3

Environmental sources of *Mycobacterium avium* linked to routes of exposure

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M. avium has been recovered from almost every environmental compartment that has been investigated including those that bring the organism into contact with susceptible species such as humans, animals, birds, and fish. The sources of *M. avium* in the environment are:

- natural water
- drinking-water
- biofilms
- aerosols
- soils
- foods
- plants and plant products
- fish.

Although a number of independent studies have shown that patient and environmental isolates of *M. avium* are either identical or similar, based on

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patterns of genetic (von Reyn *et al.* 1994; Mansfield & Lackner 1997) and physiologic (Fry *et al.* 1986) markers, it is not clear that all possible sources of *M. avium* in the environment have been identified, let alone the most important reservoirs. The diversity of sources and the heterogeneity of *M. avium* types isolated from the environment have complicated our search for the source of infection. Lack of knowledge of virulence markers has added further difficulty to the identification of the sources of *M. avium* infection. In addition, in many studies *M. avium* has not been distinguished from *M. intracellulare*. Although both are related, they are distinct species and appear to inhabit different but overlapping environments. This chapter presents a review of the environmental sources of *M. avium* and *M. intracellulare* and observations on the physiological determinants of their ecology.

3.1 ENVIRONMENTAL SAMPLE TYPES YIELDING *M. AVIUM*

3.1.1 *M. avium* in water

3.1.1.1 Methods of isolation

Isolation of *M. avium* from water requires consideration of the methods for concentration and disinfection. Filtration and centrifugation can be used to concentrate mycobacteria in water. If the water sample is suspected of containing microorganisms capable of overgrowing *M. avium* colonies, the sample can be disinfected. Disinfection relies upon the relative resistance of mycobacteria to disinfectants and detergents. If filtration is used for concentration, the sample should be disinfected before filtration (Glover *et al.* 1994). Following disinfection and filtration the filter is placed on a medium suitable for growth of mycobacteria (e.g. Middlebrook 7H10 agar). If centrifugation is used for concentration, the pelleted cells can be suspended in a small volume of water. Water is suitable for suspension of mycobacteria because the cells are resistant to lysis. Following concentration the sample can be decontaminated. Decontaminating agents include 1% NaOH, 1% Oxalic acid, 1% HCl (Brooks *et al.* 1984a), and 0.04% CPC (du Moulin & Stottmeier 1978). In addition to killing other microorganisms, the decontaminating agents also kill mycobacteria but to a lesser extent (e.g. 5% survival, Brooks *et al.* 1984). This means that only a minority of the total population of waterborne *M. avium* cells have been recovered and enumerated in the many reports of *M. avium* in water. Different combinations of concentration and disinfection for isolation of mycobacteria have been reviewed (Brooks *et al.* 1984; Schulze-Röbbecke *et al.* 1991; Kamala *et al.* 1994; Iivanainen *et al.* 1997; Neumann *et al.* 1997).

Unfortunately, there is no consensus for the method yielding the highest number of mycobacteria. For drinking-water, decontamination may not be required (Falkinham *et al.* 2001). Because of the hydrophobicity of mycobacteria it is possible that significant numbers of cells are lost during transfer.

A further discussion of analytical methods used for the detection of pathogenic waterborne mycobacteria can be found in Chapter 5.

3.1.1.2 M. avium in natural waters

A variety of natural waters throughout the world, including lakes, rivers, ponds and streams, have been shown to contain a resident *M. avium* population (Falkinham *et al.* 1980; du Moulin & Stottmeier 1986; Kirschner *et al.* 1992; von Reyn *et al.* 1993a; Covert *et al.* 1999). Whereas brackish water (1% NaCl) yields appreciable numbers of *M. avium,* seawater (> 3% NaCl) seldom has any mycobacteria (Falkinham *et al.* 1980). *M. avium* can grow in natural waters but cannot grow in waters of high salinity (George *et al.* 1980). Groundwater seldom contains *M. avium* (Martin *et al.* 1987; Falkinham *et al.* 2001). *M. avium* numbers are highest in waters of low oxygen and high organic matter content (Brooks *et al.* 1984; Kirschner *et al.* 1992). High numbers of *M. avium* are recovered from acid, brown-water swamps of the south-eastern coast of the United States (Kirschner *et al.* 1992) and acidic, brown-waters draining from peat-rich boreal forest soils in Finland (Iivanainen *et al.* 1999a).

