Mycobacteria in water, soil, plants and air: a review

K. Hruska, M. Kaevska

Veterinary Research Institute, Brno, Czech Republic

ABSTRACT: Amazingly, despite the 24 143 papers on mycobacteria, indexed in the Web of Science database during the last six years, published by 67 008 authors from 13 128 organizations located in 166 countries or territories, internationally accepted legal directives on how to control the public health risk associated with environmental mycobacteria have yet to be developed. Mycobacteria are human and animal pathogens, causing not only tuberculosis and leprosy, but mycobacterioses of skin, soft tissues and lung. Due to their cell wall composition and their adaptability mycobacteria can survive in different habitats for years. Their immunomodulatory ability has been recognised for more than 50 years and hundreds of papers published during the last two decades have demonstrated that small chemical products derived from mycobacterial cells participate in inflammatory pathways involved the pathogenesis of important human diseases like Crohn's disease, asthma, type 1 diabetes mellitus, psoriasis, arthrosis, Blau syndrom, sarcoidosis, autism etc. Mycobacteria can influence inflammatory pathways not only as live organisms, but also by means of components derived from dead cells. Pasteurisation or cooking does not affect this ability. Hence, how many mycobacterial cells are ingested, what factors play a role concurrently, and how long the harmful effect persists become important questions. This paper presents only a short review based on selected papers about mycobacteria in water, soil, plants and air with the aim of attracting attention to this significant global problem and of making the first steps towards protection of people. Selected bibliographic references of published data from 2007 to 2012 are presented in easy-to-navigate tables.

Keywords: *Mycobacterium*; water; soil; plant; vegetables; air; biofilm; sediment; determination; zoonoses; food safety

List of abbreviations

CFU = colony forming units; **DGGE** = denaturation gradient gel electrophoresis; **IMS** = immuno-magnetic separation; **IS** = insertion sequence; **ITS** = intergenic transcribed spacer; **MAC** = *Mycobacterium avium* complex; **MAP/Map** = *Mycobacterium avium* subsp. *paratuberculosis;* **MTC** = *Mycobacterium tuberculosis* complex; **MWF** = metal working fluids; **nPCR** = nested PCR; **NTM** = non-tuberculous mycobacteria; **PCR** = polymerase chain reaction; **PPM** = potentially pathogenic mycobacteria; **qPCR** = quantitative real time PCR; **RFLP** = restriction fragment length polymorphism

Contents

Tables

Supported by the Ministry of Education, Youth and Sports, Czech Republic (AdmireVet; Grant No. CZ 1.05/2.1.00/01.0006-ED 0006/01/01) and the Ministry of Agriculture of the Czech Republic (Grant No. MZE 0002716202).

1. Introduction

Potentially pathogenic mycobacteria, also referred to as non-tuberculous mycobacteria, are known pathogens of animals and can cause diseases also in humans, especially in immunocompromised persons. Mycobacterioses differ depending upon the species and hosts involved and upon ways of infection, and may present as pulmonary, skin or soft tissue lesions (Wagner and Young 2004; Griffith et al. 2007; Jarzembowski and Young 2008). Some hosts can develop a generalised mycobacteriosis. The immunomodulatory potential of mycobacteria is in the spotlight as a consequence of the composition of the mycobacterial cell wall. Bacterial cell wall components have a high immunomodulatory potential. Dead mycobacteria have been used in the complete Freund adjuvans for more than 50 years. Muramyldipeptides were discovered as the minimal structures responsible for the improved reaction to antigens (Ellouz et al. 1974; Traub et al. 2006; Coulombe et al. 2009). This ability has been proven by experiments which showed that synthetic molecules have the same effects. Coulombe et al. (2009) reported that *N*-glycolyl MDP has a greater NOD2-stimulating activity than *N*-acetyl MDP, consistent with the historical observation attributing exceptional immunogenic activity to mycobacterial cells. *N*-glycolyl MDP is produced by degradation of mycobacterial peptidoglycans. The importance of a lipid antigen in the molecular pathogenesis of ruminant paratuberculosis and human inflammatory bowel diseases are subjects of recently published data (Momotani et al. 2012; Mori and De Libero 2012). It is evident that under specific conditions mycobacteria can be zoonotic or environmental pathogens for humans and an agents that participate in foodborne autoimmune or autoinflammatory human diseases. Crohn's disease, type 1 diabetes mellitus, psoriasis, multiple sclerosis, asthma, arthrosis, autism, Blau syndrome and sarcoidosis are the most frequently mentioned diseases with respect to bacterial triggers. Nevertheless, mycobacteria are not unique in their ability to act as bacterial triggers. Some known pathogens are possible sources of components that trigger inflammatory processes as a consequence of their intensive replication during the primary infection. Non-tuberculous mycobacteria were not a focus of interest for a long time because their participation in pathogenesis need not follow the Koch's postulates completely or unequivocally. Those who cannot accept the term "pathogen" for cells unable to replicate can describe the harmful microorganism as an immunomodulator, bacterial trigger or allergen-like factor.

The difficult diagnosis by culture of slow or non-growing mycobacteria has also contributed to an underestimation of mycobacteria as a public health risk. However, the current understanding of the molecular pathogenesis of autoimmune or allergic diseases, the recognition of genetic or epigenetic components in the pathogenesis of many diseases, the expanding use of molecular biology in research on mycobacteria, and the rapidly growing number of publications and data on the distribution of mycobacteria in the environment, namely in water, air and soil, have all contributed to the evolution of a new understanding of the role of mycobacteria. *Mycobacterium avium* subsp. *paratuberculosis* plays an important role in this paradigm. Paratuberculosis (Johne's disease) in cattle and sheep was for a long time considered unimportant both for animal breeding and food safety and remained uncontrolled with regard to milk and meat contamination and in animal trade and mobility. Thus, the herd incidence increased in countries with intensive cattle and sheep industry enormously, up to an estimated 70% to 90% of all herds. The infectious agent is very resistant, can survive for a long time in water and liquid dung and can survive and replicate in amoebae. The number of mycobacteria in faeces can reach 10^8 per gram, in milk and meat 10^4 per gram and in water 10^4 per ml. It is therefore evident that humans are not absolutely protected against exposure to mycobacteria and their components. The important factors in this exposure are the numbers of mycobacteria and the age and dispositions of the hosts. Obviously some sensitisation can occur inapparently and an interval of many years can exist between the first contact and development of the clinical form of disease. The unknown sources of mycobacteria and the creeping development of health problems make the understanding of possible consequences rather difficult.

The risk of direct transmission of live tuberculous mycobacteria between humans or animals takes the form of droplet infection in open forms of pulmonary tuberculosis (*M. tuberculosis*, *M. bovis* and *M. caprae*). The risk of contracting human tuberculosis increases with the time of sharing a small room with the mycobacterial shedder, e.g. in a class room, pub or nearest neighbour during intercon-

tinental flight. Non-tuberculous mycobacteria can be transmitted in raw milk or insufficiently heat-treated meat. Water and soil are frequent sources of mycobacterial infections either in the form of direct contacts for aquarists or gardeners or by means of aerosols in showers or indoor swimming pools. Water in hospitals and dental units or metal working fluids (Falkinham 1996, 2009a,b; Primm et al. 2004; van Ingen et al. 2009) have been a recent focus of attention. A recently published book was devoted to the ecology of mycobacteria and their impact on human and animal health (Kazda et al. 2009).

Readers should pay special attention to biofilms, aerosols, resistance to disinfectants, and mycobacterioses as professional diseases. Several specific phenomenona are typical for mycobacteria: Isolation of mycobacteria from the environment is hampered by their slow or limited growth *in vitro*.

Mycobacteria are frequently overgrown by other microorganisms present in the sample. To overcome this obstacle, different decontamination methods have proven to be effective, although with negative consequences for the sensitivity of the culture.

As is the case for other microorganisms mycobacteria can be detected and identified directly and quantitatively using different molecular methods.

Mycobacteria survive for a long time in the environment and can be found in great numbers in rivers that collect water from pastures, in river and lake sediments and in soil.

The hydrophobic character of the mycobacterial cell wall is responsible for their easy aerosolisation over swimming pools and river water, by sea breakers as well as in the shower bath.

1.1. The database used

The publications on mycobacteria were retrieved from the Web of Science® (Thomson Reuters) database using the search profiles described in Table 1, and directed to water, soil, plants and air. The numbers of results retrieved from the complete database Science Citation Index Expanded (timespan: 1945 to 2012) are mentioned only to demonstrate the huge number of sources available. We acknowledge that the key words used for searching are too general and also that many inappropriate papers have been omitted. The most important papers published from 2007 to 2012 have been selected for this review using abstracts or full papers. However, certain important references published before 2007 were also included. The utility "Analyse results" was used for the selection of review articles.

1.2. The format used

The review follows the format of our recently published reviews (Eyer and Hruska 2012; Hruska and Franek 2012). Selected papers are presented in tables with the basic key words in the first column, full or shortened abstract in the second column and the link to the List of References in the third column. This format is easy-to-navigate, supplies readers with more information and minimises the misinterpretation of papers through a subjective wording by the authors of the review. The text in the tables contains several format imperfections, which exist in the Web of Science® database and are caused by transmission and copying of data between various information sources.

2. Selected review articles (Table 2)

Many reviews are devoted to mycobacteria in the environment, namely in water (Falkinham 1996, 2002, 2009a,b, 2010; Whiley et al. 2012). Attention has been also paid to non-tuberculous mycobacteria that cause human disease (Set and Shastri 2011), and guidelines on how to control its transmission is also an area of focus. *M. avium* complex members and non-tuberculous mycobacteria cause pulmonary or lung diseases and their diagnosis and treatment have been reviewed by Kasperbauer and Daley (2008) and McGrath et al. (2010). Catheter-related infections (Adekambi 2009) and nosocomial outbreaks of mycobacterial infections (Garcia-Martos and Garcia-Agudo 2012) are other fields of interest. Dental units and their waterlines are reviewed by Szymanska et al. (2008) and Szymanska and Sitkowska (2012). *M. avium* subsp. *paratuberculosis* which causes paratuberculosis in animals, namely cattle and small ruminants, should be regarded also as a bacterial trigger for Crohn's disease (Carbone et al. 2005), and as a food safety risk factor (Skovgaard 2007; Gill et al. 2011). A small number of reviews present new methods for the detection of mycobacteria using biosensors (Nayak et al. 2009), or fluorescence in-situ hybridisation (Cerqueira et al. 2008).

3. Mycobacteria in water (Table 3)

Mycobacteria are present in most natural waters and piped water supplies. The main features of mycobacteria as a public health risk have been characterized already in 1984 as evident from the sub-headings of a review published by Collins et al. (1984):

Resistance of mycobacteria to chlorination
Access, persistence and colonization in piped supplies
Is water the natural habitat of free-living mycobacteria?
Water as a vector for mycobacterial infections
Immune response to environmental mycobacteria
Mycobacteria as indicators of pollution

Most of the bacteria in drinking water distribution systems are associated with biofilms. *M. avium* has been described to survive in biofilms for more than two to four weeks in culturable forms. Lehtola et al. (2006) studied the survival of *M. avium* in drinking water biofilms after the spiking of the water using fluorescent *in situ* hybridization (FISH) with an rRNA-targeted PNA probe. They concluded that culture examination seriously underestimates the occurrence of *M. avium* in biofilms and water. The study performed by Lehtola et al. (2007) clearly proved that pathogenic bacteria entering water distribution systems can survive in biofilms for at least several weeks, even under conditions of high-shear turbulent flow, and may be a risk to water consumers. In order to understand microbial communities in drinking water biofilms, Liu et al. (2012) sequenced 16S rRNA in three faucet biofilms using 454-pyrosequencing. They found that the abundance of *Legionella* and *Mycobacterium* was affected by the residual chlorine in the water.

Most of the non-tuberculous mycobacteria not only survive in water for a long time, but can grow there as well (Kazda et al. 2009). Water, regardless of origin and quality, can be contaminated by mycobacteria and, under specific conditions, can jeopardise the users (Falkinham III 2003; Falkinham 2009a,b). Pickup et al. (2005) reported that *M. avium* subsp. *paratuberculosis* can be present in high concentrations in the river water in the catchments area of pastures. Data presented in this paper bring evidence of a higher incidence of Crohn's disease in districts bordering rivers. Exposure to waters whose catchments include heavily grazed pastures was associated with conspicuous clusters of Crohn's disease. The first of these involved a rural community of about 2000 people in England, in which 12 people developed Crohn's disease between 1960 and 1983 (Allan et al. 1986). The village, which had its own water supply from local springs, lay in a hollow surrounded by upland pastures grazed by cattle in which clinical paratuberculosis (Johne's disease) was evident. A further suspicious cluster of seven cases of Crohn's disease amongst 285 graduates of the Mankato West High School class of 1980 was reported by Van Kruiningen and Freda (2001). All seven students had been swimming in local ponds and lakes.

A novel study into the diversity of mycobacteria with regard to the physical and chemical characteristics of the water in a coastal lagoon was performed by Jacobs et al. (2009). The abundance of mycobacteria was high; their presence was detected in 96% of the stations sampled. There was a positive correlation between the number of mycobacteria and elevated temperatures, turbidity, nitrogen and phosphorus components, whereas negative correlations existed for the dissolved oxygen content, depth and salinity.

A high hydrophobicity of mycobacteria leads to their enrichment in natural ejected droplets and transfer from water to air (Blanchard and Syzdek 1970). The enrichment factor for transfer of mycobacteria from water to air ranged from 68 to 15 000 in *M. intracellulare* (Parker et al. 1983). Obviously, communal water poses a risk. Two case control epidemiological studies carried out independently in the United Kingdom each unexpectedly identified the availability of fixed hot water supplies in the early childhood home as a significant risk factor for the subsequent development of Crohn's disease (Gent et al. 1994; Duggan et al. 1998). An urban cluster of Crohn's disease possibly linked to fully treated drinking water has been described by Pierce (2009). Mycobacteria were found in 15% of bottled water in Greece, in 4% of cases at a concentration greater than 10^3 CFU/l (Papapetropoulou et al. 1997).

4. Mycobacteria in soil (Table 4)

M. avium subsp. *paratuberculosis* present on pastures or barns is the most common non-tuberculous mycobacteria detected in soil (Eisenberg et al. 2009; Pribylova et al. 2011). Soil is easily contaminated by fertilisation with manure or liquid dung or by water contaminated by animal faeces.

Survival of mycobacteria in soil for as long as one year or longer is associated with amoebae or other protozoa or with the shedding of mycobacteria by wild ruminants, wild board, hairs, rabbits and other animals. Mycobacteria from river sediments can be transferred to soil by floods or by the ejection of micro droplets forming aerosols. Any of these transfer mechanisms can explain the finding of *M. avium* subsp. *paratuberculosis* in amoebae from fields not used for grazing. (White et al. 2010).

Other *M. avium* subspecies were studied to determine sources of infection for patients (Kaevska et al. 2011). A small number of studies were concerned with the detection of *M. bovis* (Young et al. 2005) or *M. leprae* in soil. The association was observed between endemicity of leprosy in Africa and India, the distribution of mycobacteria in soil and water with respect to dry or wet season and geographical distribution. The mycobacterial isolates from soil were identified as *M. fortuitum*, whereas the uncultured sequences obtained from soil DNA fell into a few closely related groups, either *M. fortuitum* or other fast-growing mycobacteria, like *M. tokaiense*, or *M. austroafricanum* and *M. heidelbergense*. However, the method described in this study based on the sequencing of a 473 bp region of the 16SrRNA gene, cannot be used to discriminate many species that are human and animal pathogens, i.e., *M. tuberculosis, M. avium, M. bovis* and *M. leprae*, although sequences belonging to this group were identified (Chilima et al. 2006; Lavania et al. 2008; Turankar et al. 2012).

With regard to mycobacterial diversity in polycyclic aromatic hydrocarbon-contaminated soils, investigations have revealed the presence of certain species typical for that environment. Cheung and Kinkle (2001) studied the diversity of mycobacteria in petroleum-contaminated soils. 16S rRNA sequences were amplified and subjected to temperature gradient gel electrophoresis analysis. All of the sequences belonged to fast-growing mycobacteria, some of them similar to *M. monascense* and *M. chlorophenolicum*. A similar study was conducted by Leys et al. (2005). The sequences detected in the contaminated soil belonged to the species *M. frederiksbergense, M. austroafricanum, M. petroleophilum* and *M. tusciae*. In a study conducted on heavily contaminated soil in Southern Finland, Denaturation gradient gel electrophoresis revealed that 30% of the clone library of the contaminated soil belonged to the genus *Mycobacterium* (Bjorklof et al. 2009).

5. Mycobacteria in plants (Table 5)

The presence of mycobacteria in plant tissues has been a concern owing to possible transmission to animals and humans (Kazda et al. 2009). The contamination of food of plant origin with mycobacteria has been reported already several decades ago (Nassal et al. 1974). Mycobacteria were found in fruits and vegetables, such as strawberries, radish, cucumbers etc. mainly in edible parts which were close to, or beneath the soil surface. Crucially, mycobacteria were present, although in smaller numbers, even after the washing of the fruits. In the same study, the first experiments demonstrating the presence of bacteria inside fruits were reported. This finding was explained by root uptake of bacteria. In the past couple of decades, the numbers of papers which have connected mycobacteria to food contamination and which have recognised its impact on animal and human health have been increasing (Kaevska and Hruska 2010). Mycobacteria were detected also in seven out of 121 vegetable samples which posed a danger to HIV-infected individuals (Yajko et al. 1995). A later study compared the genotypes of *M. avium* isolates from patients and foods and demonstrated a link between them (Yoder et al. 1999). Mycobacteria (predominantly *M. avium*) were isolated from 46 samples of salads, leak, lettuce, mushrooms, and other vegetables as well as apple juice and twenty nine isolates were tested (Argueta et al. 2000). Zwielehner et al. (2008) studied the microbial communities present in the phylosphere of lettuce leaves. After denaturing gradient gel electrophoresis and sequencing analyses, sequences from the genus *Mycobacterium* were found on leaves as well as soil samples. The sequence obtained from conventionally grown lettuce was most similar to *M. alvei*. The adoption of the routine use of molecular biology methods, i.e., DNA isolation and PCR/real time PCR represents a major breakthrough in the detection of mycobacteria in general. These techniques enable a considerably more rapid and sensitive detection of mycobacteria, with the possibility of quantification. Most of the methods developed so far are used for the detection of *M. a. paratuberculosis* (MAP). Pribylova et al. (2011) detected MAP in grass samples using IS*900* real time quantitative PCR. *M. avium* subsp. *paratuberculosis*-specific DNA was detected in 13 out of 19 samples examined (approx. 10^2 copies/g).

6. Mycobacteria in air (Table 6)

Mycobacteria in air are associated with dust or particles originating from water or soil. Tuberculous mycobacteria can be spread by dried sputum or excrements. The transmission of *M. tuberculosis* and *M. bovis* in droplets ejected by patients suffering from open forms of pulmonary tuberculosis is a special risk. The time of exposure, quantity and virulence of the pathogen, frequency and intensity of coughing, air exchange rate in the room and many factors related to the endangered person sharing the same room play a role in the dissemination of tuberculosis. It is obvious that species and concentration of mycobacteria in the air depend on many factors. House dust samples was collected from vacuum cleaners, homogenised by vigorous shaking, and sieved. Mycobacteria were found with both qPCR and traditional culture methods in all 20 dust samples tested. The median cell count was 10^6 cells/g and the median colony count was 10^3 CFU/g. Identification of samples was not possible by qPCR, but the species isolated by culture were *M. nonchromogenicum*, *M. kumamotonense*, *M. terrae*, *M. avium* complex and *M. gordonae* (Torvinen et al. 2010). The contamination of air with mycobacteria in a peat moss processing plant was assessed by Cayer et al. (2007). A fragment of the 16S rRNA gene was amplified, cloned and sequenced. Forty-nine mycobacterial clones were obtained, most of which were *M. intracellulare* species. The other detected mycobacteria were *M. graecum*, *M. interjectum*, *M. bohemicum* and *M. smegmatis*. *M. avium* subsp. *paratuberculosis* was also detected in dust on dairy farms (Eisenberg et al. 2009).

Mycobacteria in dust do not pose a unique risk of harm for humans and animals. They only supplement the other microorganisms, allergens, mites, pesticides and other foreign bodies which may have an adverse effect and are disseminated by means of dust. Appropriate house and street cleaning technology should be thoroughly defined and required. Vacuum cleaners must be tested for efficiency and the sweeping of streets using hand held blowers should be prohibited.

Mycobacteria were detected in the air of a hospital therapy pool environment (Angenent et al. 2005). Among the indoor air sequences, there were a total of 77 belonging to mycobacterial rRNA genes. No mycobacteria were detected in the outside air sample. Perkins et al. (2009) sampled water and aerosol samples from showers in a stem cell transplantation unit. From the sequences obtained and analysed, the most notable potential pathogen identified was *M. mucogenicum*.

7. Identification of mycobacteria (Table 7)

Classical culture using solid or liquid media with the identification of colonies using different methods was a standard method for more than 100 years. The sample has to be decontaminated to prevent an overgrow by the other microorganisms. Waiting for results for several weeks or months and the inability to determine the concentrations of mycobacteria in a sample means that the popularity of culture has waned. Nowadays, sophisticated, instrumental analytical methods based on DNA or RNA specificity or determination of specific proteins is preferred. A description of these methods is outside of the scope of this review and the reader is directed elsewhere for this information (Cerqueira et al. 2008; Nayak et al. 2009).

The identification of mycobacteria in environmental samples can be achieved using different approaches. In several studies isolated mycobacterial DNA has been subjected to sequencing. Using this method, the mycobacterial diversity in different environments can be assessed. The most commonly used gene, 16S rRNA, has variable and conserved regions within the genus. For sequencing of the genus *Mycobacterium*, *hsp65, dnaJ,* or *rpoB* genes have been used (Mendum et al. 2000; Angenent et al. 2005; Feazel et al. 2009). Next generation sequencing based on pyrosequencing techniques has also been used for the identification of bacterial and mycobacterial diversity (Liu et al. 2012; Gomez-Alvarez et al. 2012). The discovery of insertion sequences which are specific for certain mycobacterial species or complexes has been crucial for their direct detection using PCR or real time PCR. IS*900* is specific for *M. avium* subsp. *paratuberculosis* and is the most widely used sequence for its detection (Pickup et al. 2005, 2006; Whan et al. 2005; Torvinen et al. 2010). For detection of *M. avium* subsp. *avium* and *M. avium* subsp. *hominissuis* IS*901* and IS*1245* are used most commonly (Kaevska et al. 2011). For direct detection of *M. ulcerans* and *M. marinum*, PCR and real time PCR system specific for the insertion sequences IS*2404* and IS*2606* has been described (Fyfe et al. 2007). For total mycobacterial DNA analysis in soil, different attempts have been made at DNA isolation.

Humic acids and other organic material in soil have been the biggest obstacle for extracting microbial DNA due to their inhibitory effects. The diversity of mycobacteria in soil was most often assessed using Denaturation gradient gel electrophoresis or T-RFLP followed by cloning and sequencing (Mendum et al. 2000; Niva et al. 2006; Kopecky et al. 2011).

Many other methods including hybridisation assays, flow cytometry or MALDI-TOF have been employed for the identification of mycobacteria, though they are so far restricted to bacterial isolates or clinical material. A suitable method should be selected according to the specific aims, the matrix to be analysed, specificity and sensitivity required, accuracy needed, time available for the determination, etc.

