish</td>
</tr>
<tr>
<td>Cerna-Cortes et al. 2015</td>
<td>Mexico City, MB cultured from ready-to-eat salads (7/100) and sprouts (12/100)</td>
</tr>
<tr>
<td>Dziedzinska et al. 2016</td>
<td>Czech Rep., MB cultured from 17/178 (9.6%) samples of raw and frozen fruits and vegetables; MB using qPCR were present in almost all 178 samples, 10(4) CE/g</td>
</tr>
<tr>
<td>Botsaris et al. 2016</td>
<td>Viable MAP detected by phage-PCR in 9, by culture in 3 and by direct PCR in 7 of 32 samples of powdered infant formula (13%, 9% and 22%, respectively)</td>
</tr>
<tr>
<td>Acharya et al. 2017</td>
<td>Australia, MAP detected by qPCR in 6/122 (4.9%) samples of powdered infant formula, &lt; 10 MAP cells/1.5 g, no viable MAP in 122 samples</td>
</tr>
<tr>
<td>Sevilla et al. 2017</td>
<td>Spain, MB DNA in 15% of 138 dairy products and 2% of 119 meat products purchased from the main supermarket chains</td>
</tr>
<tr>
<td>Grant et al. 2017</td>
<td>USA, viable MAP in calf milk replacer (similar to powdered infant formula)</td>
</tr>
</tbody>
</table>
Non-tuberculous mycobacteria: A global public health threat

Box 8
Frequently presented hypothesis and their alternative interpretation

Non-tuberculous mycobacteria act as conventional pathogens
Live non-tuberculous mycobacteria may cause inflammation, but triggers released from dead mycobacteria pose evidently another type of risk.

*M. avium* subsp. *paratuberculosis* cannot be a cause of Crohn’s disease, because it is not found in the intestines of all affected patients, and it can be detected in the intestines of healthy individuals. Chronic inflammatory diseases, Crohn’s disease included, develop more often in hosts genetically predisposed or primed earlier, hence, mycobacteria or their DNA can be found both in groups with clinical manifestations and in healthy controls. On the other hand, mycobacteria need not be cultured in all clinical cases.

Breast milk protects against immuno-mediated inflammatory and autoimmune diseases
When the epidemiological studies use well selected cohorts, it is found that formula fed infants get sick more often. Milk of cows with subclinical paratuberculosis often contains large amounts of mycobacteria, releasing triggers of immune-mediated disorders. These triggers expose children at the critical and vulnerable period of maturation of the immune system. The acquired sensitization may manifest itself in disease with considerable delay, or even in adulthood. No evidence is available that breast milk could eliminate these triggers. However, it makes more sense that the association between breastfeeding and the reduced incidence of chronic inflammatory diseases might be related to no or limited exposure to triggers, if powered infant milk and tap water are not ingested by the babies.

Hygienic hypothesis assumes insufficient environmental microbial exposure of children that would presumably downmodulate inflammatory and autoimmune responses. In this respect correlation of the incidence of these diseases with access to hot water in urban areas, and generally improved hygiene, are often mentioned.

Mycobacteria are present in tap water from communal sources and from hot water boilers. This water increases opportunities of exposure of children not only by its use in preparation of formulas, but also during bathing and showering. Increased exposure is also during “baby swimming”. Apart from the oral exposure, inhalation of aerosol exposure during showering or in indoors swimming pools can also occur.

Cold chain hypothesis proposes an association between Crohn’s disease and the use of refrigerators and freezers
While it is possible that some psychrophilic bacteria participate also as triggers of chronic inflammatory diseases, their impact is obviously overestimated. More likely, household refrigeration, increasing incidence of bovine paratuberculosis, human migration to urban setting, use of tap water for cooking, bathing and showering, and swimming in indoors swimming pools act in concert.