3.1.1.3 M. avium in drinking-waters

Drinking-water has been shown to have a resident *M. avium* population (du Moulin *et al.* 1988; von Reyn *et al.* 1993, 1994; Glover *et al.* 1994; Peters *et al.* 1995; Covert *et al.* 1999; Ristola *et al.* 1999; Falkinham *et al.* 2001). A single *M. avium* clone was isolated repeatedly from a hospital water system over a period of 18 months demonstrating that *M. avium* is not a contaminant but is a normal inhabitant of drinking-water (von Reyn *et al.* 1994). Numbers of *M. avium* in recirculating hot water systems are increased relative to numbers in the input water (du Moulin *et al.* 1988) suggesting that *M. avium* is replicating in such systems in agreement with studies in natural water (George *et al.* 1980).

The number and frequency of isolation of *M. avium* varies quite widely between drinking-water systems (Falkinham *et al.* 2001). *M. avium* numbers in raw water sources for drinking-water systems correlates with the concentration of particulate matter suggesting that *M. avium* is associated with particulates (Falkinham *et al.* 2001). Thus, one approach for reducing the number of *M. avium* in a drinking-water system is to remove particulates (e.g. turbidity) from the raw waters during treatment. Numbers of *M. avium* in drinking-water distribution systems are higher in samples collected from the mid- and endpoints of the system compared to numbers in water collected immediately after treatment (Falkinham *et al.* 2001). This suggests that mycobacterial growth occurs in the distribution system. The presence of *M. avium* in treated water is consistent with its high resistance to ozone and chlorine-based disinfectants (Taylor *et al.* 2000).

3.1.1.4 Other water samples

Due to the widespread presence of *M. avium* in drinking-water, recreational and other waters that have their origin in drinking-water yield mycobacteria. Spas and hot tubs have been shown to yield *M. avium* that have been associated with infection in people using the spas (Embil *et al.* 1997; Kahana *et al.* 1997; Khoor *et al.* 2001). Swimming pools yield *M. avium* (Havelaar *et al.* 1985; Emde *et al.* 1992) and long-term exposure to aerosols results in a granulomatous pneumonitis in lifeguards (Rose *et al.* 1998).

3.1.1.5 M. avium in biofilms

M. avium, M. intracellulare and other mycobacteria have been shown to be present in biofilms (Schulze-Röbbecke & Fischeder 1989; Iivanainen *et al.* 1999a; Falkinham *et al.* 2001). In a study of eight drinking-water distribution systems across the United States the average number of *M. avium* in biofilms was 0.3 cfu/cm^2 and the number of *M. intracellulare* was 600 cfu/cm^2 for all surfaces (Falkinham *et al.* 2001). If one considers the size of pipes used to distribute drinking-water and the length of the pipes in a system (e.g. 75 to 7100 miles) the contribution of biofilm to the microbial flora of the drinking-water is substantial.

The presence of *M. avium* in biofilms in drinking-water systems might explain why two groundwater-fed drinking-water systems did not have *M. avium* in their raw source water, but did have *M. avium* in the distribution system (Falkinham *et al.* 2001). The frequency of recovery of *M. avium* in water samples from the same systems was higher than that of *M. intracellulare* (Falkinham *et al.* 2001), suggesting that one preferred habitat of *M. intracellulare* is within a biofilm.