Table 1. Search profiles used and numbers of the results retrieved

Web of Science databases = SCI-EXPANDED, SSCI, A&HCI, Lemmatization = On

Timespan	All years	2007–2012
Water		
Topic = (mycobact* AND water AND (drinking OR potable OR tap OR surface OR river OR swimming OR plumbing OR household OR tub))		
Results	532	231
Selected		83
Cited in Table 3		55
Review articles in Table 2		12
Soil		
Topic = (mycobact* AND soil)		
Results	803	349
Selected		92
Cited in Table 4		23
Review articles in Table 2		10
Plants		
Topic = (mycobact* AND (plant* OR vegetable*))		
Results	1099	612
Selected		29
Cited in Table 5		12
Review articles in Table 2		1
Air		
Topic = (mycobact* AND (air OR aerosol))		
Results	1136	503
Selected		35
Cited in Table 6		18
Review articles in Table 2		6

Table 2. Selected review articles

Topic of review	Abstract excerpts	Reference
Air *M. tuberculosis*	WHO international guidelines for the control of tuberculosis in relation to air travel require after a risk assessment-tracing of passengers who sat for longer than 8 h in rows adjacent to people with pulmonary tuberculosis who are smear positive or smear negative. A further recommendation is that all commercial air travel should be prohibited until the person has two consecutive negative sputum smears for drug-susceptible tuberculosis or two consecutive cultures for multidrug-resistant tuberculosis. In this Review I examine the evidence put forward to support these recommendations and assess whether such an approach is justifiable. A systematic review identified 39 studies of which 13 were included. The majority of studies found no evidence of transmission. Only two studies reported reliable evidence of transmission. The analysis suggests that there is reason to doubt the value of actively screening air passengers for infection with *Mycobacterium tuberculosis* and that the resources used might be better spent addressing other priorities for the control of tuberculosis.	Abubakar 2010
Catheter related infections *M. mucogenicum*	It has become apparent that *Mycobacterium mucogenicum* isolates recovered from clinical samples are more diverse than was previously realized and include an increasing number of emerging pathogens, as depicted by multilocus sequence analysis. Most clinically significant cases of those organisms involved catheter-related infections. They are susceptible to most antimicrobial agents, but like other rapidly growing mycobacteria, they are resistant to first-line antituberculous agents. A review of the cases of *M. mucogenicum* complex infection in the literature is addressed here, as well as two additional cases of the closely related species *Mycobacterium aubagnense*.	Adekambi 2009

Metal working fluids	Potential demographic risk factors for outbreaks of respiratory disease due to water-based metalworking fluids (MWFs) were investigated through systematic review of published outbreak investigations. Search terms were selected by a multidisciplinary team, assisted by an experienced library information service. Several computerized literature databases were searched for articles published between January 1990 and October 2011, relating to ill health outbreaks due to MWFs. Papers meeting the search criteria were reviewed in detail, and their references checked for additional articles. Study design and demographic details of the outbreak were extracted from the selected articles and entered into standardized evidence tables. Thirty-five articles relating to investigations of 27 outbreaks of respiratory ill health attributed to MWF exposure were identified. The majority of reports were case series of disease or observational cross-sectional studies of symptoms and hygiene measurements. Eight of the outbreak investigations included an element of case-control analysis. Most outbreaks were from the USA, had occurred in large car- or aeronautical-manufacturing plants, and were associated with the use of central shared sumps. Hygiene studies have not demonstrated consistent risk factors for respiratory outbreaks, in terms of the type of MWF utilized, degree of microbial contamination, or levels of personal exposure. Six studies were identified that found workers with MWF exposure during outbreaks were more likely to report respiratory or systemic symptoms than unexposed control workers. Six case-control analyses were also identified that found workers with extrinsic allergic alveolitis (EAA) were more likely to demonstrate certain immune responses to microbial contaminants and/or used MWFs than workers without EAA. Despite a number of detailed workplace and immunological studies of asthma and alveolitis outbreaks in MWF-exposed workforces, our understanding of their aetiology remains limited.	Burton et al. 2012
Fluorescence in situ hybridization, peptide nucleic acids, locked nucleic acids	Fluorescence in situ hybridization (FISH) is a well-established technique that is used for a variety of purposes, ranging from pathogen detection in clinical diagnostics to the determination of chromosomal stability in stem cell research. The key step of FISH involves the detection of a nucleic acid region and as such, DNA molecules have typically been used to probe for the sequences of interest. However, since the turn of the century, an increasing number of laboratories have started to move on to the more robust DNA mimics methods, most notably peptide and locked nucleic acids (PNA and LNA). In this review, we will cover the state-of-the-art of the different DNA mimics in regard to their application as efficient markers for the presence of individual microbial cells, and consider their potential advantages and pitfalls. Available PNA probes are then reassessed in terms of sensitivity and specificity using rRNA databases. In addition, we also attempt to predict the applicability of DNA mimics in well-known techniques attempting to detect in situ low number of copies of specific nucleic acid sequences such as catalyzed reporter deposition (CARD) and recognition of individual genes (RING) FISH.	Cerqueira et al. 2008
Environmental mycobacteria	Although the environmental mycobacteria are slow growing relative to other microorganisms in water and soil which would suggest that they are poor competitors, compensating factors permit survival, growth and persistence in natural and human-engineered environments. Factors such as the hydrophobic, lipid-rich impermeable envelope, biofilm formation, acid resistance, anaerobic survival and metabolism of recalcitrant carbon compounds permit survival and growth of the environmental mycobacteria in a wide range of natural and human-engineered habitats. High numbers of environmental mycobacteria are found in coastal swamps and estuaries and boreal, peat-rich forest soils and waters. The hydrophobic surface results in concentration of the environmental mycobacteria at interfaces (air-water and surface-water) and in aerosolized droplets ejected from water. The survival and growth in protozoa and amoebae permit environmental mycobacteria to persist in habitats subject to predation and likely led to survival and growth in phagocytic cells of animals. Finally, slow growth allows time for mycobacterial cells to adapt to changing conditions before loss of viability.	Falkinham 2009b

Ecology of mycobacteria	A majority of the *Mycobacterium* species, called the nontuberculous mycobacteria (NTM), are natural inhabitants of natural waters, engineered water systems, and soils. As a consequence of their ubiquitous distribution, humans are surrounded by these opportunistic pathogens. A cardinal feature of mycobacterial cells is the presence of a hydrophobic, lipid-rich outer membrane. The hydrophobicity of NTM is a major determinant of aerosolization, surface adherence, biofilm-formation, and disinfectant-and antibiotic resistance. The NTM are oligotrophs, able to grow at low carbon levels [> 50 μg assimilable organic carbon (AOC) l^{-1})], making them effective competitors in low nutrient, and disinfected environments (drinking water). Biofilm formation and oligotrophy lead to survival, persistence, and growth in drinking water distribution systems. In addition to their role as human and animal pathogens, the widespread distribution of NTM in the environment, coupled with their ability to degrade and metabolize a variety of complex hydrocarbons including pollutants, suggests that NTM may be agents of nutrient cycling.	Falkinham 2009a
Water and mycobacteria	Nontuberculous mycobacteria (NTM) are environmental opportunistic pathogens of humans and animals. They are found in a wide variety of habitats to which humans are exposed, including drinking water distribution systems and household water and plumbing. In that regard, they are distinct from their obligate pathogenic relatives, the members of the *Mycobacterium tuberculosis* complex. Owing to the presence of NTM in the human environment, human activities have had direct impacts on their ecology and thereby their epidemiology. NTM are oligotrophic, able to grow at low organic matter concentrations and over a wide range of temperatures, and even at low oxygen concentrations. Thus, NTM are normal inhabitants of natural waters and drinking waters. Discovery of the presence of NTM-polluted soils is not surprising in light of the ability of NTM to degrade a variety of hydrocarbon pollutants. A major human activity selecting for the growth and predominance of mycobacteria in habitats is disinfection. In comparison to other bacteria, NTM are disinfectant, heavy metal and antibiotic resistant. Therefore, the use of any antimicrobial agent selects for mycobacteria. Use of disinfectant in drinking water treatment selects for mycobacteria that can grow and come to proliferate in drinking water distribution systems in the absence of disinfectant-sensitive competing microorganisms. NTM selection may also occur as a consequence of antibiotics in drinking water sources.	Falkinham 2010
Rapidly growing mycobacteria infection and treatment	Rapidly growing mycobacteria (RGM) are ubiquitous in nature and widely distributed in water, soil and animals. During the past three decades we have observed a notable increment of infections caused by RGM, both localized and disseminated, as well as nosocomial outbreaks of contaminated medical equipment. The microbiological diagnosis of RGM infections includes direct microscopic observation and culture. The taxonomic identification is performed by phenotypic, biochemical, chromatographic and molecular biology techniques. The treatment differs from that of other mycobacteriosis like tuberculosis, owing to the variable in vitro susceptibility of the species of this group. The RGM are resistant to conventional antituberculous drugs, but can be susceptible to broad spectrum antimicrobial agents. In this study we comment on the significant aspects of human infections by RGM, including their biology, epidemiology, pathology, microbiological diagnosis, taxonomic identification, antimicrobial susceptibility and treatment.	Garcia-Martos and Garcia-Agudo 2012

Water, milk and meat *M. a. paratuberculosis*	*Mycobacterium avium* subsp. *paratuberculosis* (Map) is the cause of Johne's disease, a chronic infection of the gut, in ruminant animals that provide milk and/or meat for human consumption. Map also may be involved in Crohn's disease and type I diabetes in humans. Although the role of Map in human diseases has not been established, minimizing the exposure of humans to the organism is considered desirable as a precautionary measure. 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. The presence of at least some Map in raw milk and meat and in natural waters is likely, but the numbers of Map in those foods and waters should be reduced through cooking or purification. The available information relating to Map in milk and dairy products, meats, and drinking water is reviewed here for assessment of the risks of exposure to Map from consumption of such foods and water.	Gill et al. 2011
Water, food and feed	Papers on mycobacteria in food, feed and water, published between 1945 and 2010 and indexed in the database Web of Science (R) (Thomson Reuters) were ranked according to authors, institutions, countries and source titles. The total number of papers on mycobacteria and food and mycobacteria and water were 1486 and 1419, respectively. More than 40% of papers have been published in the last five years. In addition to publications in peer reviewed journals the archives of ProMED-mail and the Rapid Alert System for Food and Feed of the European Union were also searched. It is evident that much attention is being paid to mycobacteria in food, feed and water as they likely pose a public health risk.	Kaevska and Hruska 2010
Pulmonary diseases *M. avium* complex	*Mycobacterium avium* complex (MAC) consists of nontuberculous mycobacteria that cause disease in immunocompromised and immunocompetent hosts. The organisms are ubiquitous in the environment, and acquisition occurs through ingestion or inhalation of aerosols from soil, water, or biofilms. Disease may manifest as disseminated infection, soft tissue infection, chronic pneumonia, or hypersensitivity pneumonitis. Nontuberculous mycobacteria are increasingly associated with pulmonary disease, with MAC being the most common nontuberculous mycobacteria to cause pulmonary disease in the United States. Pulmonary symptoms, nodular or cavitary opacities on a chest radiograph or high-resolution computed tomographic scan with multifocal bronchiectasis and multiple small nodules, plus positive culture results from two sputum specimens or one bronchoscopic specimen are consistent with MAC pulmonary disease. Treatment consists of a macrolide, rifamycin, and ethambutol given three times weekly for noncavitary disease and daily with or without an aminoglycoside for cavitary disease.	Kasperbauer and Daley 2008
Lung disease caused by mycobacteria	Nontuberculous mycobacteria (NTM) are resilient bacteria that grow in virtually any environment, especially those where competing microorganisms are destroyed, such as in chlorinated water. They have been discovered in soil, dust, food, water, and domestic and wild animals. Nontuberculous mycobacteria tend to infect individuals with local (e.g., damaged skin or lung) or systemic (e.g., HIV, drugs, malignancy) defects in host defence, and their incidence and prevalence have consistently increased in the last decade. Difficulty may arise in determining whether an isolated NTM from a microbiological sample is in fact a contaminant or a pathogenic organism. In this review, we discuss the important mycobacteria involved in lung disease, factors that predispose individuals to infection, and their diagnosis and treatment according to updated guidelines. English language publications in MEDLINE and references from relevant articles from January 1, 1990 to June 28, 2009 were reviewed. Keywords searched were "nontuberculous", "mycobacteria", "diagnosis", and "treatment".	McGrath et al. 2010

Contact tracing in public transport	While guidelines on contact tracing (CT) after exposure to certain infectious pathogens during air travel exist, no guidance documents are available on CT in response to potential exposure on public ground transport. We reviewed scientific and non-scientific literature on transmission of airborne pathogens in public ground transport and on factors potentially influencing transmission. We identified 32 relevant publications (15 scientific and 17 non-scientific). Most of the selected studies dealt with transmission of tuberculosis. However, the relation between travel duration, proximity to the index case and environmental factors, such as ventilation, on disease transmission in public ground transport is poorly understood. Considering the difficulty and probably limited effectiveness of CT in ground transport, our results suggest that only exceptional circumstances would justify CT. This contrasts with the high level of attention CT in air travel seems to receive in international regulations and recommendations. We question whether the indication for CT should be revisited after a risk-benefit assessment that takes into account exposure in both ground and air transport.	Mohr et al. 2012
Detection of microorganisms using biosensors	Along with useful microorganisms, there are some that cause potential damage to the animals and plants. Detection and identification of these harmful organisms in a cost and time effective way is a challenge for the researchers. The future of detection methods for microorganisms shall be guided by biosensor, which has already contributed enormously in sensing and detection technology. Here, we aim to review the use of various biosensors, developed by integrating the biological and physicochemical/mechanical properties (of tranducers), which can have enormous implication in healthcare, food, agriculture and biodefence. We have also highlighted the ways to improve the functioning of the biosensor.	Nayak et al. 2009
Free-living amoebae	*Mycobacterium* species evolved from an environmental recent common ancestor by reductive evolution and lateral gene transfer. Strategies selected through evolution and developed by mycobacteria resulted in resistance to predation by environmental unicellular protists, including free-living amoebae. Indeed, mycobacteria are isolated from the same soil and water environments as are amoebae, and experimental models using *Acanthamoeba* spp. and *Dictyostelium discoideum* were exploited to analyse the mechanisms for intracellular survival. Most of these mechanisms have been further reproduced in macrophages for mycobacteria regarded as opportunistic and obligate pathogens. Amoebal cysts may protect intracellular mycobacteria against adverse conditions and may act as a vector for mycobacteria. The latter hypothesis warrants further environmental and clinical studies to better assess the role of free-living amoebae in the epidemiology of infections caused by mycobacteria.	Salah et al. 2009

Free-living amoebae	Acanthamoebae are free-living amoebae distributed worldwide. They are among the most prevalent protozoa found in the environment, and have been isolated from a wide variety of public water supplies, swimming pools, bottled water, ventilation ducts, soil, air, surgical instruments, contact lenses, dental treatment units and hospitals. Acanthamoebae feed on bacteria by phagocytosis, but some bacteria are able to survive and sometimes multiply in the host, resulting in new properties of the bacteria. The intracellular growth of bacteria has been associated with enhanced environmental survival of the bacteria, increased virulence and increased resistance against antibiotic substances. The advantage of utilising free-living amoebae is that research can be carried out on non-mammalian cells as a model based on natural reality to study bacterial virulence and pathogenicity. Amoebae are easy to handle experimentally compared with mammalian cells and allow studies on host factors for host-parasite interactions. Bacteria are easily manipulated genetically, which creates the possibility of research on mutants to study bacteria-host interactions. Thus utilising this non-mammalian model can result in better understanding of interactions between prokaryotic and eukaryotic cells and assist in the development of new therapeutic agents to recognise and treat infections.	Sandstrom et al. 2011
Rapidly growing mycobacteria	The pathogenic potential of the rapidly growing mycobacteria (RGM) has started being recognized. This is due to more sensitive and specific techniques in the laboratory. The RGM are generally defined as nontuberculous species of mycobacteria that show visible growth on agar media within 7 days. RGM are widely distributed in nature and have been isolated from natural water, tap water, and soil. Several biochemical tests, high performance liquid chromatography, and molecular techniques have been developed for rapid identification of these species. The American Thoracic Society and the Infectious Disease Society of America recommend that RGM should be identified to the species level using a recognized acceptable methodology such as polymerase chain reaction restriction enzyme analysis or biochemical testing and routine susceptibility testing of RGM should include amikacin, imipenem, doxycycline, the fluorinated quinolones, a sulphonamide or trimethoprim-sulphamethoxazole, cefoxitin, clarithromycin, linezolid, and tobramycin. The diseases caused by these organisms have varied manifestations. They have been responsible for a number of healthcare-associated outbreaks and pseudo-outbreaks. For recognition of outbreaks, it is important to be familiar with the causative organisms like RGM which are most frequently involved in healthcare-associated outbreaks and pseudo outbreaks. It is essential to intervene as soon as possible to interrupt this transmission. Large gaps still exist in our knowledge of RGM. Unquestionably more studies are required. Through this review, we wish to emphasize that reporting of RGM from clinical settings along with their sensitivity patterns is an absolute need of the hour.	Set and Shastri 2011

Food borne pathogens	The emergence of pathogens is the result of a number of impact in all parts of the food chain. The emerging technologies in food production explain how new pathogens can establish themselves in the food chain and compromise food safety. The impact of the food technology is analysed for several bacteria, such as *Yersinia*, *Campylobacter*, *Arcobacter*, *Helicobacter pullorum*, *Enterobacter sakazakii*, *Mycobacterium avium* spp. *paratuberculosis*, prions related to vCJD and others. The importance of the ability of many microbes to form VBNC forms is elaborated on. Research on culture independent methods may address this outstanding issue to the better understanding of emerging pathogens. The "demerging" of pathogens also occur, and examples of this are explained. The reaction of bacteria to stresses and sublethal treatments, and how exposure to one stress factor can confer resistance to other stresses, literally speaking causing contagious resistance, are explained. The implication of this e.g. in modem approaches of food preservation, such as Minimally processed Foods, is considerable. Intestinal colonization of EHEC may be regulated by Quorum sensing, and this ability of microbes plays an important role in the colonization of microbes in food and on food processing equipment, an important factor in the emergence of pathogens. The emergence of *Saccharomyces cerevisiae*, as an opportunistic human pathogen, used for centuries for food and production of alcoholic beverages, calls for research in molecular tools to distinguish between probiotic and clinical strains. *Cyclospora cayetanensis* and *Norovirus outbreaks* can no longer be designated as emerging pathogens, they share however one characteristic in the epidemiology of emerging nature, the importance of the hygiene in the primary production stage, including supply of potable water, and the application of GMP and the HACCP principles in the beginning of the food chain. Hepatitis E virus is a potential emerging food borne pathogen and swine may serve as a source of infection in human, a most challenging issue in greater part of the world raising pigs. Tick-borne encephalitis virus infection, either thick borne or caused by consumption of raw milk, is an increasing trend in the industrialized part of the world. Consumer awareness, ethics of food, sustainability in food production, and trust in foods, are of growing importance to the consumer. The reaction of the consumer to new technology, such as nanotechnology, is unpredictable. Many efforts should be devoted to communication of non-biased information to both the food producers as well as the consumer.	Skovgaard 2007
Dental unit waterlines	The specific structure of dental units favours the presence of biofilm and microbial contamination of the dental unit waterlines (DUWL) water. The ability of bacteria to colonize surfaces and to form biofilm in water supply tubes, including DUWL, is a common phenomenon, which has been well documented, just as with difficulties in biofilm removal and prevention of its regrowth. Microorganisms from contaminated DUWL are transmitted with aerosol and splatter, generated by working unit handpieces. On the basis of the detailed literature review, the state-of-the art knowledge of the microflora of dental unit waterlines is presented. Most of the microorganisms isolated from DUWL are of low pathogenicity. Nevertheless, the public health significance of many of the microorganisms found in DUWL is unknown. According to current knowledge, it is not the mere presence of bacteria that is important in DUWL contamination monitoring, but their number, the presence of potential pathogens, and patients' oral cavity microflora. Numerous studies emphasize the need for effective mechanisms to reduce the microbial contamination in DUWL and highlight the risk for cross-infection in general practice, especially in view of the ever-increasing number of immunocompromised persons who present at outpatient dental clinics.	Szymanska et al. 2008

Dental office environment	Biological factors are constantly present during dental procedures. They are causes of cross-infections in a dental office, constituting occupational hazards for dental personnel exposed to them for long periods of time. The authors review the current knowledge on bacterial factors present in the environment of a dental office and look at the infectious factors spread via blood-borne route, direct contact with a patient and contaminated equipment, and aerosols emitted from both the patient's mouth and the dental unit itself. The authors describe important sources and routes of infection specific for a dental office - biofilm as a source of dental unit water contamination, microbial contamination of water in dental units and microbial contamination of air in dental offices.	Szymanska and Sitkowska 2012
Free-living amoebae in drinking water	There is an expanding body of evidence that free-living amoebae (FLA) increase both the numbers and virulence of water-based, human-pathogenic, amoeba-resisting microorganisms (ARM). *Legionella* spp., *Mycobacterium* spp., and other opportunistic human pathogens are known to be both ARM and also the etiologic agents of potentially fatal human lung infections. However, comparatively little is known about the FLA that may facilitate ARM growth in drinking water. This review examines the available literature on FLA in treated drinking water systems; in total 26 studies from 18 different countries. FLA were reported to breakthrough the water treatment barrier and enter distribution systems, in addition to the expected post-treatment system ingress. Once in the distribution system there is evidence of FLA colonization and regrowth especially in reservoirs and in-premise plumbing storage tanks. At the point of use the average FLA detection rate was 45% but highly variable (n = 16, sigma = 31) due to both differences in both assay methods and the type of water systems examined. This review reveals that FLA are consistently detected in treated drinking water systems around the world and present a yet unquantified emerging health risk. However, more research is urgently required before accurate risks assessments can be undertaken to assess the impacts on human health, in households and institutions, due to exposure to FLA facilitated pathogenic ARM.	Thomas and Ashbolt 2011
Water *M. avium* complex	*Mycobacterium avium* complex (MAC) is a group of opportunistic pathogens of major public health concern. It is responsible for a wide spectrum of disease dependent on subspecies, route of infection and patients pre-existing conditions. Presently, there is limited research on the incidence of MAC infection that considers both pulmonary and other clinical manifestations. MAC has been isolated from various terrestrial and aquatic environments including natural waters, engineered water systems and soils. Identifying the specific environmental sources responsible for human infection is essential in minimizing disease prevalence. This paper reviews current literature and case studies regarding the wide spectrum of disease caused by MAC and the role of potable water in disease transmission. Potable water was recognized as a putative pathway for MAC infection. Contaminated potable water sources associated with human infection included warm water distribution systems, showers, faucets, household drinking water, swimming pools and hot tub spas. MAC can maintain long-term contamination of potable water sources through its high resistance to disinfectants, association with biofilms and intracellular parasitism of free-living protozoa. Further research is required to investigate the efficiency of water treatment processes against MAC and into construction and maintenance of warm water distribution systems and the role they play in MAC proliferation.	Whiley et al. 2012

Table 3. Mycobacteria in water

Type of water Mycobacteria detected	Abstract excerpt	Reference
Drinking water *M.* sp. *M. gordonae* *M. kansasii* *M. fortuitum*	In the current study, we detected and quantified the presence of NTM by means of a rapid method in water samples taken from 53 cooling towers of an urban area (Barcelona, Spain). A genus-specific quantitative PCR (Q-PCR) assay with a quantification limit (QL) of 500 cells l^{-1} was used. 56% (30) of samples were positive with a concentration range from 4.6×10^3 to 1.79×10^6 cells l^{-1}. In some cases (9/30), samples were positive but with levels below the QL. The colonization rate confirmed that cooling towers could be considered as a potential reservoir for NTM. This study also evaluated Q-PCR as a useful method to detect and quantify NTM in samples coming from environmental sources.	Adrados et al. 2011
Drinking water *M.* sp. *M. fortuitum* *M. tokaiense* *M. austroafricanum* *M. vanbalenii* *M. aichiense* *M. heidelbergense*	A total of 148 soil samples and 24 water samples were collected from various locations and examined to determine the presence of mycobacteria. The detection method involved semiselective culturing and acid-fast staining, following decontamination of samples to enrich mycobacteria and reduce the numbers of other microorganisms, or PCR with primers specific for the mycobacterial 16S rRNA gene, using DNA extracted directly from soil and water samples. Mycobacteria were detected in the majority of the samples, and subsequent sequence analysis of PCR products amplified directly from soil DNA indicated that most of the products were related to known environmental mycobacteria. For both methods the rates of recovery were consistently higher for dry season samples than for wet season samples.	Chilima et al. 2006
Drinking water *M. a. paratuberculosis*	Real-time quantitative PCR assays for *Helicobacter pylori*, *Yersinia enterocolitica*, and *Mycobacterium avium* subsp. *paratuberculosis*, human pathogens with long-time survival capacity in water, and for the resistance genes ermB, mecA, blaSHV-5, ampC, tetO, and vanA were adapted or developed for water samples differing in pollutant content. The resistance genes and pathogen concentrations were determined at five or six sampling points for each recharge system. In drinking and irrigation water, none of the pathogens were detected.	Bockelmann et al. 2009
Drinking water *M.* sp. *M. peregrinum* *M. nonchromogenicum* *M. smegmatis* *M. fortuitum* *M. avium ssp. hominissuis* *M. arupense* *M. gordonae* *M. chitae*	We investigated the presence of nontuberculous mycobacteria (NTM) in three Mexican aquatic systems to evaluate the prevalence with the distribution of NTM species. Key physicochemical parameters of the water samples were determined to find correlations with the species' distributions. We used multilocus sequence analysis (MLSA) based on hsp65, rpoB, and 16S rRNA fragments to determine their taxonomic affiliations. NTM were recovered from water distribution systems and reclaimed water from the Mexico City Metropolitan Area (MCMA). The isolated species were associated with a temperature of 21 degrees C and pH > 7.7. The phylogenetic analysis showed that eight of the 14 different NTM strains were unambiguously classifiable: *Mycobacterium peregrinum*, *M. nonchromogenicum* (2), *M. smegmatis* (2), *M. fortuitum*, *M. avium* ssp. *hominissuis*, *M. arupense*, *M. gordonae*, and *M. chitae*.	Castillo-Rodal et al. 2012