Crohn’s disease is basically a genetic disease, activated by unknown environmental factor/s, stress or by another disease
Dramatic increase of Crohn’s disease in different countries and continents, in people of various ethnic and genetic backgrounds, has proven that the phenomenon is undoubtedly caused by the triggers that had spread globally during a relatively short period. This is incompatible with the proposed role of host genetics as the primary cause of these disorders. Regarding the environmental co-factors, currently no other factors, except mycobacteria, are known to have the necessary prerequisite to affect such geographically and genetically diverse populations in such a short time abd intensity. Bovine paratuberculosis, baby formula and tap water from the municipal distribution system have the potential to do it.

The increase of chronic inflammatory diseases is simply a result of the availability of more sensitive diagnostic procedures
The increase of the incidence of inflammatory diseases has been reported concurrently in countries with significant differences in the level of diagnostic capabilities. Hence, the sensitivity of diagnosis can’t be the determining factor.

Crohn’s disease is caused by other concurrent diseases
Polymorbidity is common in chronic inflammatory and autoimmune diseases (Box 3). This is compatible with the exposure to immune-modulatory triggers leading to damage of various target tissues. All different disease entities with different clinical symptoms, but very likely with similar pathogenesis, are outcomes of the consequences of the mycobacterial triggers, rather than a result of induction by different disease entities.
### Box 9
**Recommended measures to prevent human exposure to nontuberculous mycobacteria**

<table>
<thead>
<tr>
<th>A</th>
<th>Regulatory measure urgently needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obligatory testing of powdered infant milk for MAP</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Data collection by the national veterinary inspection should be used for evaluation of pathological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paratuberculosis in slaughtered cattle more than 30 months old (farm code, the number and age of the animal)</td>
<td></td>
</tr>
<tr>
<td>Mycobacterioses in slaughtered swine (farm code, the number of affected heads and livers in animals slaughtered in one day)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>Voluntary testing by the commercial producers, accompanied by “MAP free” declaration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy farms: MAP in bulk-tank milk continuously tested; if MAP count elevates, analyses of the pooled milk samples are followed by identification and immediate culling of the shedder</td>
<td></td>
</tr>
<tr>
<td>Milk processing industries: MAP in truck-tanker milk</td>
<td></td>
</tr>
<tr>
<td>Ice cream producers: MAP in milk purchased</td>
<td></td>
</tr>
<tr>
<td>Minced beef producers: MAP in final products</td>
<td></td>
</tr>
<tr>
<td>Bottlers of table and mineral water and beverages: M. spp. in water reservoirs (before processing)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>On-demand commercial testing for mycobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP in breast milk of mothers suffering from Crohn’s disease (breast feeding interruption is recommended after positive testing)</td>
<td></td>
</tr>
<tr>
<td>M. spp. in household tap water (the use of filtration unit after positive testing is recommended *)</td>
<td></td>
</tr>
<tr>
<td>M. spp. in swimming pools water and in air of indoor pools (clients should be informed about control)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E</th>
<th>Knowledge dissemination regarding the risks of the triggers **</th>
</tr>
</thead>
<tbody>
<tr>
<td>A booklet: General information *)</td>
<td></td>
</tr>
<tr>
<td>Leaflets:</td>
<td></td>
</tr>
<tr>
<td>Why Infant dry milk not tested for MAP should be avoided *)</td>
<td></td>
</tr>
<tr>
<td>Why household water filtration and exchange of shower heads is recommended *)</td>
<td></td>
</tr>
<tr>
<td>Why baby swimming in pools, not in the long term tested, should be avoided *)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F</th>
<th>Non-governmental support expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening for MAP and M. spp. in foodstuffs and water, followed by publication of the results (e.g. type and percentage of samples with more than 1000 cells per millilitre or gram)</td>
<td></td>
</tr>
<tr>
<td>Research grants for development of rapid and inexpensive analytical methods for M. spp. and MAP</td>
<td></td>
</tr>
<tr>
<td>Grants for establishment of laboratories for water and foodstuffs analysis</td>
<td></td>
</tr>
<tr>
<td>Grants for establishment and maintenance of databases and for evaluation of the data</td>
<td></td>
</tr>
<tr>
<td>Grants for research on molecular pathogenesis and biological treatment</td>
<td></td>
</tr>
</tbody>
</table>