The type of surface apparently had an effect on mycobacterial biofilm numbers. Numbers of *M. intracellulare* were 4400 cfu/cm^2 on brass or bronze surfaces compared to 70 cfu/cm^2 on plastic (Falkinham *et al.* 2001). The values for cfu/cm^2 in the drinking-water distribution systems (Falkinham *et al.* 2001) were 10-fold lower than the numbers for hot water silicon tube biofilms (Schulze-Röbbecke & Fischeder 1989). The presence of hot water would increase the growth of mycobacteria on any surface (George *et al.* 1980).

3.1.2 *M. avium* in soils

3.1.2.1 Methods of isolation

Isolation of *M. avium* from soil samples involves three steps: elution from particulate matter, concentration, and disinfection. Current methods for recovering *M. avium* from soil are inefficient; for example, Brooks and co-workers could only recover 5% of *M.avium* cells added to soil samples (Brooks *et al.* 1984). Decontamination is then required to reduce the high numbers of microorganisms whose colonies overgrow those of mycobacteria. Because decontamination reduces *M. avium* numbers by 95% and only 5% of *M. avium* cells can be separated from particulate matter (Brooks *et al.* 1984), only 0.25% of total mycobacteria in soils are recovered as cfu. As is the case for water, a variety of reports have compared different methods of isolation of mycobacteria from soils (Brooks *et al.* 1984; Portaels *et al.* 1988; Kamala *et al.* 1994; Iivanainen 1996). It has been shown that exposure of soils to polysaccharidases increases the numbers of isolates and species of mycobacteria in soils (Thorel *et al.* 1991).

3.1.2.2 M. avium in soils and peat

A wide variety of soils, sediments and peat have been reported to contain *M. avium*. The soils include those on river banks in the south-eastern United States (Brooks *et al.* 1984a), in swamps in the coastal south-eastern United States (Kirschner *et al.* 1992) and peat-rich boreal forest soils in Finland (Iivanainen *et al.* 1997a), both of which yield high numbers of *M. avium*. Characteristics that correlate with high numbers of *M. avium* include low pH, high organic matter and low oxygen (Brooks *et al.* 1984; Kirschner *et al.* 1992; Iivanainen *et al.* 1997a). The high numbers of *M. avium* reported in drinking-waters in Finland (Ristola *et al.* 1999) and in the north-eastern United States are likely due to the use of source waters rich in mycobacteria.

Yajko and co-workers reported that 55 % of soil samples from potted plants of AIDS patients contained *M. avium* which is consistent with the high numbers of *M. avium* and other mycobacteria in peat-rich boreal forest soils (Yajko *et al.* 1995). Furthermore, we have recently discovered that commercial samples of peat and potting soils sold in the United States are rich in *M. avium* and other mycobacteria (Falkinham, unpublished).

3.1.3 *M. avium* in aerosols, ejected droplets and dust

3.1.3.1 Methods of isolation

M. avium and other mycobacteria in aerosols and dusts can be recovered and enumerated. A simple method is to use the Andersen 6-Stage Cascade Sampler (Andersen 1958). This sampler separates particulates in the air on the basis of size by impacting on the surface of agar medium. Whether the particle collected is from soil (e.g. dust) or from water (e.g. droplet) cannot be determined. Airborne cells are recovered as colonies. Thus the instrument provides a measure of the number of airborne cells and the associated particle size. Particles recovered on the bottom two stages are of a size that can enter the alveoli of the human lung (Andersen 1958). Because the particles are separated by size, most fungal spores are trapped on those stages collecting larger particles. Thus decontamination may not be required unless the air or dust sample contains a substantial number of *Bacillus* spores. Malachite green at a concentration of 0.05% in Middlebrook 7H10 agar medium does not inhibit growth of mycobacteria, but is effective at preventing colony formation of other bacteria and fungi (Jones & Falkinham, in preparation).