Drinking water *M.* sp. *M. arupense* *M. gordonae*	In this study, to give insight into the bacterial diversity of biofilms from full-scale drinking water distribution systems (DWDSs), the bacterial community compositions of biofilms from two urban DWDSs (Guangzhou and Beijing, China) were determined using a 16S rRNA gene library technique. Meanwhile, the occurrence and diversity of mycobacteria were also analyzed by a *Mycobacterium*-specific hsp gene assay. The biofilms from the full-scale DWDSs have complex bacterial populations. Proteobacteria was the common and predominant group in all biofilm samples, in agreement with previous reports. The community structures of bacteria at the three sites in Guangzhou DWDS were significantly different, despite the similar physicochemical properties of portable water. Some abundant and peculiar bacterial phylotypes were noteworthy, including *Methylophilus*, *Massilia*, and *Planomicrobium*, members of which are rarely found in DWDSs and their roles in DWDS biofilms are still unclear. The diversity of *Mycobacterium* species in biofilm samples was rather low. *Mycobacterium arupense* and *Mycobacterium gordonae* were the primary *Mycobacterium* species in Guangzhou and Beijing biofilms, respectively, indicating that *M. arupense* may be more resistant to chloride than *M. gordonae*.	Liu et al. 2012
Drinking water *M. avium*	The spread of opportunistic pathogens via public water systems is of growing concern. The purpose of this study was to identify patterns of occurrence among three opportunistic pathogens (*Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas aeruginosa*) relative to biotic and abiotic factors in two representative chloraminated drinking water distribution systems using culture-independent methods. Generally, a high occurrence of *Legionella* (≥ 69.0%) and mycobacteria (100%), lower occurrence of *L. pneumophila* (≤ 20%) and M. avium (≤ 33.3%), and rare detection of *Pseudomonas aeruginosa* (≤ 13.3%) were observed in both systems according to quantitative PCR. Also, *Hartmanella vermiformis* was more prevalent than *Acanthamoeba*, both of which are known hosts for opportunistic pathogen amplification, the latter itself containing pathogenic members. Three-minute flushing served to distinguish distribution system water from plumbing in buildings (i.e., premise plumbing water) and resulted in reduced numbers of copies of *Legionella*, mycobacteria, *H. vermiformis*, and 16S rRNA genes ($P < 0.05$) while yielding distinct terminal restriction fragment polymorphism (T-RFLP) profiles of 16S rRNA genes. Within certain subgroups of samples, some positive correlations, including correlations of numbers of mycobacteria and total bacteria (16S rRNA genes), *H. vermiformis* and total bacteria, mycobacteria and *H. vermiformis*, and *Legionella* and *H. vermiformis*, were noted, emphasizing potential microbial ecological relationships. Overall, the results provide insight into factors that may aid in controlling opportunistic pathogen proliferation in real-world water systems.	Wang et al. 2012
Drinking water *M.* sp.	A metagenome-based approach was used to assess the taxonomic affiliation and function potential of microbial populations in free-chlorine-treated (CHL) and monochloramine-treated (CHM) drinking water (DW). In all, 362,640 (averaging 544 bp) and 155,593 (averaging 554 bp) pyrosequencing reads were analyzed for the CHL and CHM samples, respectively. Most annotated proteins were found to be of bacterial origin, although eukaryotic, archaeal, and viral proteins were also identified. Differences in community structure and function were noted. Most notably, *Legionella*-like genes were more abundant in the CHL samples while mycobacterial genes were more abundant in CHM samples. Genes associated with multiple disinfectant mechanisms were identified in both communities. Moreover, sequences linked to virulence factors, such as antibiotic resistance mechanisms, were observed in both microbial communities. This study provides new insights into the genetic network and potential biological processes associated with the molecular microbial ecology of DW microbial communities.	Gomez-Alvarez et al. 2012

Drinking water and ice *M.* sp. *M. porcinum*	We identified *M. porcinum* from 24 patients at a Galveston university hospital (University of Texas Medical Branch) over a 5-year period. *M. porcinum* was considered a pathogen in 11 (46%) of 24 infected patients, including 4 patients with community-acquired disease. Retrospective patient data were collected, and water samples were cultured. Molecular analysis of water isolates, clustered clinical isolates, and 15 unrelated control strains of *M. porcinum* was performed. Among samples of hospital ice and tap water, 63% were positive for RGM, 50% of which were *M. porcinum*. Among samples of water from the city of Galveston, four of five households (80%) were positive for *M. porcinum*. By pulsed-field gel electrophoresis (PFGE), 8 of 10 environmental *M. porcinum* were determined to belong to two closely related clones.	Brown-Elliott et al. 2011
Drinking water *M.* sp. *M. mucogenicum* *M. rhodesiae* *M. peregrinum* *M. fortuitum*	This study examined the frequency of occurrence of non-tuberculous mycobacteria (NTM) in potable water samples from a main trauma hospital in Mexico City. Sixty-nine potable water samples were collected, 23 from each source: cistern, kitchen tap and bathroom showers. Of the 69 samples, 36 harboured NTM species. Twenty-nine of the 36 isolates were *Mycobacterium mucogenicum*, two *Mycobacterium rhodesiae*, one *Mycobacterium peregrinum*, one *Mycobacterium fortuitum* and three were *Mycobacterium* spp. Hospital potable water harbouring NTM represents a potential source for nosocomial infections, therefore we suggest that hospital potable water microbiological guidelines should include testing for NTM species.	Fernandez-Rendon et al. 2012
Drinking water *M.* sp. *M. lentiflavum* *M. tusciae* *M. gordonae*	Drinking water distribution systems were analyzed for viable counts of mycobacteria by sampling water from waterworks and in different parts of the systems. In addition, loose deposits collected during mechanical cleaning of the main pipelines were similarly analyzed. The study covered 16 systems at eight localities in Finland. In an experimental study, mycobacterial colonization of biofilms on polyvinyl chloride tubes in a system was studied. The isolation frequency of mycobacteria increased from 35% at the waterworks to 80% in the system, and the number of mycobacteria in the positive samples increased from 15 to 140 CFU/liter, respectively. Mycobacteria were isolated from all 11 deposits with an accumulation time of tens of years and from all 4 deposits which had accumulated during a 1-year follow-up time. The numbers of mycobacteria were high in both old and young deposits (medians, 1.8×10^5 and 3.9×10^5 CFU/g [dry weight], respectively). Both water and deposit samples yielded the highest numbers of mycobacteria in the systems using surface water and applying ozonation as an intermediate treatment or posttreatment. The number and growth of mycobacteria in system waters correlated strongly with the concentration of assimilable organic carbon in the water leaving the waterworks. The densities of mycobacteria in the developing biofilms were highest at the distal sites of the systems. Over 90% of the mycobacteria isolated from water and deposits belonged to *Mycobacterium lentiflavum*, *M. tusciae*, *M. gordonae*, and a previously unclassified group of mycobacteria. Our results indicate that drinking water systems may be a source for recently discovered new mycobacterial species.	Torvinen et al. 2010
Drinking water distribution system *M. gordonae* *M. kansasii* *M. intracellulare* *M. chelonae*	Mycobacteria have emerged as a major cause of opportunistic infections. Until the present, only a few studies have characterized mycobacteria present in the water distribution system of urban areas. In this study, we characterize these microorganisms in the Lisbon water distribution system. Our results indicate a high rate of positivities (90.5%) with mainly saprophytic mycobacteria. Around 63% of these results belong to strains of *Mycobacterium gordonae* indicating a generalized proliferation of this species in the Lisbon water distribution system. A total of 21.05% of the isolates are from *M. kansasii*, *M. intracellulare*, and *M. chelonae*.	Santos et al. 2005

Drinking water	...The natural reservoirs of these non-primary pathogenic mycobacteria include aquatic and terrestrial environments. Under certain circumstances, e.g., skin lesions, pulmonary or immune dysfunctions and chronic diseases, these environmental mycobacteria (EM) may cause disease. EM such as *M. avium*, *M. kansasii*, and *M. xenopi* have frequently been isolated from drinking water and hospital water distribution systems. Biofilm formation, amoeba-associated lifestyle, and resistance to chlorine have been recognized as important factors that contribute to the survival, colonization and persistence of EM in water distribution systems. Although the presence of EM in tap water has been linked to nosocomial infections and pseudo-infections, it remains unclear if these EM provide a health risk for immunocompromised people, in particular AIDS patients. In this regard, control strategies based on maintenance of an effective disinfectant residual and low concentration of nutrients have been proposed to keep EM numbers to a minimum in water distribution systems.	Vaerewijck et al. 2005
Drinking water	Free-living amoebae have been detected in a large number of man-made water systems, including drinking water distribution systems. Some of these amoebae can host amoebae-resisting bacteria, and thus act potentially as reservoirs and vehicles for a number of pathogens. The objectives of this study were to characterize the amoebae and amoebae-resisting bacteria present in different raw waters used for drinking water production, and to assess the efficiency of different treatments applied for drinking water production in removing or inactivating these amoebae. The preliminary results of this study confirm the presence of amoebae and amoebae-resisting bacteria in raw waters used for drinking water production. Due to their capacity to encyst, most of these amoebae are extremely resistant to disinfection processes. In these conditions, preventing the dissemination of these micro-organisms through drinking water will mainly require their physical removal by clarification and filtration processes. The particular hazard that amoebae-resisting bacteria represent in drinking water production should be taken into account in any risk assessment conducted in the framework of a water safety plan, and control strategies based on physical removal rather than disinfection should be adopted where necessary.	Loret et al. 2008
Drinking and surface water *M. gordonae* *M. lentiflavum* *M. avium* complex	To investigate the occurrence and species diversity of mycobacteria in waters, surface water samples were collected monthly from the Han River and tap water samples at the terminal sites of the distribution system. Mycobacteria in each water sample were isolated by decontamination using cetylpyridinium chloride (CPC) and cultivation on Middlebrook 7H10 agar, and then identified by polymerase chain reaction-restriction fragment length polymorphism analysis (PRA) and sequencing of the 65-kDa heat-shock protein gene (hsp65 gene). Mycobacteria were detected in 59% of the surface water samples and 26% of the tap water samples. Over half of the 158 isolates could not be identified by hsp65 PRA and gene sequencing, and several identification discrepancies were observed between the two methods. The most frequently isolated species was *Mycobacterium gordonae* in surface water and *M. lentiflavum* in tap water. *M. avium* complex (MAC), the most important pathogen among environmental mycobacteria, was detected in the surface water samples but not found in the tap water samples. The result demonstrated that water is an important environmental source of mycobacteria and the combined application of hsp65 PRA and sequencing was more reliable than hsp65 PRA alone to accurately identify mycobacteria present in water.	Lee et al. 2008

Shower heads *M. avium*	Although opportunistic pathogens commonly are cultured from shower facilities, there is little knowledge of either their prevalence or the nature of other microorganisms that may be delivered during shower usage. To determine the composition of showerhead biofilms and waters, we analyzed rRNA gene sequences from 45 showerhead sites around the United States. We find that variable and complex, but specific, microbial assemblages occur inside showerheads. Particularly striking was the finding that sequences representative of non-tuberculous mycobacteria (NTM) and other opportunistic human pathogens are enriched to high levels in many showerhead biofilms, >100-fold above background water contents. We conclude that showerheads may present a significant potential exposure to aerosolized microbes, including documented opportunistic pathogens.	Feazel et al. 2009
Drinking water *M.* sp. *M. gordonae* *M. gastri/M. kansasii* *M. fortuitum* *M. simiae* *M. scrofulaceum* *M. szuigai*	This paper presents the finding of the possible cause of the high false-positive rate in acid-fast staining in histological examinations. Using acid-fast staining, culture, and PCR, acid-fast bacilli were detected in 83.7% of 49 hospital tap water samples and nontuberculous mycobacteria (NTM) were detected in 20.4% of the same 49 samples. The 10 NTM isolates were also identified to the species level using PCR-restriction fragment length polymorphism. Our findings indicate that NTM in hospital tap water are the possible cause of false positives in acid-fast staining and of nosocomial infection in immunocompromised patients.	Chang et al. 2002
Drinking water *M.* sp. *M. peregrinum* *M. chelonae* *M. abscessus* *M. gordonae*	Recently the presence of NTM in public drinking water and hospital water distribution systems has been reported. Their ability to form biofilms and their resistance to chlorine both contribute to their survival and colonization in water distribution systems. Here we analyzed thirty-two hospital tap water samples that were collected from different locations in three hospitals so as to evaluate the prevalence of NTM species. The water samples were concentrated by membrane filtration and then eluted with sterilized water following sonication. Two-step direct PCR targeting the rpoB gene, restriction fragment length polymorphism (RFLP) using the MspI restriction enzyme, and sequence analysis were performed for identification of NTM to the species level. The sequences of each PCR product were analyzed using BLASTN. Seven samples (7/32, 21.9%) were positive for NTM as determined by nested-PCR. The PCR-RFLP results indicated five different patterns among the seven positive PCR samples. The water-born NTM were identified, including *M. peregrinum*, *M. chelonae* (2 cases), *M. abscessus*, *M. gordonae* (2 cases), and *Mycobacterium s*p. JLS. The direct two-step PCR-RFLP method targeting the rpoB gene was effective for the detection and the differentiation of NTM species from hospital tap water.	Shin et al. 2008
Drinking water *M.* sp. *M. xenopi* *M. chelonae* *M. mucogenicum* *M. flavescens* *M. frederiksbergense* *M. gordonae* *M. moriokaense* *M. vaccae*	Aim of the study was to establish highly sensitive and specific techniques to detect and identify NTM in hospital drinking water. A *Mycobacterium* genus-specific 16SrDNA-based real-time LightCycler PCR assay with internal inhibition control and a *M. xenopi*-specific PCR were developed.Ninety-three water samples from 53 taps from 4 hospitals were investigated. NTM were cultured from 21 of 49 (43%) cold and 32 of 44 (73%) warm water samples. *M. chelonae*, *M. flavescens*, *M. frederiksbergense*, *M. gordonae*, *M. moriokaense*, *M. mucogenicum*, *M. vaccae*, and *M. xenopi* were identified with molecular methods. All 93 water samples were positive in the genus-specific PCR. *M. xenopi* DNA was detected in 40 of 44 (91%) warm and 33 of 49 (67%) cold water samples including 45 of 65 (69%) *M. xenopi* culture-negative samples.	Hussein et al. 2009

Drinking water biofilms *M. avium*	*Mycobacterium avium* is a potential pathogen occurring in drinking water systems. It is a slowly growing bacterium producing a thick cell wall containing mycolic acids, and it is known to resist chlorine better than many other microbes. Several studies have shown that pathogenic bacteria survive better in biofilms than in water. By using Propella biofilm reactors, we studied how factors generally influencing the growth of biofilms (flow rate, phosphorus concentration, and temperature) influence the survival of *M. avium* in drinking water biofilms. The growth of biofilms was followed by culture and DAPI (4¦6'-diamidino-2-phenylindole) staining, and concentrations of *M. avium* were determined by culture and fluorescence in situ hybridization methods. The spiked *M. avium* survived in biofilms for the 4-week study period without a dramatic decline in concentration. The addition of phosphorus (10 µg/liter) increased the number of heterotrophic bacteria in biofilms but decreased the culturability of *M. avium*. The reason for this result is probably that phosphorus increased competition with other microbes. An increase in flow velocity had no effect on the survival of *M. avium*, although it increased the growth of biofilms. A higher temperature (20 degrees C versus 7 degrees C) increased both the number of heterotrophic bacteria and the survival of *M. avium* in biofilms. In conclusion, the results show that in terms of affecting the survival of slowly growing *M. avium* in biofilms, temperature is a more important factor than the availability of nutrients like phosphorus.	Torvinen et al. 2007
Drinking water *M. a. paratuberculosis*	A "cluster" of patients refers to the geographic proximity of unrelated patients with the same disease and suggests a common environmental cause for that disease. Clusters of patients with Crohn's disease have been linked to the presence of an infectious microorganism in unpasteurized milk and cheese, untreated water supplied by wells or springs, animal manure used as fertilizer for family vegetable gardens, and bodies of water contaminated by agricultural runoff. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the suspected cause of Crohn's disease. MAP causes a disease in dairy cows and other animals that is similar to Crohn's disease, called Johne's ('Yo-knees') disease or paratuberculosis. Dairy cows with Johne's disease secrete MAP into their milk and excrete MAP into their feces. MAP is present in untreated water such as well water, in bodies of water contaminated by agricultural runoff, and in unpasteurized milk and cheese. The "treatment" of "tap" water to make it "drinkable" or "potable" by the processes of sedimentation, filtration and chlorination has little to no effect on MAP. MAP is so resistant to chlorine disinfection that such disinfection actually selects for its growth. Other subspecies of *Mycobacterium avium* grow in biofilms present on tap water pipes. Despite the documented presence of MAP in tap water and its probable growth on tap water pipes, clusters of Crohn's disease have not previously been described in relationship to tap water pipes supplying patients' homes. This report describes three unrelated individuals who lived on the same block along a street in a midwestern American city and developed Crohn's disease within four years of each other in the 1960' s. A common tap water pipe supplied their homes. This is the first reported cluster of Crohn's disease possibly linked to fully treated drinking water, and is consistent with previously reported clusters of Crohn's disease linked to an infectious microorganism in water.	Pierce 2009

Drinking water *M. chelonae* *M. kansasii* *M. fortuitum*	Non-tuberculous mycobacteria (NTM) found frequently in tap water and environment cause important opportunistic infections in immunocompromised patients. The aim of this study was to isolate and identify non-tuberculous mycobacteria in soil, raw milk and water distribution system samples in Mersin (a province located at Mediterranean region of Turkey). A total of 101 water, 124 soil and 40 milk samples collected from the central part and suburban parts of Mersin during November 2003–May 2004 period were included in the study. Water samples were collected from 29 different water distribution systems; soil samples from different parks and gardens and milk samples from raw milks sold at different districts. After the samples were processed by homogenization and decontamination, acid-fast staining and culture into Lowenstein-Jensen medium were performed. Acid-fast bacilli isolated from culture medium were identified by using conventional methods, polymerase chain reaction (PCR)-RFLP (Restriction Fragment Length Polymorphism) and INNO-LIPA Mycobacteria methods. NTM were identified from 4.9% (5/101) of water samples and 0.8% (1/124) of soil samples by culture and PCR. No NTM were detected in the raw milk samples. Three of the NTM strains isolated from water samples were defined as *Mycobacterium chelonae* type Ill and two as *Mycobacterium kansasii* type II. One NTM strain isolated from soil was defined as *Mycobacterium fortuitum*. It was of note that two of the five NTM positive water samples were tap water samples collected from hospitals. It was concluded that NTM colonization/contamination of water and environment in the hospitals was a potential risk factor in terms of nosocomial infections. Thus surveillance cultures of the water systems and the medical devices in the hospital are necessary to fix the source of NTM, to identify and type the strains and to establish effective control measures such as sterilization, disinfection, maintenance and modernization of water systems.	Cafri et al. 2010
Drinking water	Nontuberculous mycobacteria (NTM) are environmental opportunistic pathogens of humans and animals. They are found in a wide variety of habitats to which humans are exposed, including drinking water distribution systems and household water and plumbing. In that regard, they are distinct from their obligate pathogenic relatives, the members of the *Mycobacterium tuberculosis* complex. Owing to the presence of NTM in the human environment, human activities have had direct impacts on their ecology and thereby their epidemiology. NTM are oligotrophic, able to grow at low organic matter concentrations and over a wide range of temperatures, and even at low oxygen concentrations. Thus, NTM are normal inhabitants of natural waters and drinking waters. Discovery of the presence of NTM-polluted soils is not surprising in light of the ability of NTM to degrade a variety of hydrocarbon pollutants. A major human activity selecting for the growth and predominance of mycobacteria in habitats is disinfection. In comparison to other bacteria, NTM are disinfectant, heavy metal and antibiotic resistant. Therefore, the use of any antimicrobial agent selects for mycobacteria. Use of disinfectant in drinking water treatment selects for mycobacteria that can grow and come to proliferate in drinking water distribution systems in the absence of disinfectant-sensitive competing microorganisms. NTM selection may also occur as a consequence of antibiotics in drinking water sources.	Falkinham 2010

Household water	To determine whether plumbing could be a source of nontuberculous mycobacteria (NTM) infection, during 2007–2009 I isolated NTM from samples from household water systems of NTM patients. Samples from 22/37 (59%) households and 109/394 (28%) total samples yielded NTM. Seventeen (46%) of the 37 households yielded ≥ 1 *Mycobacterium* spp. isolate of the same species as that found in the patient; in 7 of those households, the patient isolate and 1 plumbing isolate exhibited the same repetitive sequence-based PCR DNA fingerprint. Households with water heater temperatures ≤125 degrees C (≤ 50 degrees C) were significantly more likely to harbor NTM compared with households with hot water temperatures ≥ 130 degrees F (≥ 55 degrees C) (p = 0.0107). Although households with water from public or private water systems serving multiple households were more likely to have NTM (19/27, 70%) compared with households with a well providing water to only 1 household (5/12, 42%), that difference was not significant (p = 0.1532).	Falkinham, III 2011
Drinking water *M. avium* **complex**	*Mycobacterium avium* complex (MAC) is a group of opportunistic pathogens of major public health concern. It is responsible for a wide spectrum of disease dependent on subspecies, route of infection and patients pre-existing conditions. Presently, there is limited research on the incidence of MAC infection that considers both pulmonary and other clinical manifestations. MAC has been isolated from various terrestrial and aquatic environments including natural waters, engineered water systems and soils. Identifying the specific environmental sources responsible for human infection is essential in minimizing disease prevalence. This paper reviews current literature and case studies regarding the wide spectrum of disease caused by MAC and the role of potable water in disease transmission. Potable water was recognized as a putative pathway for MAC infection. Contaminated potable water sources associated with human infection included warm water distribution systems, showers, faucets, household drinking water, swimming pools and hot tub spas. MAC can maintain long-term contamination of potable water sources through its high resistance to disinfectants, association with biofilms and intracellular parasitism of free-living protozoa. Further research is required to investigate the efficiency of water treatment processes against MAC and into construction and maintenance of warm water distribution systems and the role they play in MAC proliferation.	Whiley et al. 2012
Drinking water *M. a. paratuberculosis*	*Mycobacterium avium* subsp. *paratuberculosis* (Map) is the cause of Johne's disease, a chronic infection of the gut, in ruminant animals that provide milk and/or meat for human consumption. Map also may be involved in Crohn's disease and type I diabetes in humans. Although the role of Map in human diseases has not been established, minimizing the exposure of humans to the organism is considered desirable as a precautionary measure. 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. The presence of at least some Map in raw milk and meat and in natural waters is likely, but the numbers of Map in those foods and waters should be reduced through cooking or purification. The available information relating to Map in milk and dairy products, meats, and drinking water is reviewed here for assessment of the risks of exposure to Map from consumption of such foods and water.	Gill et al. 2011