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MAP: *Mycobacterium avium* subsp. *paratuberculosis*

M. spp: *Mycobacterium* spp.

*) mainly in families with infants up to 1 year age of in families with patients suffering from any immune mediated chronic inflammatory or autoimmune disease

**) with participation of family physicians, specialists and the general public.

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Box 10
Call for Action

- Relevant programs of high schools and universities should provide information regarding the risks associated with mycobacterial triggers of chronic immune mediated and autoimmune diseases
- Food and water for preterm born babies, and those babies that are not breast fed for other reasons, must be controlled for absence of mycobacteria above permitted counts
- Interest of consumers of milk, ice cream, and beef hamburgers, in products with limited mycobacterial counts, will provide the necessary pressure on the producers to reduce the public exposure to mycobacterial triggers
- Food producers should voluntarily control relevant ingredients, particularly milk and ground beef, and they should be required to include the results on labels
- Operators of public swimming pools, wellness centers and hydrotherapy facilities should also contribute to reduction of exposure

More information at www.centaur.vri.cz or www.upvav.cz
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THE HIGHEST WARNING

RURAL ANENCEPHALY CLUSTER: 48 BABIES WITH ANENCEPHALY, 1990-2017

The rural Yakima Valley community in central Washington in the United States has suffered from a long-running tragedy of a cluster of babies with anencephaly, with an initial reported rate of over 60 times the national average. The mothers of the majority of affected babies lived in an area where an aggregation of massive dairy herds known as concentrated animal feeding operations (CAFOs) or factory farms is located. Mycobacterium avium subspecies paratuberculosis (MAP), the cause of a chronic gastrointestinal disease in domestic livestock, is endemic in United States dairy cattle, affecting 100% of herds with greater than 200 cows. MAP is recognized as a probable zoonosis, involved in the pathogenesis of Crohn's disease, other autoimmune disorders and neurologic diseases including autism, multiple sclerosis and Parkinson's disease. MAP is present in an infected dairy cow's feces at the rate of over one million organisms in a few drops of manure, and the average adult cow produces 12-14 gallons of manure per day. Manure from dairy CAFOs is stored in manure lagoons the size of football fields and applied to agricultural fields at the rate of 1000 gallons per acre. MAP persists in manure and the environment for an indefinite time and is readily aerosolized. The inhalation of pathogen-contaminated feces or manure is a major route of transmission of zoonotic pathogens from infected animals to humans. The inhalation of aerosolized MAP-contaminated manure by women in the first four weeks of pregnancy, and intrauterine transmission to the embryo, may be responsible for the development of anencephaly in the fetus (Pierce 2019).

From: <promed@promedmail.org>
Date: po 4. 2. 2019 v 17:26
Subject: PRO/EDR> Central venous catheter infection - USA: (AR) new Mycobacterium sp, saline flush
To: <promed-post@promedmail.org>, <promed-edr-post@promedmail.org>

Three of 52 patients probably died due to Mycobacterium in a saline solution used to flush indwelling central venous catheters

CENTRAL VENOUS CATHETER INFECTION - USA: (ARKANSAS) NEW MYCOBACTERIUM SPECIES FVL-2018-32, SALINE FLUSH, 2018

A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the International Society for Infectious Diseases
http://www.isid.org

Date: Mon 4 Feb 2019 4:30 AM CST
Source: Arkansas Democrat Gazette [edited]

[A patient] used to travel once a week from his home in Dumas for chemotherapy treatments at Arkansas Cancer Institute. [The 56-year-old patient] had been going to the Pine Bluff clinic for about a year for treatment of his multiple myeloma, which is a cancer of the plasma cells. It had been going “all right,” he said. He’d gotten to know the other patients a little, and it was more convenient than going all the way to Little Rock [Arkansas]. Until August [2018], that is, when something went wrong.