M. avium and other mycobacteria can also be recovered from droplets that are ejected from the surface of water. Air bubbles rising through a water column collect particles, chemicals and microorganisms (Blanchard & Szydek 1970, 1978, 1982; Blanchard *et al.* 1981). Hydrophobic interaction drives the adsorption to bubbles and results in bubbles reaching the surface enriched in organic chemicals and hydrophobic microbial cells (Blanchard & Hoffman 1978; Weber *et al.* 1983). Because mycobacteria are the most hydrophobic of microorganisms (van Oss *et al.* 1975), they are enriched in the bubbles. When the bubbles reach the surface they burst and form a crater that results in the ejection of one to several droplets 8-10 cm above the water surface (Blanchard & Szydek 1978). The droplets can be collected on inverted agar medium and the droplet size calculated from the diameter of the craters formed by the droplets on an inverted Petri dish coated with MgO (Blanchard *et al.* 1981). The ejected droplets are enriched in mycobacteria (Parker *et al.* 1983). Indeed, for *M. avium*, the concentration of cfu in the ejected droplets divided by the concentration in the bulk suspension (enrichment factor) can be as high as 10 000 (Parker *et al.* 1983).

3.1.3.2 M. avium in ejected droplets

M. avium can be recovered as cfu from droplets ejected from natural waters (Wendt *et al.* 1980). For these measurements, inverted Petri dishes were held 10 cm above a river surface for 60 minutes. Immediately after collection, the liquid on the plates was spread and the plates incubated until mycobacteria colonies were recovered (Wendt *et al.* 1980). In a second study both water and ejected droplets were collected at the same site on the James River in Richmond, Virginia. The number of *M. avium* and *M. intracellulare* in ejected droplets was 2715 per ml and the number in water was 0.19 per ml (Falkinham *et al.* 1990). This data demonstrates that there is enrichment of *M. avium* and *M. intracellulare* in natural ejected droplets (~14 000-fold) resulting in considerable transfer of mycobacteria from water to air.

Laboratory experiments showed that *M. avium* and *M. intracellulare*, but not the relatively hydrophilic *M. scrofulaceum*, were preferentially aerosolized (Parker *et al.* 1983). Further studies demonstrated a correlation between enrichment factor and hydrophobicity (Falkinham 1989). Because the bubble burst jet drop mechanism of aerosol transfer of microorganisms occurs in every natural body of water, mycobacteria can be efficiently detected, enumerated and identified with few problems of contamination.

3.1.3.3 M. avium in aerosols

M. avium and other mycobacteria can be recovered from aerosols using the Andersen Cascade sampler (Falkinham *et al.* 1990). Between July 1979 and July 1980, twice monthly aerosol samples were collected at a park by the James River in Richmond, Virginia. The site was far from any agricultural activity. Numbers of *M. avium* and *M. intracellulare* averaged 25 cfu/m^3 of air and 1.4 cfu/m^3 associated with particles able to reach the alveoli (Falkinham *et al.* 1990). Based on an estimate that a human inhales 6 m^3 air per day, a person at that site would inhale 150 *M. avium* and *M. intracellulare* per day (8 reaching the alveoli). The highest number was 250 cfu/m^3 air. The numbers of *M. avium* and *M. intracellulare* were not correlated with the numbers in river water at the same site (Falkinham *et al.* 1990).

3.1.4 *M. avium* in phagocytic protozoa and amoebae

3.1.4.1 Intracellular growth and enumeration

M. avium, like other mycobacteria, are intracellular pathogens able to survive and grow in infected animal macrophages. They are also capable of survival and growth in phagocytic protozoa (*Tetrahymena pyriformis*) and amoebae (*Acanthamoeba polyphaga* and *Acanthamoeba castellanii*). In laboratory experiments it was shown that *M. avium* is readily phagocytozed by protozoa and amoebae (Strahl *et al.* 2001). Five days after infection of protozoa or amoebae, the number of intracellular *M. avium* cells increases to 5-10 per infected *T. pyriformis* cell (Strahl *et al.* 2001) or 10-15 per infected

A. castellanii cell (Cirillo *et al.* 1997). *M. avium* survives cyst formation and germination of *T. pyriformis* (Strahl *et al.* 2001) and *A. polyphaga* (Steinert *et al.* 1998). Thus protozoa and amoebae increase numbers of *M. avium* and other mycobacteria in habitats where both reside. One troubling aspect of intracellular growth of *M. avium* in protozoa and amoebae is that the resulting *M. avium* cells are more virulent in mice (Cirillo *et al.* 1997) and chickens (Falkinham, unpublished).