Drinking water	In this study, we enlarged our previous investigation focusing on the biodiversity of chlamydiae and amoebae in a drinking water treatment plant, by the inclusion of two additional plants and by searching also for the presence of legionellae and mycobacteria. Autochthonous amoebae were recovered onto non-nutritive agar, identified by 18S rRNA gene sequencing, and screened for the presence of bacterial endosymbionts. Bacteria were also searched for by *Acanthamoeba* co-culture. From a total of 125 samples, we recovered 38 amoebae, among which six harboured endosymbionts (three chlamydiae and three legionellae). In addition, we recovered by amoebal co-culture 11 chlamydiae, 36 legionellae (no *L. pneumophila*), and 24 mycobacteria (all rapid-growers). Two plants presented a similar percentage of samples positive for chlamydiae (11%), mycobacteria (20%) and amoebae (27%), whereas in the third plant the number of recovered bacteria was almost twice higher. Each plant exhibited a relatively high specific microbiota. Amoebae were mainly represented by various *Naegleria* species, *Acanthamoeba* species and *Hartmannella vermiformis*. *Parachlamydiaceae* were the most abundant chlamydiae (8 strains in total), and in this study we recovered a new genus-level strain, along with new chlamydiae previously reported. Similarly, about 66% of the recovered legionellae and 47% of the isolated mycobacteria could represent new species. Our work highlighted a high species diversity among legionellae and mycobacteria, dominated by putative new species, and it confirmed the presence of chlamydiae in these artificial water systems.	Corsaro et al. 2010
Drinking water	Data on the occurrence of non-tuberculous mycobacteria (NTM), in parallel with those obtained for bacterial indicators and amoebae, are presented with the aim to collect information on the spread of NTM in drinking water distribution systems in Italy. Samples were collected from taps of hospitals and households in Central and Southern Italy. The concentration values obtained for the more traditional microbial parameters complied with the mandatory requirements for drinking water. Conversely, moderate-to-high microbial loads (till 300 CFU/L) were observed for the NTM. Positive samples were obtained from 62% of the investigated water samples. Analogous results were observed for amoebae showing a higher percentage of positive samples (76%). In terms of public health, the presence of mycobacteria in water distribution systems may represent a potential risk especially for vulnerable people such as children, the elderly or immunocompromised individuals.	Briancesco et al. 2010
Drinking water	*Legionella* and *Mycobacterium* can proliferate within free-living amoebae (FLA) where they are protected from disinfectants at concentrations that can kill bacteria but not protozoa. Despite effective treatment of drinking water, microbes can enter water utility distribution systems (DS) and hence the plumbing within building premises. Additionally, biofilm formation may account for the persistence of microbes in the DS. In the present study a domestic water tap in north-central United States (USA) was sampled in March and September 2007 and analysed for FLA, *Legionella* and *Mycobacterium*. Identification of organisms was determined by growth on specific culture media, light and electron microscopy, and amplification of DNA probes specific for each organism. In both the spring and fall samples, amoebae, *Legionella* and *Mycobacterium* were detected. However, *Acanthamoeba* was prominent in the spring sample whereas *Vahlkampfia* and *Naegleria* were the amoebae detected in the autumn. Bacterial proliferation in laboratory cultures was noticeably enhanced in the presence of amoebae and biofilms rapidly formed in mixed amoebae and bacteria cultures. It is hypothesized that temperature affected the dynamics of FLA species population structure within the DS and that pathogenic bacteria that proliferate within FLA, which are themselves opportunistic pathogens, pose dual public health risks.	Marciano-Cabral et al. 2010

Amoeba microbial pathogens	Free-living amoebae that belong to the genus *Acanthamoeba* are widespread in the environment, including water. They are responsible for human infections and can host pathogenic microorganisms. Under unfavorable conditions, they form cysts with high levels of resistance to disinfection methods, thus potentially representing a threat to public health. In the present study we evaluated the efficacies of various biocides against trophozoites and cysts of several *Acanthamoeba* strains. We demonstrated that disinfectant efficacy varied depending on the strains tested, with environmental strains demonstrating greater resistance than collection strains. Trophozoites were inactivated by all treatments except those using glutaraldehyde as an active compound: for these treatments, we observed resistance even after 30 min exposure. Cysts resisted many treatments, including certain conditions with glutaraldehyde and other biocides. Moist heat at 55 degrees C was not efficient against cysts, whereas exposure at 65 degrees C was. Several chemical formulations containing peracetic acid, hydrogen peroxide, or ortho-phthalaldehyde presented greater efficacy than glutaraldehyde, as did ethanol and sodium hypochlorite; however, some of these treatments required relatively long incubation times to achieve cyst inactivation. Amoebal cysts can be highly resistant to some high-level disinfectants, which has implications for clinical practice. These results highlight the need to consider the effective disinfection of protozoa in their vegetative and resistant forms due to their intrinsic resistance. This is important not only to prevent the transmission of protozoa themselves but also due to the risks associated with a range of microbial pathogens that are found to be associated intracellularly with these microorganisms.	Coulon et al. 2010
Drinking water	There is an expanding body of evidence that free-living amoebae (FLA) increase both the numbers and virulence of water-based, human-pathogenic, amoeba-resisting microorganisms (ARM). *Legionella* spp., *Mycobacterium* spp., and other opportunistic human pathogens are known to be both ARM and also the etiologic agents of potentially fatal human lung infections. However, comparatively little is known about the FLA that may facilitate ARM growth in drinking water. This review examines the available literature on FLA in treated drinking water systems; in total 26 studies from 18 different countries. FLA were reported to breakthrough the water treatment barrier and enter distribution systems, in addition to the expected post-treatment system ingress. Once in the distribution system there is evidence of FLA colonization and regrowth especially in reservoirs and in-premise plumbing storage tanks. At the point of use the average FLA detection rate was 45% but highly variable (n = 16, sigma = 31) due to both differences in both assay methods and the type of water systems examined. This review reveals that FLA are consistently detected in treated drinking water systems around the world and present a yet unquantified emerging health risk. However, more research is urgently required before accurate risks assessments can be undertaken to assess the impacts on human health, in households and institutions, due to exposure to FLA facilitated pathogenic ARM.	Thomas and Ashbolt 2011
Drinking water	Culture-based methods for fecal indicator microorganisms are the standard protocol to assess potential health risk from drinking water systems. However, these traditional fecal indicators are inappropriate surrogates for disinfection-resistant fecal pathogens and the indigenous pathogens that grow in drinking water systems. There is now a range of molecular-based methods, such as quantitative PCR, which allow detection of a variety of pathogens and alternative indicators. Hence, in addition to targeting total *Escherichia coli* (i.e., dead and alive) for the detection of fecal pollution, various amoebae may be suitable to indicate the potential presence of pathogenic amoeba-resisting microorganisms, such as *Legionellae*. Therefore, monitoring amoeba levels by quantitative PCR could be a useful tool for directly and indirectly evaluating health risk and could also be a complementary approach to current microbial quality control strategies for drinking water systems.	Codony et al. 2012

Drinking water *M. mucogenicum*	Objective to better understand the mechanism of chlorine resistance of mycobacteria and evaluate the efficiency of various disinfection processes. Methods Inactivation experiments of one strain *Mycobacteria mucogenicum*, isolated from a drinking water distribution system in South China were conducted with various chlorine disinfectants. Inactivation efficiency and disinfectant residual, as well as the formation of organic chloramines, were measured during the experiments. Results: This strain of *M. mucogenicum* showed high resistance to chlorine. The CT values of 99.9% inactivation by free chlorine, monochloramine and chlorine dioxide were detected as 29.6 ± 1.46, 170 ± 6.16, and 10.9 ± 1.55 min.(mg/L) respectively, indicating that chlorine dioxide exhibited significantly higher efficiency than free chlorine and monochloramine. It was also found that *M. mucogenicum* reacted with chlorine disinfectants more slowly than *S. aureus*, but consumed more chlorine disinfectants during longer time of contact. Lipid analysis of the cell construction revealed that 95.7% of cell membrane lipid of *M. mucogenicum* was composed of saturated long chain fatty acids. Saturated fatty acids were regarded as more stable and more hydrophilic which enabled the cell membrane to prevent the diffusion of chlorine. Conclusion It was concluded that different compositions of cell membrane might endow *M. mucogenicum* with a higher chlorine resistance.	Chen et al. 2012
Therapy pool water *M.* sp.	...we conducted a multiseason survey of microorganisms present in this therapy pool water, in biofilms associated with the pool containment walls, and in air immediately above the pool. The survey used culture, microscopy, and culture-independent molecular phylogenetic analyses. Although outfitted with a state-of-the art UV-peroxide disinfection system, the numbers of bacteria in the therapy pool water were relatively high compared with the potable water used to fill the pool. Regardless of the source, direct microscopic counts of microbes were routinely 1,000 times greater than conventional plate counts. Analysis of clone libraries of small subunit rRNA genes from environmental DNA provided phylogenetic diversity estimates of the microorganisms collected in and above the pool. A survey of >1,300 rRNA genes yielded a total of 628 unique sequences, the most common of which was nearly identical to that of *M. avium* strains. The high proportion of clones with different *Mycobacterium* spp. rRNA genes suggested that such organisms comprised a significant fraction of microbes in the pool water (to >30%) and preferentially partition into aerosols (to >80%) relative to other waterborne bacteria present.	Angenent et al. 2005
Hot tub aerosol *M.* sp.	Human activities associated with aerosol-generating hot water sources are increasingly popular. Recently, a hypersensitivity pneumonitis (HP)-like granulomatous lung disease, with non-tuberculous mycobacteria from exposure to hot water aerosols from hot tubs/spas, showers, and indoor swimming pools, has been described in immunocompetent individuals (also called "hot tub lung"). Our objective in this study was to examine four additional cases of hot tub lung and compare these cases with others reported in the English print literature on this disease. We retrospectively reviewed all cases (n = 4) of presumptively diagnosed hot tub lung in immunocompetent individuals at the various physician practices in Springfield, Illinois, during 2001–2005. In addition, we searched MEDLINE for cases of hot tub lung described in the literature. We summarized the clinical presentation and investigations of four presumptive cases and reviewed previously reported cases of hot tub lung. There is a debate in the literature whether hot tub lung is an HP or a direct infection of the lung by nontuberculous mycobacteria. Primary prevention of this disease relies on ventilation and good use practices. Secondary prevention of this disease requires education of both the general public and clinicians to allow for the early diagnosis of this disease.	Sood et al. 2007

Hot tubs *M. avium*	To assess the current spectrum of causes and clinical features associated with hypersensitivity pneumonitis (HP). We studied consecutive patients with HP diagnosed at the Mayo Clinic in Rochester, Minn, from January 1, 1997, through December 31, 2002. Diagnostic criteria for HP included the following: (1) presence of respiratory symptoms, (2) radiologic evidence of diffuse lung disease, (3) known exposure or a positive serologic test result to an inciting antigen, and (4) no other identifiable cause for the lung disease. If there was no identifiable inciting antigen, I of the following 2 criteria was required: (1) lung biopsy specimen that demonstrated features of HP or (2) bronchoalveolar lavage lymphocytosis and high-resolution computed tomographic evidence of ground-glass opacities or centrilobular nodules bilaterally. The meant SO age of the 85 study patients was 53 ± 14 years; 53 patients (62%) were women. Only 2 patients (2%) were current smokers. Chronic (A months) respiratory symptoms were present in 66 patients (78%). Histopathologic confirmation was obtained in 64 patients (75%). The cause was identified in 64 patients (75%), and the most common causes were avian anti-gens (34%) and *Mycobacterium avium* complex in hot tub water (21%). Farmer's lung disease accounted for 3.1% of cases, and an additional 9% were related to household mold exposure. The inciting antigen was not identifiable in 25% of patients. Most patients with HP seen at this tertiary care referral center in the Midwest region of the United States had chronic HIP, and the most common causes were exposure to birds and exposure to hot tubs.	Hanak et al. 2007
Hot tubs *M. avium*	The objective of our study was to describe the CT features of "hot tub lung" caused by exposure to *Mycobacterium avium* complex (MAC) organisms in contaminated water Chart review was performed to identify all patients with a histologic diagnosis of granulomatous pneumonitis and positive cultures for MAC between January 1, 1995, and July 1, 2004. Individuals identified who also had a hot tub were included in the study. Twelve patients, seven females and five males with an average age of 50 years (range, 13–66 years), who had a CT scan were identified. The CT images were reviewed by two thoracic radiologists who assessed the images for the presence of any parenchymal abnormalities, including nodules, areas of ground-glass attenuation, reticular opacities, and air trapping, on expiratory images. When nodules, reticular opacities, areas of ground-glass attenuation, or a combination of these findings was present, the reviewers visually determined the extent of involvement of the lungs using a scale of < 10%, 10–40%, or > 40%. They also recorded the distribution of the involvement both cephalocaudal and transaxial. Decisions were reached by consensus of the reviewers. Nodules were present in 10 (83%) of 12 patients. In eight (80%) of 10 patients, the nodules were diffuse with a centrilobular distribution. In the other two, the nodules were randomly distributed with an upper lung predominance. In five patients the nodules showed areas of ground-glass attenuation, whereas in the other five the nodules were solid. Areas of ground-glass attenuation were present in eight (75%) of 12 patients and were bilateral in all cases. The areas of ground-glass attenuation were diffuse in the cephalocaudal plane with a random distribution in the transaxial plane in seven (88%) of eight cases. In the remaining case, the areas of ground-glass attenuation had a lower lung predominance with a random distribution in the transaxial plane. Expiratory images showed evidence of air trapping in all seven cases for which these images were available. In one patient, air trapping was the only abnormality identified. The CT findings in patients with hot tub lung include areas of ground-glass attenuation, centrilobular nodules, and air trapping on expiratory images. These findings are similar to previously published findings of subacute hypersensitivity pneumonitis. Therefore, in cases in which CT findings suggest hypersensitivity pneumonitis, hot tub lung should also be a diagnostic consideration.	Hartman et al. 2007

Shower water and aerosol *M.* sp. *M. mucogenicum* *M. phocaicum*	To quantify the microbial load in shower water and aerosol samples, we used culture, microscopic, and quantitative PCR methods to investigate four shower stalls in a stem cell transplant unit at Barnes-Jewish Hospital in St. Louis, MO. We also tested membrane-integrated showerheads as a possible mitigation strategy. In addition to quantification, a 16S rRNA gene sequencing survey was used to characterize the abundant bacterial populations within shower water and aerosols. The average total bacterial counts were 2.2×10^7 cells/liter in shower water and 3.4×10^4 cells/m^3 in shower aerosol, and these counts were reduced to 6.3×10^4 cells/liter (99.6% efficiency) and 8.9×10^3 cells/m^3 (82.4% efficiency), respectively, after membrane-integrated showerheads were installed. Potentially pathogenic organisms were found in both water and aerosol samples from the conventional showers. Most notable was the presence of *Mycobacterium mucogenicum* (99.5% identity) in the water and Pseudomonas aeruginosa (99.3% identity) in the aerosol samples.	Perkins et al. 2009
Hot tub air and water *M.* sp.	Hot tub exposure has been causally associated with a steroid-responsive, granulomatous lung disease featuring nontuberculous mycobacterial (NTM) growth in both clinical and environmental samples. Little is known regarding prevalence of and risk factors for NTM-contamination and associated illness in these settings. In this study, the frequency of NTM growth and aerosolization in 18 public hot tubs and warm water therapy pools and the factors associated with mycobacterial growth were analyzed. Each site was characterized by water chemistry analysis; a questionnaire on maintenance, disinfection, and water quality; and air and water sampling for quantitative NTM culture. NTM were detected in air or water from 13/18 (72%) sites; a strong correlation was found between the maximum air and water NTM concentrations (rho 0.49, $p = 0.04$). Use of halogen (chlorine or bromine) disinfection was associated with significantly lower air and water concentrations of NTM compared with disinfection using ultraviolet light and hydrogen peroxide ($p = 0.01$-0.04). Higher water turnover rates were also associated with lower air and water NTM concentrations ($p = 0.02$-0.03). These findings suggest that NTM are frequently detectable in the air and water of spas and therapy pools and that particular maintenance and disinfection approaches affect NTM bioaerosol concentrations in these settings.	Glazer et al. 2007
Whirlpool spa water *M.* sp.	An outbreak of occupational hot tub lung necessitated quantitative analysis of mycobacteria in water samples. We combined procedures for cultivation of mycobacteria in urine and quantitative analyses of dialysis water. Whirlpool spa water samples were analyzed showing promising results. In conclusion, quantitative mycobacterial culture of water is possible by adapting methods routinely used in clinical laboratories.	Svensson et al. 2011

Bottled water *M. avium*	Adhesion of the bacteria *Campylobacter jejuni* and *Mycobacterium avium* onto polyethylene terephtalate (PET), a polymer widely used within the bottled water industry was measured in two different groundwater solutions. From this, it was found that whilst the percentage cell adhesion for a given strain did not change between groundwater types, substantial variation was obtained between the two bacterial species tested: *M. avium* (10–30% adhered cells) and C. jejuni (1–2%) and no major variations were measured as a function of groundwater composition for a given strain. To explain this, the interfacial electro-hydrodynamic properties of the bacteria were investigated by microelectrophoresis, with the resultant data analysed on the basis of electrokinetic theory for soft biocolloidal particles. The results obtained showed that *M. avium* carries a significant volume charge density and that its peripheral layer exhibits limited hydrodynamic flow permeation compared to that of *C. jejuni*. It was also demonstrated that steric hindrance to flow penetration and the degree of hydrophobicity within/of the outer bacterial interface are larger for *M. avium* cells. In line with this, the larger amount of *M. avium* cells deposited onto PET substrates as compared to that of *C. jejuni* can be explained by hydrophobic attraction and chemical binding between hydrophobic PET and outer soft surface layer of the bacteria. Hydrophobicity of PET was addressed by combining contact angle analyses and force spectroscopy using CH_3-terminated AFM tip.	Tatchou-Nyamsi-Konig et al. 2008
Bottled water *M. avium*	The main objective of our study was to assess the persistence of *Mycobacterium avium* in an oligotrophic environment such as bottled groundwater. Filtered groundwater samples were spiked with washed *Myco. avium* suspension and stored in dark and under static conditions, at 20 degrees C, for 3 months in 500 ml PET bottles. The loss of *Myco. avium* cultivability was slow in water. On the contrary, after a 3-month storage at 20 degrees C, growth of attached cells was observed and cell adhesiveness to the PET wall increased with time. It could probably be because of the presence of an extracellular matrix. This study has shown the great stability of *Myco. avium* in bulk water as well as their adhesiveness and their growth on a PET bottle wall in an oligotrophic environment. Slowly growing mycobacteria are well adapted to oligotrophic environments such as groundwater. As they stick very well to surfaces, they could be used for determining the efficiency of the cleaning of contaminated surfaces.	Tatchou-Nyamsi-Konig et al. 2009
Industry water *M.* sp.	Bacteria were enumerated by conventional culture method and fluorescent vital staining. Activated carbon treatment and storage in a tank provided favourable environments for bacterial growth. The bacterial population of the water in both the post-activated carbon treatment and the tank was analysed by denaturing gradient gel electrophoresis (DGGE) with PCR-amplified 16S rDNA fragments including V6, -7, and -8 regions. The bacterial community structure in activated carbon treated water was stable throughout the year. Several kinds of bacteria such as genus *Aquaspirillum* and *Methylobacterium* were found in the water after activated carbon treatment. The bacterial community structure was changed and other bacteria such as mycobacteria were detected after storage. Mycobacteria were quantified in water samples using real-time PCR targeting the 16S rDNA gene. Mycobacteria were also detected in tap water and their number was increased 10^3–10^4-fold higher after storage.	Kawai et al. 2004
Industry water *M. a. paratuberculosis*	Culture-independent techniques were used for the detection of pathogenic bacteria in drinking water at potentially critical control points along the production lines at a German dairy company and a Spanish dry cured ham company. Denaturing gradient gel electrophoresis (DGGE) was used to describe bacterial population shifts indicating biological instability in the drinking water samples. Autochthonous bacteria were identified by sequencing the excised DGGE DNA bands. More specifically, real-time PCR was applied to detect a number of pathogenic bacteria, i.e. *Listeria monocytogenes*, *Mycobacterium avium* subsp. *paratuberculosis*, *Campylobacter jejuni*, *Enterococcus* spp., *Salmonella* spp., *Escherichia coli*, and *Pseudomonas aeruginosa*.	Villarreal et al. 2010

Drinking water Free-living amoebae	Free-living amoebae constitute reservoirs for many bacteria including not only well-known pathogens but also emerging pathogens responsible for respiratory diseases, and contribute to the protection, survival and dissemination of these bacteria in water systems, despite the application of disinfection or thermal treatments. In this article we review the available information on the presence of free-living amoebae and amoebae-resisting bacteria in drinking water systems, on the factors that contribute to their presence in the water and/or the biofilms, on the possible control measures and their effectiveness, and we identify some gaps in current knowledge needing further research.	Loret and Greub 2010
Water used for swimming	The first of this three-part series on water-related dermatoses involving the athlete will include sports occurring with the majority of time spent in the water. These sports include swimming, diving, scuba, snorkeling and water polo. Numerous authors have described dermatologic conditions commonly seen in swimmers. This series provides an updated and comprehensive review of these water dermatoses. In order to organize the vast number of skin conditions related to water exposure, we divided the skin conditions into groupings of infectious and organism-related dermatoses, irritant and allergic dermatoses and miscellaneous dermatoses. The vast majority of skin conditions involving the water athlete result from chemicals and microbes inhabiting each environment. When considering the effects of swimming on one's skin, it is also useful to differentiate between exposure to freshwater (lakes, ponds and swimming pools) and exposure to saltwater. This review will serve as a guide for dermatologists, sports medicine physicians and other medical practitioners in recognition and treatment of these conditions.	Tlougan et al. 2010a,b,c
Watering troughs *M. a. paratuberculosis*	*Mycobacterium avium* subsp. *paratuberculosis* (Map) is the causative agent of Johne's disease, a chronic enteric infection that affects ruminants. Despite the ubiquitous occurrence of *Mycobacterium* sp. in nature and the fact that Johne's disease has been reported worldwide, little research has been done to assess its survival in agricultural environments. The goal of this 365-day study was to evaluate the ability of Map to persist in mixed-community biofilms on materials commonly used to construct livestock watering troughs. Map was inoculated into 32 l of trough water containing either concrete, plastic, galvanized OF stainless steel trough materials. The concentration of Map was determined by using quantitative, real-time PCR to target the IS*900* sequence in DNA extracts. High concentrations of Map were detected on all trough materials after 3 days (around 1×10^5 cells/cm^2). Based on the best-fit slopes, the time required for a 99% reduction (t_{99}) in biofilm-associated Map cells was 144 and 115 days for plastic and stainless steel trough materials, respectively. Map concentrations did not decrease on concrete and galvanized steel trough materials. These results suggest that Map survives well in biofilms present on livestock watering trough materials. To inhibit spread of this organism and exposure of susceptible animals to Map on infected farms, best management practices aimed at maintaining biofilm-free trough surfaces should be included in any Johne's control plan.	Cook et al. 2010
Natural water (river or lake) *M.* sp. *M. avium*	Water, sediment, and stems and roots of common reed (*Phragmites australis*) and greater reedmace (*Typha latifolia*) were taken from 15 locations within the reed bed plus sites upstream and downstream. Samples were analysed for mycobacteria using PCR and specifically for *M. avium* using nested PCR. Environmental mycobacteria were found throughout the entire reed bed but *M. avium* was not found downstream of the first vegetation growth. The reed bed was found to effectively remove *M. avium* from the water through a combination of sedimentation and adsorption onto vegetation stems.	Drewe et al. 2009

Lake water *M.* sp.	The actinobacterial communities present in two Finnish lakes and in the surrounding conifer forest soil were investigated using DNA based methods. The dominant actinobacteria in the soil were found to belong to genus *Mycobacterium*. Therefore specific primers were designed and tested to study the mycobacterial communities in boreal environment more closely. The denaturing gradient gel electrophoresis (DGGE) and sequencing analysis showed that the microbial populations in lakes were different from those in the surrounding soil. Thus, each of the environments had their own actinobacterial and mycobacterial populations. The majority of the obtained mycobacterial sequences were closely related to the described species of environmental mycobacteria, some of which are pathogenic.	Niva et al. 2006
Natural water *M. a. paratuberculosis*	A limited survey was undertaken in Northern Ireland to test for *M. avium* subsp. *paratuberculosis* in untreated water entering nine water treatment works (WTWs) over a 1-year period. Three detection methods were employed, viz., immunomagnetic separation-PCR and culture on Herrold's egg yolk medium (HEYM) and BACTEC 12B medium, the latter both supplemented with mycobactins. Of the 192 untreated water samples tested, 15 (8%) tested *M. avium* subsp. *paratuberculosis* positive by one or more of the three detection methods.	Whan et al. 2005
River water and sediment *M. shottsii* *M. pseudoshotsii*	Striped bass (*Morone saxatilis*) in the Chesapeake Bay are currently experiencing a very high prevalence of mycobacteriosis associated with newly described *Mycobacterium* species, *Mycobacterium pseudoshottsii* and *M. shottsii*. The ecology of these mycobacteria outside the striped bass host is currently unknown. In this work, we developed quantitative real-time PCR assays for *M. pseudoshottsii* and *M. shottsii* and applied these assays to DNA extracts from Chesapeake Bay water and sediment samples, as well as to tissues from two dominant prey of striped bass, Atlantic menhaden (*Brevoortia tyrannus*) and bay anchovy (*Anchoa mitchilli*). *Mycobacterium pseudoshottsii* was found to be ubiquitous in water samples from the main stem of the Chesapeake Bay and was also present in water and sediments from the Rappahannock River, Virginia. *M. pseudoshottsii* was also detected in menhaden and anchovy tissues. In contrast, *M. shottsii* was not detected in water, sediment, or prey fish tissues. In conjunction with its nonpigmented phenotype, which is frequently found in obligately pathogenic mycobacteria of humans, this pattern of occurrence suggests that *M. shottsii* may be an obligate pathogen of striped bass.	Gauthier et al. 2010
River water *M. a. paratuberculosis*	In South Wales, United Kingdom, a populated coastal region lies beneath hill pastures grazed by livestock in which *Mycobacterium avium* subsp. *paratuberculosis* is endemic. The Taff is a spate river running off the hills and through the principal city of Cardiff. We sampled Taff water above Cardiff twice weekly from November 2001 to November 2002. *M. avium* subsp. *paratuberculosis* was detected by IS*900* PCR and culture. Thirty-one of 96 daily samples (32.3%) were IS*900* PCR positive, and 12 grew *M. avium* subsp. *paratuberculosis* bovine strains.	Pickup et al. 2005