The trouble began with an unusually bad reaction to a treatment, which put him in bed for a few days with chills and stomach pain. He told a nurse about it when he went back for his next treatment -- after which he was taken away in an ambulance. “I felt like I was dying,” he wrote in a statement about the incident. “My whole body was in pain.” He wrote that he was hot, cold, throwing up, and had “severe
weakness." [The patient] was transported to Jefferson Regional Medical Center, where he spent 7 days fighting a mysterious infection that he says left him bedridden for 2 weeks after he got out of the hospital. He later learned that he was one of more than 50 people, mostly cancer patients, who were infected in a bacterial outbreak at Arkansas Cancer Institute.

Months have passed since the peak of that outbreak, during which the Arkansas Department of Health, the federal Centers for Disease Control and Prevention [CDC], the clinic and nearby Jefferson Regional Medical Center engaged in rapid-fire correspondence as the health organizations worked to respond to the situation, emails and meeting and call records show. Those records were obtained by the Arkansas Democrat-Gazette through an Arkansas Freedom of Information Act request. More recently, the state Health Department's investigation of the outbreak, which identified a new species of bacteria, has moved into an academic phase that could lead to publication of a scientific article.

Alarm bells didn't go off at the Health Department initially. Health officials at first suspected that a Jefferson Regional Medical Center laboratory's discovery of an unusual bacterial infection was an error. _Mycobacterium parafortuitum_ is rare and wasn't thought to cause disease in people, state epidemiologist Dr Dirk Haselow said in a recent interview. "It was somewhat discounted, because no one knew what to do with it," Haselow said. "As we started seeing more infections, we realized something was wrong." On [5 Sep 2018], Jefferson Regional Medical Center staff members reported to the Health Department a cluster of infections among patients that had appeared during the last 2 weeks of August [2018]. The reported constellation raised the number of cultures showing the unusual bacteria to 13.

Within days of that report, investigators were able to trace the outbreak to Arkansas Cancer Institute and identify its source: saline flushes, which the clinic used on patients who had chemotherapy ports, devices implanted under the skin that help in giving medications. In a [6 Sep 2018] email to other officials, Kelley Garner, program coordinator and epidemiology supervisor for the state's health care-associated infections program, wrote: "Yesterday [5 Sep 2018], [Arkansas Cancer Institute] requested blood cultures be performed on 11 patients who were having fever, chills, and nausea and as such missed their oncology appointments. We spoke with the medical director of the clinic, who reported that patients would come into the clinic ok, but after 30 mins after accessing the port would become severely ill and were taken to the Emergency Department at the acute care hospital at which point some were hospitalized," Garner wrote.

The next day, [Fri 7 Sep 2018], a Health Department team conducted site visits at Jefferson Regional Medical Center and Arkansas Cancer Institute. A written recap of those visits describes, "a few non-standard practices observed at [Arkansas Cancer Institute]," including the saline flushes. According to Health Department documents and correspondence, the cancer clinic was preparing saline flushes by filling syringes from a large bag of saline fluid and storing them for up to 7 days for later use, rather than using factory-sealed, single-use syringes pre-filled with saline.

On [10 Sep 2018], an infection-control nurse at Jefferson Regional Medical Center wrote to the Health Department to say that the hospital's lab had tested saline bags and syringes from Arkansas Cancer Institute. She wrote: "4 of the 5 syringes showed growth very similar to what we have seen from the blood cultures, leading [the lab] to believe that it is the mycobacterium."

On [13 Sep 2018], a letter of formal recommendations to control the outbreak went out from the Health Department to Arkansas Cancer Institute. Topping the list of recommendations was an advisement to "replace saline flushes drawn from a larger bag of saline with sealed, pre-drawn saline flushes. Discontinue future use of locally-filled flushes," 2 officials wrote. "This appears to have taken place on [Tue 11 Sep 2018], but we would like confirmation from you that no more homemade flushes are being used."