Recovery of intracellular *M. avium* from protozoa or amoebae can be performed using lysis with 1% SDS (Cirillo *et al.* 1997). The detergent does not reduce colony formation by *M. avium* or other mycobacteria (Cirillo *et al.* 1997). Protozoa and amoebae can be recovered from water samples by centrifugation (1000 x g for 10 minutes), and mycobacteria recovered after lysis or simply by spreading the low speed pellet on Middlebrook 7H10 agar. Eventually the protozoa or amoebae lyse. Individual protozoa or amoebae can be isolated using micropipettes and grown in medium to determine numbers of intracellular mycobacteria.

There are no published reports of any systematic study of numbers of *M. avium* and other mycobacteria in protozoa or amoebae. Protozoa and amoebae offer an environmental sample likely enriched for *M. avium* and mycobacteria. In one study, we compared the recovery of mycobacteria from water with recovery from *T. pyriformis* that had been added to the water sample and incubated one week. More species and higher numbers of *Mycobacterium* were recovered from the protozoa (Falkinham, unpublished). An investigation of intracellular mycobacteria in protozoa and amoebae is important because those environmental habitats yielding high numbers of *M. avium* also harbour high numbers of protozoa and amoebae.

3.1.5 Other sources of *M. avium*

M. avium and other mycobacteria have been isolated from foods (Yajko *et al.* 1995; Yoder *et al.* 1999; Argueta *et al.* 2000), fish (Mediel *et al.* 2000; Rhodes *et al.* 2001) and tobacco products (Eaton *et al.* 1995) all of which come into contact with humans and thus can serve as a source of infection. In one study, 25 of 121 (21%) food samples yielded *M. avium* (Argueta *et al.* 2000). Isolation from tobacco products was attempted because of the report that smokers who suffered from pulmonary alveolar proteinosis were also infected with *M. avium* whereas non-smokers were not infected (Witty *et al.* 1994).

3.2 IMPACT OF UNIDENTIFIED MYCOBACTERIAL ISOLATES

Depending upon the study, a significant proportion of mycobacteria recovered from different environments do not belong to established mycobacterial species. For example, in a study of brook waters in Finland, 15% of isolates belonged to established species and that percentage was raised to only 28% if isolates that were “like” established species were included (Iivanainen *et al.* 1993). In studies of drinking-water the percentage of isolates that did not belong to established species varied from 15-50% (Covert *et al.* 1999; Falkinham *et al.* 2001; Le Dantec *et al.* 2002). Because different computer programs for calculating similarity index values for isolates from the same 16SrRNA gene sequence data yield different values (Drancourt *et al.* 2000), the program used has a direct impact on the diversity of mycobacterial species isolated. The method of recovery directly influences the spectrum of *Mycobacterium* species isolated from environmental samples. Decontamination reduces the number and range of species and colony variants differ in their susceptibility to disinfection (Brooks *et al.* 1984). In addition, the diversity of species recovered from a sample is related to the total number of isolates recovered. For example, polyclonal infection in AIDS patients can only be detected when a sufficient number of isolates are recovered (Slutsky *et al.* 1994). The same holds for diversity in environmental samples.

The impact of these factors means that we are isolating, identifying and characterizing only a minority of cells that are present in the environment. That in turn tempers the impact of any conclusions that can be made concerning the ecology of *M. avium, M. intracellulare* or other mycobacteria.