River water *M. a. paratuberculosis*	We studied the River Tywi in South Wales, United Kingdom, whose catchment comprises 1,100 km^2 containing more than a million dairy and beef cattle and more than 1.3 million sheep. The River Tywi is abstracted for the domestic water supply. Between August 2002 and April 2003, 48 of 70 (68.8%) twice-weekly river water samples tested positive by IS*900* PCR. In river water, the organisms were associated with a suspended solid which was depleted by the water treatment process. Disposal of contaminated slurry back onto the land established a cycle of environmental persistence. A concentrate from 100 liters of finished water tested negative, but 1 of 54 domestic cold water tanks tested positive, indicating the potential for these pathogens to access domestic outlets. In the separate English Lake District region, with hills up to 980 m, tests for *M. avium* subsp. *paratuberculosis* in the high hill lakes and sediments were usually negative, but streams and sediments became positive lower down the catchment. Sediments from 9 of 10 major lakes receiving inflow from these catchments were positive, with sediment cores indicating deposition over at least 40 to 50 years. Two of 12 monthly 1-liter samples of effluent and a single 100-liter sample from the Ambleside sewage treatment works were positive for *M. avium* subsp. *paratuberculosis*. Since Lake Ambleside discharges into Lake Windermere, which is available for domestic supply, there is a potential for these organisms to cycle within human populations.	Pickup et al. 2006
Coastal lagoon water *M.* sp.	This study uses indirect gradient analysis to illustrate the strong relationships that exists between coastal water quality and the abundance of *Mycobacterium* spp. within a U.S. mid-Atlantic embayment. *Mycobacterium* species abundance and water quality conditions (based on 16 physical and chemical variables) were examined simultaneously in monthly samples obtained at 18 Maryland and Virginia coastal bay stations from August 2005 to November 2006 (n = 212). A quantitative molecular assay for *Mycobacterium* spp. was evaluated and applied, allowing for rapid, direct enumeration. By using indirect gradient analysis (environmental principal components analysis), a strong linkage between eutrophic conditions, characterized by low dissolved-oxygen levels and elevated nutrient concentrations, and mycobacteria was determined. More specifically, a strong nutrient response was noted, with all nitrogen components and turbidity measurements correlating positively with abundance (r values of > 0.30; P values of < 0.001), while dissolved oxygen showed a strong negative relationship (r = –0.38; P = 0.01). Logistic regression models developed using salinity, dissolved oxygen, and total nitrogen showed a high degree of concordance (83%). These results suggest that coastal restoration and management strategies designed to reduce eutrophication may also reduce total mycobacteria in coastal waters.	Jacobs et al. 2009
Surface water *M. ulcerans*	This study reports the first successful application of real-time PCR for the detection of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer (BU), in Ghana, a BU-endemic country. Environmental samples and organs of small mammals were analyzed. The real-time PCR assays confirmed the presence of *M. ulcerans* in a water sample collected in a BU-endemic village in the Ashanti Region.	Vandelannoote et al. 2010

Table 4. Mycobacteria in soil

Type of soil Mycobacteria detected	Abstract excerpts	Reference
Arable soil *M.* sp. *M. lentiflavum* *M. heidelbergense* *M. austroafricanum*	PCR primers designed to amplify part of the mycobacterial 16S rRNA gene were applied to DNA extracted from cultured organisms and soil. The PCR products from soil contained sequences with similarity to slow growing mycobacteria similar to *Mycobacterium lentiflavum*, and to fast growing mycobacteria such as the xenobiotic degraders PYR-I and RJGII.	Mendum et al. 2000
Boreal forest soil *M.* sp.	We applied a quantitative sandwich hybridization approach for direct detection of mycobacterial 16S rRNA in soil without a nucleic acid amplification step. The numbers of mycobacterial 16S rRNA molecules found in the soil indicated the presence of up to 10^7 to 10^8 mycobacterial cells per gram of soil. These numbers exceed by factor of 10 to 100 × the previous estimates of mycobacteria in soil based on culture methods. When real-time PCR with mycobacteria targeting primers was used to estimate the number of 16S rDNA copies in soil, one copy of 16S rDNA was detected per 10^4 copies of 16S rRNA. This is close to the number of 16S rRNA molecules detected per cell by the same method in laboratory pure cultures of *M. chlorophenolicum*.	Nieminen et al. 2006
Household soil *M.* sp.	A total of 148 soil samples and 24 water samples were collected from various locations and examined to determine the presence of mycobacteria. The detection method involved semiselective culturing and acid-fast staining, following decontamination of samples to enrich mycobacteria and reduce the numbers of other microorganisms, or PCR with primers specific for the mycobacterial 16S rRNA gene, using DNA extracted directly from soil and water samples. Mycobacteria were detected in the majority of the samples, and subsequent sequence analysis of PCR products amplified directly from soil DNA indicated that most of the products were related to known environmental mycobacteria. For both methods the rates of recovery were consistently higher for dry season samples than for wet season samples.	Chilima et al. 2006
Surface soil *M. ulcerans*	This paper describes the development of a TaqMan assay targeting IS*2404* multiplexed with an internal positive control to monitor inhibition with a detection limit of less than 1 genome equivalent of DNA. The assay improves the turnaround time for diagnosis and replaces conventional gel-based PCR as the routine method for laboratory confirmation of *M. ulcerans* infection in Victoria, Australia. Following analysis of 415 clinical specimens, the new test demonstrated 100% sensitivity and specificity compared with culture. Another multiplex TaqMan assay targeting IS*2606* and the ketoreductase-B domain of the *M. ulcerans* mycolactone polyketide synthase genes was designed to augment the specificity of the IS*2404* PCR for the analysis of a variety of environmental samples. Assaying for these three targets enabled the detection of *M. ulcerans* DNA in soil, sediment, and mosquito extracts collected from an area of endemicity for Buruli ulcer in Victoria with a high degree of confidence. Final confirmation was obtained by the detection and sequencing of variable-number tandem repeat (VNTR) locus 9, which matched the VNTR locus 9 sequence obtained from the clinical isolates in this region. This suite of new methods is enabling rapid progress in the understanding of the ecology of this important human pathogen.	Fyfe et al. 2007

Surface soil *M. bovis*	PCR primers specific for the *Mycobacterium tuberculosis* complex were used to detect the presence of *Mycobacterium bovis* BCG (Pasteur) in soil microcosms and *Mycobacterium bovis* in environmental samples taken from a farm in Ireland with a history of bovine tuberculosis. *M. bovis* genes were detected in soil at 4 and 21 months after possible contamination. Gene levels were found in the range of 1×10^3 to 3.6×10^3 gene copies per g of soil, depending on the sampling area. Areas around badger setts had the highest levels of detectable genes and were shown to have the highest levels of gene persistence. *M. bovis*-specific 16S rRNA sequences were detected, providing evidence of the presence of viable cells in Irish soils. Studies of DNA turnover in soil microcosms proved that dead cells of *M. bovis* BCG did not persist beyond 10 days.	Young et al. 2005
Surface soil (Antarctic) *M.* sp.	The diversity of actinomycetes was estimated with two different strategies that use PCR-denaturing gradient gel electrophoresis. First, a PCR was applied, using a group-specific primer that allows selective amplification of actinomycete sequences. Second, a nested-PCR approach was used that allows the estimation of the relative abundance of actinomycetes within the bacterial community. Molecular identification, which was based on 16S rDNA sequence analysis, revealed eight genera of actinomycetes, *Actinobacterium*, *Actinomyces*, an uncultured *Actinomycete*, *Streptomyces, Leifsonia*, *Frankineae*, *Rhodococcus*, and *Mycobacterium*.	Learn-Han et al. 2012
Aquifer send *M. a. paratuberculosis*	To investigate the processes controlling the transport of *Mycobacterium avium* subsp. *paratuberculosis* (Map) through aquifer materials. We measured two important surface characteristics known to affect bacterial attachment to sediment surfaces: surface charge and hydrophobicity. We then measured the transport of Map through laboratory columns packed with aquifer sand with varying ionic strength solutions and sediment surface charge. We found that Map has a strong negative charge and is highly hydrophobic and that the transport of Map through positively charged Fe-coated sands was reduced compared with transport through negatively charged clean quartz sand, although Map transport for all treatments was low compared with the transport behaviour reported in the literature for other bacteria. Our results suggest that the potential for groundwater contamination by Map is low; however, the organism may remain bound to the soil near the surface where it can be ingested by grazing animals or be released during run off events. This is the first study looking at the surface characteristics and transport behaviour of Map through aquifer materials and therefore provides important information for understanding the movement of Map in the environment.	Bolster et al. 2009
Forest soil *M.* sp.	Total metagenomic DNA was isolated from high Andean forest soil and subjected to taxonomical and functional composition analyses by means of clone library generation and sequencing . Most clone sequences were classified as Bacteria belonging to phlya *Actinobacteria, Proteobacteria* and *Acidobacteria*. Among the most represented orders were Actinomycetales (34% average), Rhizobiales, Burkholderiales and Myxococcales and with a greater number of sequences in the genus *Mycobacterium* (7% average), *Frankia, Streptomyces* and *Bradyrhizobium*.	Montana et al. 2012

Acidic forest soil *M.* sp. *M. simiae* *M. conspicuum* *M. cookii* *M. hodleri* *M. aichiense* *M. holsaticum* *M. tusciae* *M. pallens* *M. gadium*	The diversity of environmental mycobacteria was studied in water-logged acidic forest soil. Mycobacteria were assessed in upper and lower soil horizons and summer and winter seasons using T-RFLP and sequencing of 16S rRNA gene produced with Mycobacterium-specific primers. Mycobacteria diversity differed between both the two seasons and soil horizons. Cloning revealed the presence of mycobacteria belonging to three major clusters recognized within the genus, i.e. fast-growing, intermediate, and slow-growing species, with unprecedented abundance and diversity of the latter. Two novel clusters of sequences unrelated to the known mycobacteria were identified. This study raises the possibility that forest wetlands could serve as environmental reservoirs for an unexplored diversity of mycobacteria including those related to pathogenic species.	Kopecky et al. 2011
Garden/House soil (4"deep) *M. leprae*	In the present study, we have tried to detect viable *M. leprae* from soil samples in endemic areas by using molecular methods. Eighty soil samples were collected from villages of this area, DNA and RNA of *M. leprae* extracted and identified using specific M. leprae primers. PCR amplification was done and real-time RT-PCR was used to detect viable *M. leprae.* DNA targeting the 16S region of *M. leprae* was detected in 37.5%, whereas *M. leprae* RNA targeting the same region was detected in 35% of these samples. Of the total 80 samples, 40 were collected from residential areas of leprosy patients whereas 40 samples were from no-patient areas. Fifty-five percent positivity for 16S rRNA of *M. leprae* was observed from the "patient" area in comparison to 15% positivity from the "no-patient" area ($p < 0.001$). This study thus provides valuable information of presence of viable *M. leprae* in soil specimens, which would be of use in investigating the transmission dynamics in leprosy.	Lavania et al. 2008
Household soil *M. leprae*	...Evidence suggests that humidity may favor survival of *M. leprae* in the environment. Several reports show that non-human sources like 'naturally' infected armadillos or monkeys could act as reservoir for *M. leprae*. Inanimate objects or fomites like articles used by infectious patients may theoretically spread infection. However, it is only through detailed knowledge of the biodiversity and ecology that the importance of this mode of transmission can be fully assessed. Our study focuses here to decipher the role of environment in the transmission of the disease. Two hundred and seven soil samples were collected from a village in endemic area where active cases also resided at the time of sample collection. Slit skin smears were collected from 13 multibacillary (MB) leprosy patients and 12 household contacts of the patients suspected to be hidden cases. DNA and RNA of *M. leprae* were extracted and amplified using M. leprae specific primers. Seventy-one soil samples showed presence of *M. leprae* DNA whereas 16S rRNA could be detected in twenty-eight of these samples. Samples, both from the environment and the patients, exhibited the same genotype when tested by single nucleotide polymorphism (SNP) typing. Genotype of *M. leprae* found in the soil and the patients residing in the same area could help in understanding the transmission link in leprosy.	Turankar et al. 2012

Polycyclic aromatic hydrocarbons – Contaminated soil *M.* sp. *M. tusciae* *M. frederiksbergense* *M. austroafricanum* *M. petroleophylum*	To study the natural role and diversity of the *Mycobacterium* community in contaminated soils, a culture-independent fingerprinting method based on PCR combined with denaturing gradient gel electrophoresis (DGGE) was developed. New PCR primers were selected which specifically targeted the 16S rRNA genes of fast-growing mycobacteria, and single-band DGGE profiles of amplicons were obtained for most *Mycobacterium* strains tested. Strains belonging to the same species revealed identical DGGE fingerprints, and in most cases, but not all, these fingerprints were typical for one species, allowing partial differentiation between species in a *Mycobacterium* community. *Mycobacterium* strains inoculated in soil were detected with a detection limit of 10^6 CFU/g of soil using the new primer set as such, or approximately 10^2 CFU/g in a nested PCR approach combining eubacterial and the *Mycobacterium* specific primers. Using the PCR-DGGE method, different species could be individually recognized in a mixed *Mycobacterium* community.	Leys et al. 2005
Polycyclic aromatic hydrocarbons – Contaminated soil *M.* sp. *M. monascense* *M. chlorophenolicum*	In the present study 16S rRNA genes were PCR amplified using *Mycobacterium*-specific primers and separated by temperature gradient gel electrophoresis (TGGE), and prominent bands were sequenced to compare the indigenous *Mycobacterium* community structures in four pairs of soil samples taken from heavily contaminated and less contaminated areas at four different sites.	Cheung and Kinkle 2001
Stable floor *M. a. paratuberculosis*	The objectives of the present study were to estimate the correlation and association between Ct and CFU in fresh and thawed pooled fecal and environmental samples. Results of HEYM culture of 1,997 pooled fecal samples from cows in 14 herds, and 802 environmental samples from 109 dairies nationwide were negatively (inversely) correlated with their respective real-time qPCR results. The Spearman's rank correlation between Ct and CFU was good (–0.66) in fresh and thawed pooled fecal samples, and excellent (–0.76) and good (–0.61) in fresh and thawed environmental samples, respectively. The correlation varied from good (–0.53) to excellent (–0.90) depending on the number of samples in a fecal pool. Truncated regression models indicated a significant negative association between Ct and CFU in fecal pools and environmental samples. The use of real-time qPCR instead of HEYM can yield rapid, quantitative estimates of MAP load and allow for incorporation of real-time qPCR results of pooled and environmental samples in testing strategies to identify dairy cow groups with the highest MAP shedding.	Aly et al. 2010
Stable floor dust *M. a. paratuberculosis*	Environmental samples were collected to investigate the spatial and temporal spread of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in a dairy cattle barn before and after the introduction of two groups of MAP-shedding animals. Samples collected off the floor of the barn reflected the moment of sampling whereas samples collected by microfiber wipes at a minimal of 3 m height contained the accumulated settled dust over a 3-week period. Samples were analysed by IS*900* qPCR for the presence of MAP DNA and by culture for viable MAP bacteria. MAP DNA was detected in a large number of sites both before and after introduction cattle. MAP DNA was detected inside the barn in floor and dust samples from cubicles and slatted floors and in settled dust samples located above the slatted floors and in the ventilation ridge opening. Outside the barn MAP DNA was detected by PCR in samples reflecting the walking path of the farmer despite hygiene measures. No viable MAP was detected before the introduction of shedder cattle. Three weeks later viable MAP was found inside the barn at 7/49 locations but not outside. Fifteen weeks later viable MAP was also detected in environmental samples outside the barn. In conclusion, introduction of MAP shedding cattle lead to widespread contamination of the internal and external environment of a dairy barn, including the presence of viable MAP in settled dust particles suggesting potential transmission of MAP infection through bio-aerosols.	Eisenberg et al. 2009

Potting and garden soil *M. a. avium* *M. a. hominissuis*	In order to trace the source of infection from the environment, a method of DNA isolation from soil and other environmental samples, such as dust, cobwebs, and compost, was developed. The triplex qPCR examination revealed the presence of *M. avium* subsp. *hominissuis* in a high proportion of the environmental samples (42.8% in the first patient's house and 47.6% in the second patient's house). Both patients were also exposed to *M. avium* subsp. *avium*, which was present due to the breeding of infected domestic hens. The high infectious dose of *M. avium* subsp. *hominissuis* or the increased susceptibility of humans to *M. avium* subsp. *hominissuis* compared to *M. avium* subsp. *avium* could be the reason why the children were infected with *M. avium* subsp. *hominissuis*.	Kaevska et al. 2011
Potting and garden soil *M. avium* complex	Background: *Mycobacterium avium*-intracellulare complex (MAC) is a ubiquitous pathogen found in soil and water. Environmental exposure is the primary route for MAC infection. However, specific environmental risk factors have been poorly determined in immunocompetent patients with pulmonary MAC disease. Methods: A case-control study was performed with 106 patients with pulmonary MAC disease (men [women], 23 [83]; age, 64.3 ± 9.2 years) and 53 age-matched control patients with bronchiectasis but not pulmonary MAC infection (men [women], 7[46]; age, 63.0 ± 11.0 years). All participants completed a standardized questionnaire that included questions about medical history, smoking history, alcohol usage, age at menopause, and environment exposures. Environment exposures included soil exposure from farming or gardening; water exposure from bathing, showering, hot tub use, dishwashing, swimming, and drinking water; and pet exposure. Results: No differences were identified in the patient characteristics and underlying diseases. More case patients experienced high soil exposure (≥ 2 per week) than control patients (23.6% vs 9.4%, P = .032); this remained significant after multivariate analysis (OR, 5.9; 95% CI, 1.4–24.7; P = .015). There were no significant differences in other environmental exposures. Case patients with high soil exposure were significantly older than those with low soil exposure (67.3 ± 7.3 years vs 64.3 ± 9.5 years, P = .037). Other characteristics, underlying diseases, and mycobacterial species did not differ between the two groups. Conclusions: Patients with pulmonary MAC disease had significantly more soil exposure than noninfected control patients, which suggests that environmental soil exposure is a likely risk factor for the development of pulmonary MAC disease.	Maekawa et al. 2011
Different soil types *M. a. paratuberculosis*	Attachment of *Mycobacterium avium* subsp. *paratuberculosis* to soil particles could increase their availability to farm animals, as well as influence the transportation of *M. avium* subsp. *paratuberculosis* to water sources. To investigate the possibility of such attachment, we passed a known quantity of *M. avium* subsp. *paratuberculosis* through chromatography columns packed with clay soil, sandy soil, pure silica, clay-silica mixture, or clay-silica complexes and measured the organisms recovered in the eluent using culture or quantitative PCR. Experiments were repeated using buffer at a range of pH levels with pure silica to investigate the effect of pH on *M. avium* subsp. *paratuberculosis* attachment. Linear mixed-model analyses were conducted to compare the proportional recovery of *M. avium* subsp. *paratuberculosis* in the eluent between different substrates and pH levels. Of the organisms added to the columns, 83 to 100% were estimated to be retained in the columns after adjustment for those retained in empty control columns. The proportions recovered were significantly different across different substrates, with the retention being significantly greater ($P < 0.05$) in pure substrates (silica and clay-silica complexes) than in soil substrates (clay soil and sandy soil). However, there were no significant differences in the retention of *M. avium* subsp. *paratuberculosis* between silica and clay-silica complexes or between clay soil and sandy soil. The proportion retained decreased with increasing pH in one of the experiments, indicating greater adsorption of *M. avium* subsp. *paratuberculosis* to soil particles at an acidic pH ($P < 0.05$). The results suggest that under experimental conditions *M. avium* subsp. *paratuberculosis* adsorbs to a range of soil particles, and this attachment is influenced by soil pH.	Dhand et al. 2009b

Farm soil *M. a. paratuberculosis*	Speculation about the association of soil characteristics with the expression of ovine Johne's disease (OJD) prompted this cross-sectional study. We enrolled 92 sheep flocks in Australia during 2004–2005 and in each enrolled flock collected pooled faecal samples from an identified cohort (group of same age and sex) of sheep and soil samples from the paddocks grazed by this cohort of sheep. Faecal pools were cultured to create three outcome variables: positive or negative status of faecal pools (pool OJD status, binary): the log number of viable *Mycobacterium avium* subsp. *paratuberculosis* (MAP) organisms per gram of faeces (log pool MAP number, continuous): and the prevalence of faecal shedders (cohort OJD prevalence level, ordinal: low <2%, medium 2–10% and high >10%). Separate statistical models were then developed to investigate the association between soil characteristics and each outcome variable. Sheep raised on soils with a higher percentage of organic carbon and clay had a higher OJD prevalence whereas, sheep grazing on soils with a higher content of sand and nitrogen had a lower OJD prevalence. Iron content of the soil was positively associated with OJD infection but the association between soil pH and OJD was inconclusive. Parent soil type, the only farm level factor, was not significant in any of the final models. Study results indicate a higher risk of OJD in sheep raised on soils with greater organic matter and clay content. We hypothesise that this is due to adsorption of MAP to clay and the consequent retention of the bacteria in the topsoil, thus making them available in higher numbers to grazing sheep.	Dhand et al. 2009a
Pasture soil *M. a. paratuberculosis*	The aim of this study was to demonstrate the persistence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in soil and colonization of different plant parts after deliberate exposure to mouflon feces naturally contaminated with different amounts of MAP. Samples of aerial parts of plants, their roots, and the soil below the roots were collected after 15 weeks and examined using IS900 real-time quantitative PCR (qPCR) and cultivation. Although the presence of viable MAP cells was not demonstrated, almost all samples were found to be positive using qPCR. MAP IS*900* was not only found in the upper green parts, but also in the roots and soil samples (from 1.00×10^0 to 6.43×10^3). The level of soil and plant contamination was influenced mainly by moisture, clay content, and the depth from which the samples were collected, rather than by the initial concentration of MAP in the feces at the beginning of the experiment.	Pribylova et al. 2011
Peat *M.* sp. *M. a. hominissuis* *M. fortuitum* *M. gordonae* *M. chelonae* *M. terrae* *M. xenopi* *M. flavescens* *M. phlei*	Examination of 118 samples from various types of commercially available peat (natural peat, packed peat for horticulture and specially processed peat intended for piglet feeding) showed that PPM were present in 84 (71.1%) samples. *Mycobacterium avium* subsp. *hominissuis* (82.1%) was the most frequent mycobacterial isolate. In addition, from a natural locality where peat is mined and stored in large piles for up to four months, mycobacteria were detected in peat samples collected from the surface and from up to 25 cm in depth.	Matlova et al. 2012

Floor soil in pheasant farms *M.* sp. *M. a. hominissuis* *M. chelonae* *M. fortuitum* *M. scrofulaceum* *M. terrae*	Although avian mycobacteriosis is not prevalent among domestic fowl used for intensive husbandry, it has been described in both free living birds and birds in captivity, e.g., zoological gardens and small fowl flocks. In this study, we examined 305 pheasants from six flocks as well as 70 other birds belonging to 14 species and 97 other vertebrates caught in a closed area. We also investigated the prevalence of mycobacteria in non-vertebrates (earthworms) and soil in two pheasant flocks. *Mycobacterium avium* subsp. *avium* (*M. a. avium*) was isolated in four flocks from 17 (5.6%) pheasants. In one *M. a. avium*-infected pheasant co-infection with *M. a. hominissuis* was diagnosed. Granulomatous inflammatory lesions were observed in liver and spleen in only four *M. a. avium*-infected pheasants originating from two flocks. From the other 38 pheasants other mycobacterial species were isolated, such as *M. fortuitum*, *M. terrae*, *M. triviale*, *M. chelonae*, *M. scrofulaceum*, *M. smegmatis*, *M. flavescens*, *M. diernhoferi* and non-identifiable mycobacterial species. In the group of 70 birds of other species, we identified *M. a. avium* in two (2.9%) goshawks (*Accipiter gentilis*). We did not isolate *M. a. avium* from any of the other 97 vertebrates, the 391 environment samples or 97 earthworms.	Moravkova et al. 2011
Floor soil on cattle farm *M. a. paratuberculosis*	The aim of this study was to monitor the persistence of *Mycobacterium avium* subsp. *paratuberculosis* in environmental samples taken from a Holstein farm with a long history of clinical paratuberculosis. A herd of 606 head was eradicated, and mechanical cleaning and disinfection with chloramine B with ammonium (4%) was carried out on the farm; in the surrounding areas (on the field and field midden) lime was applied. Environmental samples were collected before and over a period of 24 months after destocking. Only one sample out of 48 (2%) examined on the farm (originating from a waste pit and collected before destocking) was positive for *M. avium* subsp. *paratuberculosis* by cultivation on solid medium (Herrold's egg yolk medium). The results using real-time quantitative PCR (qPCR) showed that a total of 81% of environmental samples with an average mean *M. avium* subsp. *paratuberculosis* cell number of 3.09×10^3 were positive for *M. avium* subsp. *paratuberculosis* before destocking compared to 43% with an average mean *M. avium* subsp. *paratuberculosis* cell number of 5.86×10^2 after 24 months. *M. avium* subsp. *paratuberculosis*-positive samples were detected in the cattle barn as well as in the calf barn and surrounding areas. *M. avium* subsp. *paratuberculosis* was detected from different matrices: floor and instrument scrapings, sediment, or scraping from watering troughs, waste pits, and cobwebs. *M. avium* subsp. *paratuberculosis* DNA was also detected in soil and plants collected on the field midden and the field 24 months after destocking. Although the proportion of positive samples decreased from 64% to 23% over time, the numbers of *M. avium* subsp. *paratuberculosis* cells were comparable.	Moravkova et al. 2012