In an email exchange with Arkansas Cancer Institute administrator Michael Legate on [18 Sep 2018], Arkansas-based CDC epidemic intelligence service officer Dr Sarah Labuda explained that neither the federal agency's nor the Association for Professionals in Infection Control and Epidemiology's guidelines recommend pre-drawing of saline for flushes, and that syringes of that type would be considered good for only 24 hours in a pharmacy. Officials continued to study the outbreak in subsequent days and weeks, looping in infectious-disease specialists from the University of Arkansas for Medical Sciences and the CDC.
A new wrinkle appeared when a CDC analysis clarified that the bacterium was not _M. parafortuitum_, but a new, related organism temporarily dubbed _Mycobacterium_ sp. FVL 201832.

As the investigation into the cause of the infections continued, Arkansas Cancer Institute worked to contact and collect samples from more than 150 patients thought to have been exposed to the bacteria, though only about a third of those ended up testing positive for the infection.

Legate and clinic president Dr Omar Atiq declined interview requests for this article, referring questions to an attorney. Records show that the clinic’s formal communications with affected patients did not always make clear the source of the infection. A conference call invitation set for [14 Sep 2018] between Jefferson Regional Medical Center, the Health Department and Arkansas Cancer Institute calls for “each organization to have people on the phone that can discuss risk management strategies and make high level communication decisions.”

A patient notification letter from the clinic dated [20 Sep 2018] went through several revisions and ultimately did not mention saline flushes, citing work with state and federal health regulators “to determine the cause of the infection and to put in place steps to prevent it in the future.” An undated, 11-step script for calls to summon patients for screening (“How are you doing today? Have you had any fever or shakes lately?”) also does not refer to the investigation into the cause of the infection.

Health Department officials had been investigating the outbreak for almost 2 months by the time the 1st news report about it, a segment on Little Rock television station KARK, appeared around Halloween [31 Oct 2018]. By that time, 3 patients who had been infected with the bacteria had died, though their deaths could not be explicitly linked to the infection.

In interviews and statements at that time, the clinic began acknowledging the saline flush issue to media. “While the specific cause of contamination is still unknown, the source has been determined to be the saline flushes that are no longer in use. The process Arkansas Cancer Institute used for 27 years without any complications has been replaced with a different process to prevent future infections,” an October [2018] news release said.

[Byline: Kat Stromquist]

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Communicated by:
ProMED-mail from HealthMap Alerts
<promed@promedmail.org>

[A previous ProMED-mail post reported on this outbreak at a single cancer therapy facility (we now learn was the Arkansas Cancer Institute in Pine Bluff, Arkansas), where 52 patients with indwelling central venous access catheters undergoing cancer chemotherapy developed a bloodstream infection due to the same bacterium not previously known to be a pathogen; 3 of the patients died. The bacterium, initially thought erroneously to be _Mycobacterium parafortuitum_, is now identified as _Mycobacterium_ sp. FVL 201832. This organism has been traced to a saline solution used to flush indwelling central venous catheters; syringes were filled with saline from a large bag of saline fluid and stored for up to 7 days for later use, rather than using factory-sealed, single-use syringes pre-filled with saline.

Cancer treatment frequently requires access to the bloodstream on an ongoing basis for administration of fluids, cancer chemotherapeutic drugs, blood transfusions, and antibiotics, which requires an indwelling catheter or port that has been tunneled under the skin and then inserted into a large, central vein. These indwelling, tunneled central venous catheters must be flushed frequently with either heparin or saline solution to keep them clear of blood and prevent clotting.

Pine Bluff, Arkansas, with a population of about 50,000 residents, is a city about 45 miles (72 km) south of Little Rock, the state capital (<https://en.wikipedia.org/wiki/Pine_Bluff,_Arkansas>); a map showing the location of Pine Bluff can be found at https://www.google.com/maps/dir/Little+Rock,+AR/Pine+Bluff,+AR

HealthMap/ProMED map of Arkansas, United States: http://healthmap.org/promed/p/37474

[See Also:
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