Table 5. Mycobacteria in plants

Type of plants Mycobacteria detected	Abstract excerpts	Reference
Reed *M. avium*	Water, sediment, and stems and roots of common reed (*Phragmites australis*) and greater reedmace (*Typha latifolia*) were taken from 15 locations within the reed bed plus sites upstream and downstream. Samples were analysed for mycobacteria using PCR and specifically for *M. avium* using nested PCR. Environmental mycobacteria were found throughout the entire reed bed but *M. avium* was not found downstream of the first vegetation growth. The reed bed was found to effectively remove *M. avium* from the water through a combination of sedimentation and adsorption onto vegetation stems.	Drewe et al. 2009
Salad *M.* sp.	Food associated indigenous microbial communities exert antagonistic effects on pathogens and may routinely deliver health relevant microorganisms to the GI tract. By using molecular, culture independent methods including PCR-DGGE of 16S rDNA-coding regions and real-time PCR (RT-PCR) as well as BIOLOG metabolic fingerprinting, microbial communities on lettuce were analyzed in samples from fields, from supermarkets and soil. Amplified 16S rRNA gene sequences (57.7%) could be assigned to species previously reported as typical for the phyllosphere including *Pantoea agglomerans*, *Pseudomonas flavescens*, *Moraxella* spp., and *Mycobacterium* spp. 71.8% of the sequences obtained represented so far undescribed taxa.	Zwielehner et al. 2008
Silage *M. a. paratuberculosis*	*Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the causative agent of paratuberculosis (Johne's disease) in ruminants. Paratuberculosis can cause severe economic losses and is acknowledged as one of the most important diseases of ruminants today. High amounts of MAP can be shed in the faeces of infected individuals and can survive for a long period in the environment. In the presented trial, baled grass silage was inoculated with a MAP-suspension, and the viability of MAP was studied over time. Samples from the bales were taken at increasing intervals and subsequently tested for the presence of MAP by solid culture on Herrold's Egg Yolk Media (HEYM), liquid culture and real time Polymerase Chain Reaction (PCR) for the IS900 and F57 fragments. No growth of MAP was observed at any time on solid or in liquid cultures, except at the time of inoculation; PCR detections were positive in the majority of the bales. From the results of the presented study, baled grass silage can be classed as a minor risk for the transmission of MAP.	Khol et al. 2010
Plants growing in contaminated soils *M. a. paratuberculosis*	The aim of this study was to demonstrate the persistence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in soil and colonization of different plant parts after deliberate exposure to mouflon feces naturally contaminated with different amounts of MAP. Samples of aerial parts of plants, their roots, and the soil below the roots were collected after 15 weeks and examined using IS900 real-time quantitative PCR (qPCR) and cultivation. Although the presence of viable MAP cells was not demonstrated, almost all samples were found to be positive using qPCR. MAP IS900 was not only found in the upper green parts, but also in the roots and soil samples (from 1.00×10^0 to 6.43×10^3. The level of soil and plant contamination was influenced mainly by moisture, clay content, and the depth from which the samples were collected, rather than by the initial concentration of MAP in the feces at the beginning of the experiment.	Pribylova et al. 2011

Maize *M. phlei*	An understanding of ecological conditions effecting on bacterial inoculants is important when introducing microbes for increasing plant growth and productivity. In this study the influence of two different soil types on the stimulatory effect of plant growth promoting rhizobacteria for maize was investigated. The investigations were carried out in pot experiments with calcareous calcisol soil taken from Sirdarya, Uzbekistan and loamy sand from Muencheberg, Germany. The bacteria strains *Pseudomonas alcaligenes* PsA15, *Bacillus polymyxa* BcP26 and *Mycobacterium phlei* MbP18 had a much better stimulatory effect on plant growth and nitrogen (N), phosphorus (P) and potassium (K) uptake of maize in nutrient deficient calcisol soil. Their stimulatory efficiency reduced in relatively rich loamy sand soil where bacterial inoculants stimulated only root growth and N, K uptake of root. These results suggest that plant growth stimulating efficiency of bacterial inoculants affected by soil nutritional condition. The bacterial inoculation has a much better stimulatory effect on plant growth in nutrient deficient soil than in nutrient rich soil.	Egamberdiyeva 2007
Rice *M. bolletii*	In this study, the effects of plant genotype, soil type and nutrient use efficiency on the composition of different bacterial communities associated with rice roots were investigated. Thus, total bacteria, Alpha- and Beta-proteobacteria, *Pseudomonas* and Actinobacteria were studied using PCR, followed by denaturing gradient gel electrophoresis (PCR-DGGE). Rice genotype determined, to a large extent, the composition of the different bacterial communities across cultivars. Several cultivars belonging to *Oryza sativa* ssp. *indica* tended to select similar bacterial communities, whereas those belonging to subspecies *japonica* and *aromatica* selected ones with divergent community structures. An effect of soil type was pronounced for the *Actinobacteria communities*, while a small effect of 'improved' and 'traditional' plants was noted for all communities analyzed. A few dominant bands in PCR-DGGE, affiliated with *Rhizobium radiobacter*, *Dickeya zeae*, *Mycobacterium bolletii* and with members of the Rhizobiales, *Rhodospirillaceae* and *Paenibacillaceae*, were spread across cultivars. In contrast, a majority of bands (e.g. affiliated with *Enterobacter cloacae* or *Burkholderia kururiensis*) was only present in particular cultivars or was erratically distributed among rice replicates. These findings suggested that both bacterial adaptation and plant genotype contribute to the shaping of the dynamic bacterial communities associated with roots of rice plants.	Hardoim et al. 2011
Wheat rhizosphere *M. phlei*	One of the natural reservoirs of potentially human-pathogenic bacteria is believed to be the rhizosphere. The aim of the present work was to test nontuberculous mycobacterium *Mycobacterium phlei* MbP18 for its ability to colonize the rhizosphere of wheat and to evaluate its effect on plant growth under saline conditions. In competitive wheat root tip colonization assays, *M. phlei* MbP18 showed poor competitive colonization of the wheat rhizosphere compared to the reference strain. The strain produced lipase, amylase, cellulase, and pectinase and grew well in the presence of high salt (up to 4% NaCl) and at high temperatures (up to 40 degrees C). It was also able to utilize a wide range of carbohydrates for growth. The strain produced indole-3-acetic acid and proved to be very efficient in promoting a significant increase in the shoot and root of wheat under saline conditions. In conclusion, the results of this study indicate that *M. phlei* MbP18 has beneficial effects on plant growth under saline conditions through its ability to produce different biologically active compounds such as cell wall-degrading enzymes and the phytohormone auxin. However, its competitive colonization abilities in the rhizosphere are poor. In light of this observation, attempts should be made to manage the rhizosphere in order to prevent colonization of the rhizosphere by pathogens. This will help remove mycobacteria from habitats where humans or animals can be exposed.	Egamberdieva 2012

Lettuce	Transgenic lettuce plants (*Lactuca sativa* L.) with genes coding the synthesis of tuberculous antigens were obtained using the Agrobacterium-mediated transformation procedure. Cotyledonary leaves of in vitro lettuce seedlings (cvs. Eralash, Snezhinka, and Rubinovoe Kruzhevo) were transformed with plasmids containing nptII, a selective neomycin phosphotransferase II gene, and ESAT6, Ag85B (-TMD), and ESAT6:Ag85B (-TMD) target genes. A PCR analysis of the genome's DNA confirmed the presence of both selective and target genes in all plants examined. At the same time, RT-PCR analysis showed that, in the case of stable transcription of the nptII gene, both the presence and absence of transcription of the ESAT6 gene are possible.	Matvieieva et al. 2009
Chicory cotyledons	Transgenic plants containing either ifn-alpha 2b gene encoding human leukocytic interferon or esxA::fbpB $^{\Delta TMD}$ genes encoding *Mycobacterium tuberculosis* antigens ESAT6 and Ag85B were regenerated from hairy root cultures after transformation of chicory cotyledons (*Cichorium intybus* L. var. *Foliosum Hegi*) with a wild-type *A. rhizogenes* A4 strain. The direct shoot regeneration from transgenic roots without callus formation phase on growth regulator-free nutrient medium was demonstrated. The transgenes transfer and transcription in the plants were confirmed by the results of RT-PCR and PCR analyses.	Matvieieva et al. 2011
Tuberculosis transmission by ecological factors	In this paper, the cumulative effect of ecological factors in the habitat on the spread of tuberculosis (TB) in human population is modeled and analyzed. The total human population is divided into two classes, susceptibles and infectives. It is assumed that TB is not only spread by direct contacts with infectives in the population but also indirectly by bacteria which are emitted by infectives in the habitat. It is assumed further that bacteria survive due to conducive ecological factors such as flower pots, plants, grasses, human clothes, etc. in the habitat. The cumulative density of ecological factors in the habitat is assumed to be governed by a population density dependent logistic model. The analysis of the model shows that as parameters governing the conducive ecological factors in the habitat increase, the spread of TB increases. The same result is also found with the increase in the parameter governing the survival and accumulation of bacteria in the habitat. It is further found that due to immigration of the population TB becomes more endemic. A numerical study of the model is also carried out to support the analytical results.	Naresh et al. 2009
Protozoa-plant symbiosis	The survival of *Salmonella enterica* was recently shown to increase when the bacteria were sequestered in expelled food vacuoles (vesicles) of *Tetrahymena*. Because fresh produce is increasingly linked to outbreaks of enteric illness, the present investigation aimed to determine the prevalence of protozoa on spinach and lettuce and to examine their interactions with *S. enterica*, *Escherichia coli* O157:117, and *Listeria monocytogenes*. *Glaucoma* sp., *Colpoda steinii*, and *Acanthamoeba palestinensis* were cultured from store-bought spinach and lettuce and used in our study. A strain of *Tetrahymena pyriformis* previously isolated from spinach and a soil-borne *Tetrahymena* sp. were also used. Washed protozoa were allowed to graze on green fluorescent protein-or red fluorescent protein-labeled enteric pathogens. Significant differences in interactions among the various protist-enteric pathogen combinations were observed. Vesicles were produced by Glaucoma with all of the bacterial strains, although *L. monocytogenes* resulted in the smallest number per ciliate. Vesicle production was observed also during grazing of *Tetrahymena* on E. coli O157:117 and *S. enterica* but not during grazing on *L. monocytogenes*, in vitro and on leaves. All vesicles contained intact fluorescing bacteria. In contrast, *C. steinii* and the amoeba did not produce vesicles from any of the enteric pathogens, nor were pathogens trapped within their cysts. Studies of the fate of *E. coli* O157:117 in expelled vesicles revealed that by 4 It after addition of spinach extract, the bacteria multiplied and escaped the vesicles. The presence of protozoa on leafy vegetables and their sequestration of enteric bacteria in vesicles indicate that they may play an important role in the ecology of human pathogens on produce.	Gourabathini et al. 2008

Table 6. Mycobacteria in air

Type of samples Mycobacteria detected	Abstract excerpts	Reference
Therapy pool aerosol and air *M.* sp.	...we conducted a multiseason survey of microorganisms present in this therapy pool water, in biofilms associated with the pool containment walls, and in air immediately above the pool. The survey used culture, microscopy, and culture-independent molecular phylogenetic analyses. Although outfitted with a state-of-the art UV-peroxide disinfection system, the numbers of bacteria in the therapy pool water were relatively high compared with the potable water used to fill the pool. Regardless of the source, direct microscopic counts of microbes were routinely approximate to 1,000 times greater than conventional plate counts. Analysis of clone libraries of small subunit rRNA genes from environmental DNA provided phylogenetic diversity estimates of the microorganisms collected in and above the pool. A survey of >1,300 rRNA genes yielded a total of 628 unique sequences, the most common of which was nearly identical to that of *M. avium* strains. The high proportion of clones with different Mycobacterium spp. rRNA genes suggested that such organisms comprised a significant fraction of microbes in the pool water (to >30%) and preferentially partition into aerosols (to >80%) relative to other waterborne bacteria present.	Angenent et al. 2005
Shower aerosol *M.* sp. *M. mucogenicum*	To quantify the microbial load in shower water and aerosol samples, we used culture, microscopic, and quantitative PCR methods to investigate four shower stalls in a stem cell transplant unit at Barnes-Jewish Hospital in St. Louis, MO. We also tested membrane-integrated showerheads as a possible mitigation strategy. In addition to quantification, a 16S rRNA gene sequencing survey was used to characterize the abundant bacterial populations within shower water and aerosols. The average total bacterial counts were 2.2×10^7 cells/liter in shower water and 3.4×10^4 cells/m^3 in shower aerosol, and these counts were reduced to 6.3×10^4 cells/liter (99.6% efficiency) and 8.9×10^3 cells/m^3 (82.4% efficiency), respectively, after membrane-integrated showerheads were installed. Potentially pathogenic organisms were found in both water and aerosol samples from the conventional showers. Most notable was the presence of *Mycobacterium mucogenicum* (99.5% identity) in the water and *Pseudomonas aeruginosa* (99.3% identity) in the aerosol samples.	Perkins et al. 2009
Dental unit waterlines aerosol	Dental unit waterlines (DUWL) support growth of a dense microbial population that includes pathogens and hypersensitivity-inducing bacteria, such as *Legionella s*pp. and non-tuberculous mycobacteria (NTM). Dynamic dental instruments connected to DUWL generate aerosols in the work environment, which could allow waterborne pathogens to be aerosolized. The use of the real-time quantitative polymerase chain reaction (qPCR) provides a more accurate estimation of exposure levels compared with the traditional culture approach. Bioaerosol sampling was performed 13 times in an isolated dental treatment room according to a standardized protocol that included four dental prophylaxis treatments. Inhalable dust samples were taken at the breathing zone of both the hygienist and patient and outside the treatment room (control). Total bacteria as well as *Legionella* spp. and NTM were quantified by qPCR in bioaerosol and DUWL water samples. Dental staff and patients are exposed to bacteria generated during dental treatments (up to 4.3 E + 05 bacteria per m^3 of air). Because DUWL water studied was weakly contaminated by *Legionella* spp. and NTM, their aerosolization during dental treatment was not significant. As a result, infectious and sensitization risks associated with legionellae and NTM should be minimal.	Dutil et al. 2007

Air from peat moss processing plants *M.* sp. *M. malmoense* *M. smegmatis* *M. graceum* *M. bohemicum* *M. interjectum*	We evaluated the presence of mycobacteria in air samples from peat moss processing plants using molecular biology approaches (cloning-sequencing and polymerase chain reaction (PCR)) and the workers exposure using immunoglobulin G (IgG) complexes to mycobacteria. In addition, species detected in air samples and in peat moss were compared. Two peat moss processing plants were chosen among 14 previously studied. A total of 49 clones were sequenced. Real-time PCR was also performed on the same air samples to evaluate the airborne concentration of mycobacteria and estimate exposure levels. Several *Mycobacterium* species were present in the air samples (*M. malmoense*, *M. smegmatis*, *M. graceum*, *M. bohemicum*, and *M. interjectum*). Mycobacterium avium was recovered by culture in peat moss but not in the air using the molecular approach. Total airborne *Mycobacterium* concentration was estimated at $8.2 \times 10^8/m^3$. Workers had IgG against the mycobacterial mix and *M. avium*, suggesting significant exposure. The findings from air samples, supported by IgG measurements, demonstrate that peat moss processing plant workers are exposed to mycobacteria in addition to other biological agents.	Cayer et al. 2007
Aerosol-generating activities *M. avium* complex	Rationale: *Mycobacterium avium* complex lung disease is an increasingly common and chronically debilitating problem. Several host traits have been suggested or confirmed as risk factors. Potential environmental and behavioral risk factors have also been proposed. Few have been evaluated in comparative studies. Objectives: To determine if aerosol-generating activities in the home and garden, features of the home water supply, or several pulmonary and immune-compromising conditions are associated with *Mycobacterium avium* complex lung disease. Methods: Cases were recruited from academic medical centers and by informal referrals from nonuniversity practices in Washington and Oregon. Control subjects were recruited by random-digit dialing and matched to cases by age, sex, and partial telephone number. Associations were measured as odds ratios (OR) estimated using conditional logistic regression. Measurements and Main Results: Known and potential risk factors were measured by in-home interview. Fifty-two matched pairs were studied. Six of 12 examined host traits were associated with disease, including history of chronic obstructive pulmonary disease (OR, 10; 95% confidence interval [CI], 1.2–80), pneumonia hospitalization (OR, 3.4; 95% Cl, 1.1–11), and steroid use (OR, 8; 95% CI, 1.6–41). In contrast, 11 of the 14 aerosol-generating activities and all five features of home water supply studied bore little or no association with disease. Conclusions: Aerosol-generating activities seem not to be risk factors for *Mycobacterium avium* complex lung disease in HIV-negative adults, but prior lung disease and immune-suppressing drugs seem to be associated with susceptibility.	Dirac et al. 2012
Aerosol infection *M. avium*	In a mouse model of mycobacteria-induced immunopathology, wild-type C57BL/6 (WT), IL-18-knockout (KO) and IFN-alpha beta receptor-KO mice developed circumscript, centrally necrotizing granulomatous lesions in response to aerosol infection with *M. avium*, whereas mice deficient in the IFN-gamma receptor, STAT-1 or IRF-1 did not exhibit granuloma necrosis. Comparative, microarray-based gene expression analysis in the lungs of infected WT and IRF-1-KO mice identified a set of genes whose differential regulation was closely associated with granuloma necrosis, among them cathepsin K, cystatin F and matrix metalloprotease 10. Further microarray-based comparison of gene expression in the lungs of infected WT, IFN-gamma-KO and IRF-1-KO mice revealed four distinct clusters of genes with variable dependence on the presence of IFN-gamma, IRF-1 or both. In particular, IRF-1 appeared to be directly involved in the differentiation of a type I immune response to mycobacterial infection. In summary, IRF-1, rather than being a mere transcription factor downstream of IFN-gamma, may be a master regulator of mycobacteria-induced immunopathology.	Aly et al. 2009

Hot tub exposure Non-tuberculous mycobacteria	Hot tub exposure has been causally associated with a steroid-responsive, granulomatous lung disease featuring non-tuberculous mycobacterial (NTM) growth in both clinical and environmental samples. Little is known regarding prevalence of and risk factors for NTM-contamination and associated illness in these settings. In this study, the frequency of NTM growth and aerosolization in 18 public hot tubs and warm water therapy pools and the factors associated with mycobacterial growth were analyzed. Each site was characterized by water chemistry analysis; a questionnaire on maintenance, disinfection, and water quality; and air and water sampling for quantitative NTM culture. NTM were detected in air or water from 13/18 (72%) sites; a strong correlation was found between the maximum air and water NTM concentrations (rho 0.49, P = 0.04). Use of halogen (chlorine or bromine) disinfection was associated with significantly lower air and water concentrations of NTM compared with disinfection using ultraviolet light and hydrogen peroxide (P = 0.01–0.04). Higher water turnover rates were also associated with lower air and water NTM concentrations (P = 0.02–0.03). These findings suggest that NTM are frequently detectable in the air and water of spas and therapy pools and that particular maintenance and disinfection approaches affect NTM bioaerosol concentrations in these settings.	Glazer et al. 2007
Hot water aerosols Non-tuberculous mycobacteria	Objective: Human activities associated with aerosol-generating hot water sources are increasingly popular. Recently, a hypersensitivity pneumonitis (HP)-like granulomatous lung disease, with non-tuberculous mycobacteria from exposure to hot water aerosols from hot tubs/spas, showers, and indoor swimming pools, has been described in immunocompetent individuals (also called "hot tub lung"). Our objective in this study was to examine four additional cases of hot tub lung and compare these cases with others reported in the English print literature on this disease. Data sources and extraction: We retrospectively reviewed all cases (n = 4) of presumptively diagnosed hot tub lung in immunocompetent individuals at the various physician practices in Springfield, Illinois, during 2001–2005. In addition, we searched Medline for cases of hot tub lung described in the literature. Data synthesis: We summarized the clinical presentation and investigations of four presumptive cases and reviewed previously reported cases of hot tub lung. Conclusions: There is a debate in the literature whether hot tub lung is an HP or a direct infection of the lung by nontuberculous mycobacteria. Primary prevention of this disease relies on ventilation and good use practices. Secondary prevention of this disease requires education of both the general public and clinicians to allow for the early diagnosis of this disease.	Sood et al. 2007
Airborne transmission *M. bovis*	Despite years of study the principle transmission route of bovine tuberculosis to cattle remains unresolved. The distribution of pathological lesions, which are concentrated in the respiratory system, and the very low dose of *Mycobacterium bovis* needed to initiate infection from a respiratory tract challenge suggest that the disease is spread by airborne transmission. Critical to the airborne transmission of a pathogenic microorganism is its ability to survive the stresses incurred whilst airborne. This study demonstrates that *M. bovis* is resistant to the stresses imposed immediately after becoming airborne, 94% surviving the first 10 min after aerosolisation. Once airborne the organism is robust, its viability decreasing with a half-life of approximately 1.5 hours. These findings support the hypothesis that airborne transmission is the principle route of infection for bovine tuberculosis.	Gannon et al. 2007

Air *M. bovis*	Background: Evidence has recently emerged indicating that in addition to large airborne droplets, fine aerosol particles can be an important mode of influenza transmission that may have been hitherto underestimated. Furthermore, recent performance studies evaluating airborne infection isolation (AII) rooms designed to house infectious patients have revealed major discrepancies between what is prescribed and what is actually measured. Methods: We conducted an experimental study to investigate the use of high-throughput in-room air decontamination units for supplemental protection against airborne contamination in areas that host infectious patients. The study included both intrinsic performance tests of the air-decontamination unit against biological aerosols of particular epidemiologic interest and field tests in a hospital AII room under different ventilation scenarios. Results: The unit tested efficiently eradicated airborne H5N2 influenza and *Mycobacterium bovis* (a 4- to 5-log single-pass reduction) and, when implemented with a room extractor, reduced the peak contamination levels by a factor of 5, with decontamination rates at least 33% faster than those achieved with the extractor alone. Conclusion: High-throughput in-room air treatment units can provide supplemental control of airborne pathogen levels in patient isolation rooms.	Bergeron et al. 2011
Air *M. pinnipedii*	Objectives: Ail outbreak of tuberculosis (TB)) in sea lions occurred recently in a zoo in the Netherlands. The disease was detected in a captive colony consisting of 29 animals kept in an open air basin with an indoor night house. Approximately 25 animal keepers were in close contact with the animals. Methods: The sea lions were Investigated Using the tuberculin skin test (TST) with avian and bovine purified protein derivative (PPD) and, in case of positivity, necropsied. A survey was conducted among the animal keepers Including TSTs with *Mycobacterium tuberculosis* complex PPD tuberculin, a chest X-ray and an interferon-gamma release assay (IGRA). Results: Necropsy was positive for TB in 13 of the 29 sea lions. Three cases of pulmonary involvement were found. Only one of these was infectious and it was therefore regarded as the source case. The causative *Mycobacterium* was identified as *M. pinnipedii*. Six of the 25 animal keepers were TST-positive; in five of these, infection was confirmed by a positive IGRA. Conclusion: Transmission of *M. pinnipedii* infection from sea lions to humans was established by TST. IGRA results largely agreed with the TST results. Nebulisation when cleaning the sea lions' enclosure was most likely the main cause of transmission to humans.	Kiers et al. 2008
Aerosol *M. a. hominissuis*	*Mycobacterium avium* ssp. *hominissuis*, hereafter referred to as *M. avium*, forms biofilm, a property that, in mice, is associated with lung infection via aerosol. As *M. avium* might co-inhabit the respiratory tract with other pathogens, treatment of the co-pathogen-associated infections, such as in bronchiectasis, would expose *M. avium* to therapeutic compounds that may have their origin in other organisms sharing the natural environments. Incubation of *M. avium* with two compounds produced by environmental organisms, streptomycin and tetracycline, in vitro at subinhibitory concentrations increased biofilm formation in a number of *M. avium* strains, although exposure to ampicillin, moxifloxacin, rifampin and trimethoprim-sulphamethoxazole had no effect on biofilm formation. No selection of genotypically resistant clones was observed. Although incubation of bacteria in the presence of streptomycin upregulates the expression of biofilm-associated genes, the response to the antibiotics had no association with the expression of a regulator (LysR) linked to the formation of biofilm in *M. avium*. Biofilms are composed of planktonic and sessile bacteria. Whereas planktonic *M. avium* is susceptible to clarithromycin and ethambutol (clinically used antimicrobials), sessile bacteria are at least three-fold to four-fold more resistant to antibiotics. The sessile phenotype, however, is reversible, and no selection of resistant clones was observed. Mice infected through the airway with both phenotypes were infected with a similar number of bacteria, demonstrating no phenotype advantage. *M. avium* biofilm formation is enhanced by commonly used compounds and, in the sessile bacterial phenotype, is resistant to clarithromycin and ethambutol, in a reversible manner.	McNabe et al. 2011

Aerosol *M. tuberculosis*	The large reservoir of human latent tuberculosis (TB) contributes to the global success of the pathogen, *Mycobacterium tuberculosis* (Mtb). We sought to test whether aerosol infection of rabbits with Mtb H37Rv could model paucibacillary human latent TB. The lung burden of infection peaked at 5 weeks after aerosol infection followed by host containment of infection that was achieved in all rabbits. One-third of rabbits had at least one caseous granuloma with culturable bacilli at 36 weeks after infection suggesting persistent paucibacillary infection. Corticosteroid-induced immunosuppression initiated after disease containment resulted in reactivation of disease. Seventy-two percent of rabbits had culturable bacilli in the right upper lung lobe homogenates compared to none of the untreated controls. Discontinuation of dexamethasone led to predictable lymphoid recovery, with a proportion of rabbits developing multicentric large caseous granuloma. The development and severity of the immune reconstitution inflammatory syndrome (IRIS) was dependent on the antigen load at the time of immunosuppression and subsequent bacillary replication during corticosteroid-induced immunosuppression. Clinically, many aspects were similar to IRIS in severely immunosuppressed HIV-infected patients who have functional restoration of T cells in response to effective (highly active) antiretroviral therapy. This corticosteroid model is the only animal model of the IRIS. Further study of the rabbit model of TB latency, reactivation and IRIS may be important in understanding the immunopathogenesis of these poorly modelled states as well as for improved diagnostics for specific stages of disease.	Manabe et al. 2008
Aerosol indused infection *M. a. paratuberculosis*	A challenge experiment was performed to investigate whether administration of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) via the respiratory route leads to MAP infection in calves. Eighteen calves from test negative dams were randomly allocated to four groups. Six calves were challenged with MAP nasally and six calves were challenged by transtracheal injection; three orally challenged calves served as positive controls, and three non-challenged calves as negative controls. The challenge was performed as a nine-fold trickle dose, 10^7 CFU in total. Blood and faecal samples were collected frequently. Calves were euthanized three months post-challenge and extensively sampled. Blood samples were tested for the presence of antibodies and interferon gamma producing cells by ELISA. Faecal and tissue samples were cultured in a liquid culture system and the presence of MAP was confirmed by IS*900* realtime PCR. Fourteen out of fifteen calves had no MAP antibody response. The negative controls remained negative; all positive controls became infected. Two nasally challenged calves showed a Purified Protein Derivative Avian (PPDA) specific interferon gamma response. In all nasally challenged calves, MAP positive intestinal samples were detected. In three calves of the nasal group MAP positive retropharyngeal lymph nodes or tonsils were detected. In all calves of the transtracheal group MAP positive intestinal tissues were detected as well and three had a MAP positive tracheobronchial lymph node. These findings indicate that inhalation of MAP aerosols can result in infection. These experimental results may be relevant for transmission under field conditions since viable MAP has been detected in dust on commercial dairy farms.	Eisenberg et al. 2011

Desert dust *M.* sp.	A previously developed resequencing microarray, "Tropical and Emerging Infections (RPM-TEI v. 1.0 chip)", designed to identify and discriminate between tropical diseases and other potential biothreat agents, their near-neighbor species, and/or potential confounders, was used to characterize the microbes present in the silt/clay fraction of surface soils and airborne dust collected from the Middle East. Local populations and U. S. military personnel deployed to the Middle East are regularly subjected to high levels of airborne desert dust containing a significant fraction of inhalable particles and some portion require clinical aid. Not all of the clinical symptoms can be directly attributed to the physical action of material in the human respiratory tract. To better understand the potential health effects of the airborne dust, the composition of the microbial communities associated with surface soil and/or airborne dust (air filter) samples from 19 different sites in Iraq and Kuwait was identified using RPM-TEI v. 1.0. Results indicated that several microorganisms including a class of rapidly growing *Mycobacterium*, *Bacillus*, *Brucella*, *Clostridium* and *Coxiella burnetti*, were present in the samples. The presence of these organisms in the surface soils and the inhalable fraction of airborne dust analyzed may pose a human health risk and warrants further investigation. Better understanding of the factors influencing the composition of these microbial communities is important to address questions related to human health and is critical to achieving Force Health Protection for the Warfighter operating in the Middle East, Afghanistan, North Africa and other arid regions.	Leski et al. 2010
Dust *M.* sp. *M. barrassiae* *M. gilvum* *M. vanbalenii*	Although the link between airborne particulate inhalation and a variety of respiratory diseases has long been established, little is known about the pathogenic role of the microbial component of the dust. In this study, we applied highly multiplexed PCR and a high-density resequencing microarray (RPM-TEI version 1.0) to screen samples of fine topsoil particles and airborne dust collected in 19 locations in Iraq and Kuwait for the presence of a broad range of human pathogens. The results indicated the presence of potential human pathogens, including *Mycobacterium*, *Brucella*, *Coxiella burnetii*, *Clostridium perfringens*, and *Bacillus.*	Leski et al. 2011
Metal working fluids *M. immunogenum*	Hypersensitivity pneumonitis, also known as "machine operator's lung" (MOL), has been related to microorganisms growing in metalworking fluids (MWFs), especially *Mycobacterium immunogenum*. We aimed to (i) describe the microbiological contamination of MWFs and (ii) look for chemical, physical, and environmental parameters associated with variations in microbiological profiles. We microbiologically analyzed 180 MWF samples from nonautomotive plants (e.g., screw-machining or metal-cutting plants) in the Franche-Comte region in eastern France and 165 samples from three French automotive plants in which cases of MOL had been proven. Our results revealed two types of microbial biomes: the first was from the nonautomotive industry, showed predominantly Gram-negative rods (GNR), and was associated with a low risk of MOL, and the second came from the automotive industry that was affected by cases of MOL and showed predominantly Gram-positive rods (GPR). Traces of *M. immunogenum* were sporadically detected in the first type, while it was highly prevalent in the automotive sector, with up to 38% of samples testing positive. The use of chromium, nickel, or iron was associated with growth of Gram-negative rods; conversely, growth of Gram-positive rods was associated with the absence of these metals. Synthetic MWFs were more frequently sterile than emulsions. Vegetable oil-based emulsions were associated with GNR, while mineral ones were associated with GPR. Our results suggest that metal types and the nature of MWF play a part in MWF contamination, and this work shall be followed by further in vitro simulation experiments on the kinetics of microbial populations, focusing on the phenomena of inhibition and synergy.	Murat et al. 2012

Metal working fluids *M. immunogenum*	Purpose of review: To highlight advances in understanding the respiratory disease associated with metal machining, a common work process involving approximately 1.2 million workers in the USA. Recent findings: Recent studies emphasize that work-related asthma and hypersensitivity pneumonitis continue to be caused by exposure to metalworking fluid. Identification of an individual patient indicates the need for follow-up investigations at the work site to prevent additional disease and/or identify additional effected individuals. Identification of the causal agent for hypersensitivity pneumonitis has centered on microbial contamination of metalworking fluids with a number of studies focusing on *Mycobacterium immunogenum*. Summary: Both asthma and hypersensitivity pneumonitis occur among workers exposed to metalworking fluid. The incidence of these diseases among such workers is unknown. Outbreaks of these conditions continue to be identified among metal machinists. Whether these are true outbreaks associated with some breakdown in workplace controls or, rather the recognition of ongoing endemic disease that is typically misdiagnosed as pneumonia or common adult onset asthma, needs further evaluation. Further work to elucidate the specific causal agent(s) is necessary to affect effective workplace controls. Treating an identified individual case as an index case with a follow-up workplace investigation will only be possible if practicing physicians interact with public health authorities to report newly diagnosed cases.	Rosenman 2009

Table 7. Different methods for detection and identification of mycobacteria in the environment

Detection method	Mycobacteria detected	Examined/Positive samples	Quantification	Reference
Culture	*M*. sp.	24/18 water 148/76 soil	up to 10^4 CFU	Chilima et al. 2006
	M. sp.	49/10 water	n-a	Chang et al. 2002
	M. sp.	49/21 cold water 44/32 warm water	up to 10^3 CFU/500 ml	Hussein et al. 2009
	M. sp.	50/8 air	n-a	Cayer et al. 2007
	M. sp.	34/28 water	n-a	Perkins et al. 2009
	M. sp.	40/34 water	n-a	Castillo-Rodal et al. 2012
	M. sp. *M. porcinum*	63% water	n-a	Brown-Elliott et al. 2011
	M. sp.	69/36 water	n-a	Fernandez-Rendon et al. 2012
	M. avium	n-a	up to 10^4 CFU/ml	Svensson et al. 2011
	M. a. paratuberculosis	192/8 water	n-a	Whan et al. 2005
	M. a. paratuberculosis	96/12 water	n-a	Pickup et al. 2005
	M. sp.	118/84 peat	n-a	Matlova et al. 2012
	M. sp.	391/26 soil	n-a	Moravkova et al. 2011

PCR	*M*. sp.	49/0 water	n-a	Chang et al. 2002
	M. sp.	24/13 water 148/75 soil	n-a	Chilima et al. 2006
	IS*2404*, IS*2606*/*M. ulcerans*	35/2 water 14/2 detritus 2/1 vegetation	n-a	Stinear et al. 2000
	M. bovis/*mpb64, mpb70*	11/4 sites soil	10^3/g	Young et al. 2005
	M. leprae/16S rDNA	80/30 soil	n-a	Lavania et al. 2008
	M. leprae/16S rDNA	207/71 soil	n-a	Turankar et al. 2012
	IS*900*/*M. a. paratuberculosis*	96/31 water	n-a	Pickup et al. 2005
	IS*900*/*M. a. paratuberculosis*	70/48 water 10/9 sediment	n-a	Pickup et al. 2006
IMS-PCR	IS*900*/*M. a. paratuberculosis*	192/9 water	n-a	Whan et al. 2005
Nested PCR	*rpo*B/ *M*. sp.	32/7 water	n-a	Shin et al. 2008
	M. sp. *M. avium*	45/25 water 45/5 sediment	n-a	Drewe et al. 2009
Real time PCR	IS*900*/*M. a. paratuberculosis*	366/219 dust	n-a	Eisenberg et al. 2011
	M. a. paratuberculosis	81 % water midwest, 0% national survey	up to 10^2/400 ml	Beumer et al. 2010
	M. a. paratuberculosis	16/0 water	n-a	Bockelmann et al. 2009
	16S rRNA/*M*. sp.	4/4 bedding	up to 10^{10}/g	Pakarinen et al. 2007
	16S rRNA/*M*. sp.	20/20 dust	n-a	Torvinen et al. 2010
	M. avium	14/13 water 32/25 biofilm	n-a	Feazel et al. 2009
	M. sp.	53/30 water	10^3–10^6/l	Adrados et al. 2011
	M. sp.	21/12 water	up to 10^4/ml	Kawai et al. 2004
	16S rRNA/*M*. sp. *M. xenopi*	93/93 water 93/73 water	n-a	Hussein et al. 2009
	IS*900*/*M. a.* paratuberculosis	22/0 water	n-a	Villarreal et al. 2010
	IS*900*, *f57*/*M. a. paratuberculosis*	19/10 sillage	up to 10^7/g	Khol et al. 2010
	IS*900*/*M. a. paratuberculosis*	19/11 soil 57/43 plants	up to 10^3/g	Pribylova et al. 2011
	IS*901*/*M. a. avium* IS*1245*/*M. a. hominissuis*	28/10 soil 28/13 soil	up to 10^7/g	Kaevska et al. 2011
	ITS/*M*. sp.	96% water	up to 10^3/ml	Jacobs et al. 2009
	IS*2404*, IS*2606*/*M. ulcerans*	1/148 water	n-a	Vandelannoote et al. 2010
	M. leprae/16S rRNA	80/28 soil	up to 10^5/g	Lavania et al. 2008
	M. leprae/16S rRNA	207/28 soil	n-a	Turankar et al. 2012

Hybridization	16S rRNA/*M.* sp.	4/4 bedding	n-a	Pakarinen et al. 2007
	16S rRNA/*M.* sp.	3/3 soil	up to 10^{10}/g	Nieminen et al. 2006
	16S rRNA/*M.* sp.	10/10 soil	n-a	Mendum et al. 2000
	16S rRNA/*M.* sp.	12/12 soil	n-a	Kopecky et al. 2011
	16S rRNA/*M.* sp.	482/1306 clones air	n-a	Angenent et al. 2005
	16S rRNA/*M.* sp.	50/32 air	up to 10^8/m^3	Cayer et al. 2007
	16S rRNA/*M.* sp.	88/247 clones aerosol	n-a	Perkins et al. 2009
Cloning-DGGE-Sequencing	16S rRNA/*M.* sp.	96 clones water	n-a	Liu et al. 2012
	16S rRNA/*M.* sp.	7% soil	n-a	Montana et al. 2012
	16S rRNA/*M.* sp.	84 clones soil	n-a	Kopecky et al. 2011
	16S rRNA/*M.* sp.	6/7 sites soil	n-a	Leys et al. 2005
	16S rRNA/*M.* sp.	8/8 soil	n-a	Cheung and Kinkle 2001
	16S rRNA	70% of clones sponges	n-a	Xin et al. 2008
Microarray	*rpo*B	n-a dust	n-a	Leski et al. 2011

n-a = not available

8. Acknowledgements

The critical comments of Professor I. Pavlik, Veterinary Research Institute, Brno, Czech Republic, are greatly appreciated.

9. REFERENCES

Abubakar I (2010): Tuberculosis and air travel: a systematic review and analysis of policy. Lancet Infectious Diseases 10, 176–183.

Adekambi T (2009): Mycobacterium mucogenicum group infections: a review. Clinical Microbiology and Infection 15, 911–918.

Adrados B, Julian E, Codony F, Torrents E, Luquin M, Morato J (2011): Prevalence and concentration of nontuberculous mycobacteria in cooling towers by means of quantitative PCR: a prospective study. Current Microbiology 62, 313–319.

Allan RN, Pease P, Ibbotson JP (1986): Clustering of Crohn's disease in a Cotswold village. Quarterly Journal of Medicine 59, 473–478.

Aly S, Mages J, Reiling N, Kalinke U, Decker T, Lang R, Ehlers S (2009): Mycobacteria-induced granuloma necrosis depends on IRF-1. Journal of Cellular and Molecular Medicine 13, 2069–2082.

Aly SS, Mangold BL, Whitlock RH, Sweeney RW, Anderson RJ, Jiang J, Schukken YH, Hovingh E, Wolfgang D, Van Kessel JA, Karns JS, Lombard JE, Smith JM, Gardner IA (2010): Correlation between Herrold egg yolk medium culture and real-time quantitative polymerase chain reaction results for Mycobacterium avium subspecies paratuberculosis in pooled fecal and environmental samples. Journal of Veterinary Diagnostic Investigation 22, 677–683.

Angenent LT, Kelley ST, St Amand A, Pace NR, Hernandez MT (2005): Molecular identification of potential pathogens in water and air of a hospital therapy pool. Proceedings of the National Academy of Sciences of U.S.A. 102, 4860–4865.

Argueta C, Yoder S, Holtzman AE, Aronson TW, Glover N, Berlin OG, Stelma GN, Jr., Froman S, Tomasek P (2000): Isolation and identification of nontuberculous mycobacteria from foods as possible exposure sources. Journal of Food Protection 63, 930–933.

Bergeron V, Chalfine A, Misset B, Moules V, Laudinet N, Carlet J, Lina B (2011): Supplemental treatment of air in airborne infection isolation rooms using high-throughput in-room air decontamination units. American Journal of Infection Control 39, 314–320.

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

Bjorklof K, Karlsson S, Frostegard A, Jorgensen KS (2009): Presence of actinobacterial and fungal communities in clean and petroleum hydrocarbon contaminated subsurface soil. Open Microbiology Journal 3, 75–86.

Blanchard DC, Syzdek L (1970): Mechanism for the water-to-air transfer and concentration of bacteria. Science 170, 626–628.

Bockelmann U, Dorries HH, Ayuso-Gabella MN, de Marcay MS, Tandoi V, Levantesi C, Masciopinto C, Van Houtte E, Szewzyk U, Wintgens T, Grohmann E (2009): Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European Artificial Groundwater Recharge Systems. Applied and Environmental Microbiology 75, 154–163.

Bolster CH, Cook KL, Haznedaroglu BZ, Walker SL (2009): The transport of Mycobacterium avium subsp. paratuberculosis through saturated aquifer materials. Letters in Applied Microbiology 48, 307–312.

Briancesco R, Semproni M, Della LS, Sdanganelli M, Bonadonna L (2010): Non-tuberculous mycobacteria and microbial populations in drinking water distribution systems. Annali dell'Istituto Superiore di Sanita 46, 254–258.

Brown-Elliott BA, Wallace RJ, Tichindelean C, Sarria JC, McNulty S, Vasireddy R, Bridge L, Mayhall CG, Turenne C, Loeffelholz M (2011): Five-Year outbreak of community- and hospital-acquired Mycobacterium porcinum infections related to public water supplies. Journal of Clinical Microbiology 49, 4231–4238.

Burton CM, Crook B, Scaife H, Evans GS, Barber CM (2012): Systematic review of respiratory outbreaks associated with exposure to water-based metalworking fluids. Annals of Occupational Hygiene 56, 374–388.

Cafri U, Aslan G, Direkel S, Tarhan G, Ceyhan I, Emekdas G (2010): Identification and isolation of non-tuberculous mycobacteria from environmental samples (in Bulgarian). Mikrobiyoloji Bulteni 44, 395–403.

Carbone KM, Luftig RB, Buckley MR (2005): Microbial triggers of chronic human ilness. American Academy of Microbiology Colloquium, 1–14.

Castillo-Rodal AI, Mazari-Hiriart M, Lloret-Sanchez LT, Sachman-Ruiz B, Vinuesa P, Lopez-Vidal Y (2012): Potentially pathogenic nontuberculous mycobacteria found in aquatic systems. Analysis from a reclaimed water and water distribution system in Mexico City. European Journal of Clinical Microbiology and Infectious Diseases 31, 683–694.

Cayer MP, Veillette M, Pageau P, Hamelin R, Bergeron MJ, Meriaux A, Cormier Y, Duchaine C (2007): Identification of mycobacteria in peat moss processing plants: application of molecular biology approaches. Canadian Journal of Microbiology 53, 92–99.

Cerqueira L, Azevedo NF, Almeida C, Jardim T, Keevil CW, Vieira MJ (2008): DNA mimics for the rapid identification of microorganisms by fluorescence in situ hybridization (FISH). International Journal of Molecular Sciences 9, 1944–1960.

Chang CT, Wang LY, Liao CY, Huang SP (2002): Identification of nontuberculous mycobacteria existing in tap water by PCR-restriction fragment length polymorphism. Applied and Environmental Microbiology 68, 3159–3161.

Chen YQ, Chen C, Zhang XJ, Zheng Q, Liu YY (2012): Inactivation of resistant Mycobacteria mucogenicum in water: chlorine resistance and mechanism analysis. Biomedical and Environmental Sciences 25, 230–237.

Cheung PY, Kinkle BK (2001): Mycobacterium diversity and pyrene mineralization in petroleum-contaminated soils. Applied and Environmental Microbiology 67, 2222–2229.

Chilima BZ, Clark IM, Floyd S, Fine PEM, Hirsch PR (2006): Distribution of environmental mycobacteria in Karonga District, northern Malawi. Applied and Environmental Microbiology 72, 2343–2350.

Codony F, Perez LM, Adrados B, Agusti G, Fittipaldi M, Morato J (2012): Amoeba-related health risk in drinking water systems: could monitoring of amoebae be a complementary approach to current quality control strategies? Future Microbiology 7, 25–31.

Collins CH, Grange JM, Yates MD (1984): Mycobacteria in water. Journal of Applied Bacteriology 57, 193-211.

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

Corsaro D, Pages GS, Catalan V, Loret JF, Greub G (2010): Biodiversity of amoebae and amoeba-associated bacteria in water treatment plants. International Journal of Hygiene and Environmental Health 213, 158–166.

Coulombe F, Divangahi M, Veyrier F, de LL, Gleason JL, Yang Y, Kelliher MA, Pandey AK, Sassetti CM, Reed MB, Behr MA (2009): Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide. Journal of Experimental Medicine 206, 1709–1716.

Coulon C, Collignon A, McDonnell G, Thomas V (2010): Resistance of Acanthamoeba cysts to disinfection treatments used in health care settings. Journal of Clinical Microbiology 48, 2689–2697.

Dhand NK, Eppleston J, Whittington RJ, Toribio JA (2009a): Association of farm soil characteristics with ovine Johne's disease in Australia. Preventive Veterinary Medicine 89, 110–120.

Dhand NK, Toribio JA, Whittington RJ (2009b): Adsorption of Mycobacterium avium subsp. paratuberculosis to soil particles. Applied and Environmental Microbiology 75, 5581–5585.

Dirac MA, Horan KL, Doody DR, Meschke JS, Park DR, Jackson LA, Weiss NS, Winthrop KL, Cangelosi GA (2012): Environment or host? A case-control study of risk factors for Mycobacterium avium complex lung disease. American Journal of Respiratory and Critical Care Medicine 186, 684–691.

Drewe JA, Mwangi D, Donoghue HD, Cromie RL (2009): PCR analysis of the presence and location of Mycobacterium avium in a constructed reed bed, with implications for avian tuberculosis control. Fems Microbiology Ecology 67, 320–328.

Duggan AE, Usmani I, Neal KR, Logan RF (1998): Appendicectomy, childhood hygiene, Helicobacter pylori status, and risk of inflammatory bowel disease: a case control study. Gut 43, 494–498.

Dutil S, Veillette M, Meriaux A, Lazure L, Barbeau J, Duchaine C (2007): Aerosolization of mycobacteria and legionellae during dental treatment: low exposure despite dental unit contamination. Environmental Microbiology 9, 2836–2843.

Egamberdiyeva D (2007): The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Applied Soil Ecology 36, 184–189.

Egamberdieva D (2012): Colonization of Mycobacterium phlei in the rhizosphere of wheat grown under saline conditions. Turkish Journal of Biology 36, 487–492.

Eisenberg SW, Nielen M, Santema W, Houwers DJ, Heederik D, Koets AP (2009): Detection of spatial and temporal spread of Mycobacterium avium subsp. paratuberculosis in the environment of a cattle farm through bio-aerosols. Veterinary Microbiology 143, 284–292.

Eisenberg SW, Koets AP, Nielen M, Heederik D, Mortier R, De BJ, Orsel K (2011): Intestinal infection following aerosol challenge of calves with Mycobacterium avium subspecies paratuberculosis. Veterinary Research 42, 117.

Ellouz F, Adam A, Ciorbaru R, Lederer E (1974): Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. Biochemical and Biophysical Research Communications 59, 1317–1325.

Eyer L, Hruska K (2012): Single-domain antibody fragments derived from heavy-chain antibodies: a review. Veterinarni Medicina 57, 439–513.

Falkinham JO (1996): Epidemiology of infection by nontuberculous mycobacteria. Clinical Microbiology Reviews 9, 177–215.

Falkinham JO (2002): Nontuberculous mycobacteria in the environment. Clinics in Chest Medicine 23, 529–551.

Falkinham JO, III (2003): Mycobacterial aerosols and respiratory disease. Emerging Infection Diseases 9, 763–767.

Falkinham JO (2009a): Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. Journal of Applied Microbiology 107, 356–367.

Falkinham JO (2009b): The biology of environmental mycobacteria. Environmental Microbiology Reports 1, 477–487.

Falkinham JO (2010): Impact of human activities on the ecology of nontuberculous mycobacteria. Future Microbiology 5, 951–960.

Falkinham JO, III (2011): Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. Emerging Infection Diseases 17, 419–424.

Feazel LM, Baumgartner LK, Peterson KL, Frank DN, Harris JK, Pace NR (2009): Opportunistic pathogens enriched in showerhead biofilms. Proceedings of the National Academy of Sciences of the United States of America 106, 16393–16398.

Fernandez-Rendon E, Cerna-Cortes JF, Ramirez-Medina MA, Helguera-Repetto AC, Rivera-Gutierrez S, Estrada-Garcia T, Merchand JA (2012): Mycobacterium mucogenicum and other non-tuberculous mycobacteria in potable water of a trauma hospital: a potential source for human infection. Journal of Hospital Infection 80, 74–76.

Fyfe JAM, Lavender CJ, Johnson PDR, Globan M, Sievers A, Azuolas J, Stinear TP (2007): Development and application of two multiplex real-time PCR assays for the detection of Mycobacterium ulcerans in clinical and environmental samples. Applied and Environmental Microbiology 73, 4733–4740.

Gannon BW, Hayes CM, Roe JM (2007): Survival rate of airborne Mycobacterium bovis. Research in Veterinary Science 82, 169–172.

Garcia-Martos P, Garcia-Agudo L (2012): Infections due to rapidly growing mycobacteria. Enfermedades Infecciosas y Microbiologia Clinica 30, 192–200.

Gauthier DT, Reece KS, Xiao J, Rhodes MW, Kator HI, Latour RJ, Bonzek CF, Hoenig JM, Vogelbein WK (2010): Quantitative PCR assay for Mycobacterium pseudoshottsii and Mycobacterium shottsii and application to environmental samples and fishes from the Chesapeake Bay. Applied and Environmental Microbiology 76, 6171–6179.

Gent AE, Hellier MD, Grace RH, Swarbrick ET, Coggon D (1994): Inflammatory bowel disease and domestic hygiene in infancy. Lancet 343, 766–767.

Gill CO, Saucier L, Meadus WJ (2011): Mycobacterium avium subsp paratuberculosis in dairy products, meat, and drinking water. Journal of Food Protection 74, 480–499.

Glazer CS, Martyny JW, Lee B, Sanchez TL, Sells TM, Newman LS, Murphy J, Heifets L, Rose CS (2007): Nontuberculous mycobacteria in aerosol droplets and bulk water samples from therapy pools and hot tubs. Journal of Occupational and Environmental Hygiene 4, 831–840.

Gomez-Alvarez V, Revetta RP, Domingo JWS (2012): Metagenomic analyses of drinking water receiving different disinfection treatments. Applied and Environmental Microbiology 78, 6095–6102.

Gourabathini P, Brandl MT, Redding KS, Gunderson JH, Berk SG (2008): Interactions between food-borne pathogens and protozoa isolated from lettuce and spinach. Applied and Environmental Microbiology 74, 2518–2525.

Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Winthrop K (2007): An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. American Journal of Respiratory and Critical Care Medicine 175, 367–416.

Hanak V, Golbin JM, Ryu JH (2007): Causes and presenting features in 85 consecutive patients with hypersensitivity pneumonitis. Mayo Clinic Proceedings 82, 812–816.

Hardoim PR, Andreote FD, Reinhold-Hurek B, Sessitsch A, van Overbeek LS, van Elsas JD (2011): Rice root-associated bacteria: insights into community structures across 10 cultivars. Fems Microbiology Ecology 77, 154–164.

Hartman TE, Jensen E, Tazelaar HD, Hanak V, Ryu JH (2007): CT findings of granulomatous pneumonitis secondary to Mycobacterium avium-intracellulare inhalation: "hot tub lung". AJR American Journal of Roentgenology 188, 1050–1053.

Hruska K, Franek M (2012): Sulfonamides in the environment: a review and a case report. Veterinarni Medicina 57, 1–35.

Hussein Z, Landt O, Wirths B, Wellinghausen N (2009): Detection of non-tuberculous mycobacteria in hospital water by culture and molecular methods. International Journal of Medical Microbiology 299, 281–290.

Jacobs J, Rhodes M, Sturgis B, Wood B (2009): Influence of environmental gradients on the abundance and distribution of Mycobacterium spp. in a coastal lagoon estuary. Applied and Environmental Microbiology 75, 7378–7384.

Jarzembowski JA, Young MB (2008): Nontuberculous mycobacterial infections. Archives of Pathology and Laboratory Medicine 132, 1333–1341.

Kaevska M, Hruska K (2010): Mycobacteria in water, feedstocks and food: analysis of publications. Veterinarni Medicina 55, 571–580.

Kaevska M, Slana I, Kralik P, Reischl U, Orosova J, Holcikova A, Pavlik I (2011): "Mycobacterium avium subsp. hominissuis" in neck lymph nodes of children and their environment examined by culture and triplex quantitative real-time PCR. Journal of Clinical Microbiology 49, 167–172.

Kasperbauer SH, Daley CL (2008): Diagnosis and treatment of infections due to Mycobacterium avium complex. Seminars in Respiratory and Critical Care Medicine 29, 569–576.

Kawai M, Yamagishi J, Yamaguchi N, Tani K, Nasu M (2004): Bacterial population dynamics and community structure in a pharmaceutical manufacturing water supply system determined by real-time PCR and PCR-denaturing gradient gel electrophoresis. Journal of Applied Microbiology 97, 1123–1131.

Kazda J, Pavlik I, Falkinham III JO, Hruska K (Eds.) (2009): The Ecology of Mycobacteria: Impact on Animal's and Human's Health. Springer, 1st ed., xviii+522 pp. http://link.springer.com/book/10.1007/978-1-4020-9413-2/page/1

Khol JL, Beran V, Kralik P, Trckova M, Pavlik I, Baumgartner W (2010): Grass silage contaminated with Mycobacterium avium subspecies paratuberculosis (MAP): a possible source of paratuberculosis infection in ruminants? Veterinarni Medicina 55, 225–232.

Kiers A, Klarenbeek A, Mendelts B, van Soolingen D, Koeter G (2008): Transmission of Mycobacterium pinnipedii to humans in a zoo with marine mammals. International Journal of Tuberculosis and Lung Disease 12, 1469–1473.

Kopecky J, Kyselkova M, Omelka M, Cermak L, Novotna J, Grundmann G, Moenne-Loccoz Y, Sagova-Mareckova M (2011): Environmental mycobacteria closely related to the pathogenic species evidenced in an acidic forest wetland. Soil Biology and Biochemistry 43, 697–700.

Koskimaki JJ, Hankala E, Suorsa M, Nylund S, Pirttila AM (2010): Mycobacteria are hidden endophytes in the shoots of rock plant [Pogonatherum paniceum (Lam.) Hack.] (Poaceae). Environmental Microbiology Reports 2, 619–624.

Lavania M, Katoch K, Katoch VM, Gupta AK, Chauhan DS, Sharma R, Gandhi R, Chauhan V, Bansal G,

Sachan P, Sachan S, Yadav VS, Jadhav R (2008): Detection of viable Mycobacterium leprae in soil samples: insights into possible sources of transmission of leprosy. Infection, Genetics and Evolution 8, 627–631.

Learn-Han L, Yoke-Kqueen C, Shiran MS, Vui-Ling CMW, Nurul-Syakima AM, Son R, Andrade HM (2012): Identification of actinomycete communities in Antarctic soil from Barrientos Island using PCR-denaturing gradient gel electrophoresis. Genetics and Molecular Research 11, 277–291.

Lee ES, Lee MY, Han SH, Ka JO (2008): Occurrence and molecular differentiation of environmental mycobacteria in surface waters. Journal of Microbiology and Biotechnology 18, 1207–1215.

Lehtola MJ, Torvinen E, Miettinen LT, Keevil CW (2006): Fluorescence in situ hybridization using peptide nucleic acid probes for rapid detection of Mycobacterium avium subsp avium and Mycobacterium avium subsp paratuberculosis in potable-water biofilms. Applied and Environmental Microbiology 72, 848–853.

Lehtola MJ, Torvinen E, Kusnetsov J, Pitkanen T, Maunula L, von Bonsdorff CH, Martikainen PJ, Wilks SA, Keevil CW, Miettinen IT (2007): Survival of Mycobacterium avium, Legionella pneumophila, Escherichia coli, and caliciviruses in drinking water-associated biofilms grown under high-shear turbulent flow. Applied and Environmental Microbiology 73, 2854–2859.

Leski TA, Gregory MJ, Malanoski AP, Smith JP, Glaven RH, Wang Z, Stenger DA, Lin B (2010): Analysis of dust samples from the middle east using high density resequencing microarray "RPM-TEI". Sensors, and command, control, communications, and intelligence (c3i) technologies for homeland security and homeland defense IX Book Series: Proceedings of SPIE 76661E, Article Number: 76661E DOI: 10.1117/12.853119.

Leski TA, Malanoski AP, Gregory MJ, Lin BC, Stenger DA (2011): Application of a broad-range resequencing array for detection of pathogens in desert dust samples from Kuwait and Iraq. Applied and Environmental Microbiology 77, 4285–4292.

Leys NM, Ryngaert A, Bastiaens L, Wattiau P, Top EM, Verstraete W, Springael D (2005): Occurrence and community composition of fast-growing Mycobacterium in soils contaminated with polycyclic aromatic hydrocarbons. Fems Microbiology Ecology 51, 375–388.

Liu RY, Yu ZS, Zhang HX, Yang M, Shi BY, Liu XC (2012): Diversity of bacteria and mycobacteria in biofilms of two urban drinking water distribution systems. Canadian Journal of Microbiology 58, 261–270.

Loret JF, Greub G (2010): Free-living amoebae: Biological by-passes in water treatment. International Journal of Hygiene and Environmental Health 213, 167–175.

Loret JF, Jousset M, Robert S, Saucedo G, Ribas F, Thomas V, Greub G (2008): Amoebae-resisting bacteria in drinking water: risk assessment and management. Water Science and Technology 58, 571–577.

Maekawa K, Ito Y, Hirai T, Kubo T, Imai S, Tatsumi S, Fujita K, Takakura S, Niimi A, Iinuma Y, Ichiyama S, Togashi K, Mishima M (2011): Environmental risk factors for pulmonary Mycobacterium avium-intracellulare complex disease. Chest 140, 723–729.

Manabe YC, Kesavan AK, Lopez-Molina J, Hatem CL, Brooks M, Fujiwara R, Flochstein K, Pitt MLM, Tufariello J, Chan J, McMurray DN, Bishai WR, Dannenberg AM, Mendez S (2008): The aerosol rabbit model of TB latency, reactivation and immune reconstitution inflammatory syndrome. Tuberculosis 88, 187–196.

Marciano-Cabral F, Jamerson M, Kaneshiro ES (2010): Free-living amoebae, Legionella and Mycobacterium in tap water supplied by a municipal drinking water utility in the USA. Journal of Water and Health 8, 71–82.

Matlova L, Kaevska M, Moravkova M, Beran V, Shitaye JE, Pavlik I (2012): Mycobacteria in peat used as a supplement for pigs: failure of different decontamination methods to eliminate the risk. Veterinarni Medicina 57, 212–217.

Matvieieva NA, Vasylenko MY, Shakhovsky AM, Kuchuk NV (2009): Agrobacterium-mediated transformation of lettuce (Lactuca sativa L.) with genes coding bacterial antigens from Mycobacterium tuberculosis. Cytology and Genetics 43, 94–98.

Matvieieva NA, Kishchenko OM, Potrochov AO, Shakhovsky AM, Kuchuk MV (2011): Regeneration of transgenic plants from hairy roots of Cichorium intybus L. var. Foliosum Hegi. Cytology and Genetics 45, 277–281.

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

McNabe M, Tennant R, Danelishvili L, Young L, Bermudez LE (2011): Mycobacterium avium ssp hominissuis biofilm is composed of distinct phenotypes and influenced by the presence of antimicrobials. Clinical Microbiology and Infection 17, 697–703.

Mendum TA, Chilima BZ, Hirsch PR (2000): The PCR amplification of non-tuberculous mycobacterial 16S rRNA sequences from soil. Fems Microbiology Letters 185, 189–192.

Mohr O, Askar M, Schink S, Eckmanns T, Krause G, Poggensee G (2012): Evidence for airborne infectious disease transmission in public ground transport – a literature review. Eurosurveillance 17, 12–22.

Momotani E, Ozaki H, Hori M, Yamamoto S, Kuribayashi T, Eda S, Ikegami M (2012): Mycobacterium avium subsp. paratuberculosis lipophilic antigen causes Crohn's disease-type necrotizing colitis in mice. SpringerPlus 1, 47-doi:10.1186/2193-1801-1-47.

Montana JS, Jimenez DJ, Hernandez M, Angel T, Baena S (2012): Taxonomic and functional assignment of cloned sequences from high Andean forest soil metagenome. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 101, 205–215.

Moravkova M, Babak V, Kralova A, Pavlik I, Slana I (2012): Culture- and quantitative IS900 real-time PCR-based analysis of the persistence of Mycobacterium avium subsp. paratuberculosis in a controlled dairy cow farm environment. Applied and Environmental Microbiology 78, 6608–6614.

Moravkova M, Lamka J, Kriz P, Pavlik I (2011): The presence of Mycobacterium avium subsp avium in common pheasants (Phasianus colchicus) living in captivity and in other birds, vertebrates, non-vertebrates and the environment. Veterinarni Medicina 56, 333–343.

Mori L, De Libero G (2012): T cells specific for lipid antigens. Immunologic Research 53, 191–199.

Murat JB, Grenouillet F, Reboux G, Penven E, Batchili A, Dalphin JC, Thaon I, Millon L (2012): Factors influencing the microbial composition of metalworking fluids and potential implications for machine operator's lung. Applied and Environmental Microbiology 78, 34–41.

Naresh R, Pandey S, Shukla JB (2009): Modeling the cumulative effect of ecological factors in the habitat on the spread of tuberculosis. International Journal of Biomathematics 2, 339–355.

Nassal J, Breunig W, Schnedelbach U (1974): Atypical mycobacteria in furit, vegetables, and cereals (in German). Praxis der Pneumologie 28, 667–674.

Nayak M, Kotian A, Marathe S, Chakravortty D (2009): Detection of microorganisms using biosensors – A smarter way towards detection techniques. Biosensors and Bioelectronics 25, 661–667.

Nieminen T, Pakarinen J, Tsitko I, Salkinoja-Salonen M, Breitenstein A, Ali-Vehmas T, Neubauer P (2006): 16S rRNA targeted sandwich hybridization method for direct quantification of mycobacteria in soils. Journal of Microbiological Methods 67, 44–55.

Niva M, Hernesmaa A, Haahtela K, Salkinoja-Salonen M, Sivonen K, Haukka K (2006): Actinobacterial communities of boreal forest soil and lake water are rich in mycobacteria. Boreal Environment Research 11, 45–53.

Pakarinen J, Nieminen T, Tirkkonen T, Tsitko I, li-Vehmas T, Neubauer P, Salkinoja-Salonen MS (2007): Proliferation of mycobacteria in a piggery environment revealed by mycobacterium-specific real-time quantitative PCR and 16S rRNA sandwich hybridization. Veterinary Microbiology 120, 105–112.

Papapetropoulou M, Tsintzou A, Vantarakis A (1997): Environmental mycobacteria in bottled table waters in Greece. Canadian Journal of Microbiology 43, 499–502.

Parker BC, Ford MA, Gruft H, Falkinham JO, III (1983): Epidemiology of infection by nontuberculous mycobacteria. IV. Preferential aerosolization of Mycobacterium intracellulare from natural waters. American Review of Respiratory Diseases 128, 652–656.

Perkins SD, Mayfield J, Fraser V, Angenent LT (2009): Potentially pathogenic bacteria in shower water and air of a stem cell transplant unit. Applied and Environmental Microbiology 75, 5363–5372.

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

Pickup RW, Rhodes G, Bull TJ, Arnott S, Sidi-Boumedine K, Hurley M, Hermon-Taylor J (2006): Mycobacterium avium subsp. paratuberculosis in lake catchments, in river water abstracted for domestic use, and in effluent from domestic sewage treatment works: Diverse opportunities for environmental cycling and human exposure. Applied and Environmental Microbiology 72, 4067–4077.

Pierce ES (2009): Possible transmission of Mycobacterium avium subspecies paratuberculosis through potable water: lessons from an urban cluster of Crohn's disease. Gut Pathogens 1, Article Number: 17 DOI: 10.1186/1757-4749-1-17 Published: 2009.

Pribylova R, Slana I, Kaevska M, Lamka J, Babak V, Jandak J, Pavlik I (2011): Soil and plant contamination with Mycobacterium avium subsp paratuberculosis after exposure to naturally contaminated mouflon feces. Current Microbiology 62, 1405–1410.

Primm TP, Lucero CA, Falkinham JO (2004): Health impacts of environmental mycobacteria. Clinical Microbiology Reviews 17, 98–106.

Rosenman KD (2009): Asthma, hypersensitivity pneumonitis and other respiratory diseases caused by metalworking fluids. Current Opinion in Allergy and Clinical Immunology 9, 97–102.

Salah IB, Ghigo E, Drancourt M (2009): Free-living amoebae, a training field for macrophage resistance of mycobacteria. Clinical Microbiology and Infection 15, 894–905.

Sandstrom G, Saeed A, Abd H (2011): Acanthamoeba-bacteria: A model to study host interaction with human pathogens. Current Drug Targets 12, 936–941.

Santos R, Oliveira F, Fernandes J, Goncalves S, Macieira F, Cadete M (2005): Detection and identification of mycobacteria in the Lisbon water distribution system. Water Science and Technology 52, 177–180.

Set R, Shastri J (2011): Laboratory aspects of clinically significant rapidly growing mycobacteria. Indian Journal of Medical Microbiology 29, 343–352.

Shin JH, Lee HK, Cho EJ, Yu JY, Kang YH (2008): Targeting the rpoB gene using nested PCR-restriction fragment length polymorphism for identification of nontuberculous mycobacteria in hospital tap water. Journal of Microbiology 46, 608–614.

Skovgaard N (2007): New trends in emerging pathogens. International Journal of Food Microbiology 120, 217–224.

Sood A, Sreedhar R, Kulkarni P, Nawoor AR (2007): Hypersensitivity pneumonitis-like granulomatous lung disease with nontuberculous mycobacteria from exposure to hot water aerosols. Environmental Health Perspectives 115, 262–266.

Stinear T, Davies JK, Jenkin GA, Hayman JA, Oppedisano F, Johnson PD (2000): Identification of Mycobacterium ulcerans in the environment from regions in Southeast Australia in which it is endemic with sequence capture-PCR. Applied and Environmental Microbiology 66, 3206–3213.

Svensson E, Akerstrom M, Andersson E (2011): Quantitative analyses of mycobacteria in water: Adapting methods in clinical laboratories. Journal of Microbiological Methods 87, 114–115.

Szymanska J, Sitkowska J (2012): Bacterial hazards in a dental office: An update review. African Journal of Microbiology Research 6, 1642–1650.

Szymanska J, Sitkowska J, Dutkiewicz J (2008): Microbial contamination of dental unit waterlines. Annals of Agricultural and Environmental Medicine 15, 173–179.

Tatchou-Nyamsi-Konig JA, Dague E, Mullet M, Duval JF, Gaboriaud F, Block JC (2008): Adhesion of Campylobacter jejuni and Mycobacterium avium onto polyethylene terephtalate (PET) used for bottled waters. Water Research 42, 4751–4760.

Tatchou-Nyamsi-Konig JA, Dailloux M, Block JC (2009): Survival of Mycobacterium avium attached to polyethylene terephtalate (PET) water bottles. Journal of Applied Microbiology 106, 825–832.

Thomas JM, Ashbolt NJ (2011): Do Free-living Amoebae in treated drinking water systems present an emerging health risk? Environmental Science and Technology 45, 860–869.

Tlougan BE, Podjasek JO, Adams BB (2010a): Aquatic sports dematoses. Part 2 – in the water: saltwater dermatoses. International Journal of Dermatology 49, 994–1002.

Tlougan BE, Podjasek JO, Adams BB (2010b): Aquatic sports dermatoses: part 1. In the water: freshwater dermatoses. International Journal of Dermatology 49, 874–885.

Tlougan BE, Podjasek JO, Adams BB (2010c): Aquatic sports dermatoses: Part 3. On the water. International Journal of Dermatology 49, 1111–1120.

Torvinen E, Lehtola MJ, Martikainen PJ, Miettinen IT (2007): Survival of Mycobacterium avium in drinking water biofilms as affected by water flow velocity, availability of phosphorus, and temperature. Applied of Environmental Microbiology 73, 6201–6207.

Torvinen E, Torkko P, Nevalainen A, Rintala H (2010): Real-time PCR detection of environmental mycobacteria in house dust. Journal of Microbiological Methods 82, 78–84.

Traub S, von Aulock S, Hartung T, Hermann C (2006): MDP and other muropeptides – direct and synergistic effects on the immune system. Journal of Endotoxin Research 12, 69–85.

Turankar RP, Lavania M, Singh M, Sai KSRS, Jadhav RS (2012): Dynamics of Mycobacterium leprae transmission in environmental context: Deciphering the role of environment as a potential reservoir. Infection Genetics and Evolution 12, 121–126.

Vaerewijck MJM, Huys G, Palomino JC, Swings J, Portaels F (2005): Mycobacteria in drinking water distribution systems: ecology and significance for human health. Fems Microbiology Reviews 29, 911–934.

van Ingen J, Boeree MJ, Dekhuijzen PNR, van Soolingen D (2009): Environmental sources of rapid growing nontuberculous mycobacteria causing disease in humans. Clinical Microbiology and Infection 15, 888–893.

Van Kruiningen HJ, Freda BJ (2001): A clustering of Crohn's disease in Mankato, Minnesota. Inflammatory Bowel Diseases 7, 27–33.

Vandelannoote K, Durnez L, Amissah D, Gryseels S, Dodoo A, Yeboah S, Addo P, Eddyani M, Leirs H, Ablordey A, Portaels F (2010): Application of real-time PCR in Ghana, a Buruli ulcer-endemic country, confirms the presence of Mycobacterium ulcerans in the environment. Fems Microbiology Letters 304, 191–194.

Villarreal JV, Schwartz T, Obst U (2010): Culture-independent techniques applied to food industry water

surveillance – A case study. International Journal of Food Microbiology 141, S147–S155.

Wagner D, Young LS (2004): Nontuberculous mycobacterial infections: A clinical review. Infection 32, 257–270.

Wang H, Edwards M, Falkinham JO, Pruden A (2012): Molecular survey of the occurrence of Legionella spp., Mycobacterium spp., Pseudomonas aeruginosa, and Amoeba hosts in two chloraminated drinking water distribution systems. Applied and Environmental Microbiology 78, 6285–6294.

Whan L, Ball HJ, Grant IR, Rowe MT (2005): Development of an IMS-PCR assay for the detection of Mycobacterium avium ssp. paratuberculosis in water. Letters in Applied Microbiology 40, 269–273.

Whiley H, Keegan A, Giglio S, Bentham R (2012): Mycobacterium avium complex – the role of potable water in disease transmission. Journal of Applied Microbiology 113, 223–232.

White CI, Birtles RJ, Wigley P, Jones PH (2010): Mycobacterium avium subspecies paratuberculosis in free-living amoebae isolated from fields not used for grazing. Veterinary Record 166, 401–402.

Xin Y, Huang J, Deng M, Zhang W (2008): Culture-independent nested PCR method reveals high diversity of actinobacteria associated with the marine sponges Hymeniacidon perleve and Sponge sp. Antonie Van Leeuwenhoek 94, 533–542.

Yajko DM, Chin DP, Gonzalez PC, Nassos PS, Hopewell PC, Reingold AL, Horsburgh Jr. CR, Yakrus MA, Ostroff SM, Hadley WK (1995): Mycobacterium avium complex in water, food, and soil samples collected from the environment of HIV-infected individuals. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology 9, 176–182.

Yoder S, Argueta C, Holtzman A, Aronson T, Berlin OG, Tomasek P, Glover N, Froman S, Stelma G, Jr. (1999): PCR comparison of Mycobacterium avium isolates obtained from patients and foods. Applied and Environmental Microbiology 65, 2650–2653.

Young JS, Gormley E, Wellington EMH (2005): Molecular detection of Mycobacterium bovis and Mycobacterium bovis BCG (Pasteur) in soil. Applied and Environmental Microbiology 71, 1946–1952.

Zwielehner J, Handschur M, Michaelsen A, Irez S, Demel M, Denner EBM, Hasiberger AG (2008): DGGE and real-time PCR analysis of lactic acid bacteria in bacterial communities of the phyllosphere of lettuce. Molecular Nutrition and Food Research 52, 614–623.

Received: 2011–07–25
Accepted after corrections: 2012–12–29

Corresponding Author:

Prof. MVDr. Karel Hruska, CSc., Veterinary Research Institute, Hudcova 70, 621 00 Brno, Czech Republic
Tel. +420 533 332 014, E-mail: hruska@vri.cz