3.3 ENVIRONMENTS WITH HIGH NUMBERS OF *M. AVIUM*

Because *M. avium* is an opportunistic human pathogen it is important to point out those environmental sources that have high numbers. Boreal, peat-rich forest soils and waters yield high numbers of *M. avium* and other mycobacteria (Iivanainen *et al.* 1997a). Such forests are found in Finland, across northern Europe, the northern United States, and Canada. The presence of high numbers of *M. avium* in Finnish drinking-water was associated with a high frequency of *M. avium* infection in Finnish AIDS patients (Ristola *et al.* 1999). Waters and soils of the acid, brown-water swamps of the south-eastern coastal United States also yield high numbers of *M. avium* (Kirschner *et al.* 1992). Although located in different parts of the world, both share low pH, low oxygen and presence of high concentrations of humic and fulvic acids.

Peat and potting soils yield high numbers of *M. avium* (Yajko *et al.* 1995). This is not unexpected because peat-rich boreal forest soils have high numbers of *M. avium* and other mycobacteria. Commercial peat-rich potting soils and samples of soil in pots with plants have numbers of *M. avium* approaching 10^6 per gram (Falkinham, unpublished).

Recirculating hot water systems, spas, and hot tubs yield high numbers of *M. avium*. The presence of *M. avium* in spas and hot tubs has been associated with *M. avium* infection in humans in a number of independent studies (Embil *et al.* 1997; Kahana *et al.* 1997). Recirculating hot water systems in hospitals were shown to have higher numbers of *M. avium* than the source water for the system (du Moulin *et al.* 1988).

Biofilms contain *M. avium, M. intracellulare* and other mycobacteria. Biofilms in drinking-water distribution systems appear to be a preferred habitat for *M. intracellulare* (600 cfu/cm^2) compared to *M. avium* (0.3 cfu/cm^2) (Falkinham *et al.* 2001). Shower heads collect a biofilm and are good sites to sample for *M. avium*. The ability of *M. avium, M. intracellulare* and other mycobacteria to form biofilms means that they can populate in-line water filters used for water purification (Ridgway *et al.* 1984). In fact, it has been shown that *M. avium* was capable of populating and growing to 100 000 cfu in an inline filter (Rodgers *et al.* 1999). This observation suggests another method for collection of samples to detect or monitor *M. avium* numbers: place in-line filters in the water distribution system and sample them at intervals.

3.4 ROUTES OF EXPOSURE

Routes of *M. avium* exposure include ingestion, inhalation and surface or traumatic exposure. Because of the widespread distribution of *M. avium* in many environments, human and mycobacterial habitats overlap leading to exposure.

3.4.1 Ingestion

There is long-term evidence that ingestion is a route of *M. avium* infection. Cervical lymphadenitis caused by *M. avium* is found in children from six months to two years old (Wolinsky 1995). In addition, *M. avium* infection in simian immunodeficiency virus infected macaques was traced to the water drunk by the monkeys (Mansfield & Lackner 1997). Ingestion of mycobacteria could also occur through eating fish or other foods that are colonized (infected) with *M. avium* or other mycobacteria.

3.4.2 Inhalation

The results of studies of *M. tuberculosis* transmission and evidence of pulmonary infection have been used to postulate an aerosol route of infection for *M. avium, M. intracellulare* and other mycobacteria. Demonstration that *M. avium* and *M. intracellulare* cells are readily aerosolized from water, are highly hydrophobic and can be recovered from aerosols, certainly support that route of infection. However, there has been no demonstration of aerosol transmission of *M. avium* infection to laboratory animals, possibly because of the low infectivity of *M. avium*. Both water droplets and particulates in the atmosphere are likely vehicles of *M. avium* transmission. Not only are *M. avium* cells enriched in water droplets ejected from surfaces (Parker *et al.* 1983), but *M. avium* numbers are associated with particulate matter in water (Falkinham *et al.* 2001). Droplets enriched for *M. avium* (and other mycobacteria) would be generated from any body of water and are formed in large numbers in domestic showers. Because hot water systems can be enriched for mycobacteria, showers may be rich sources for mycobacterial-enriched aerosols. Dust particles may also serve as vectors for transmission of *M. avium*. Discovery that peat soils are rich in *M. avium* and other mycobacteria led us to measure the mycobacteria in aerosols generated by dropping 100 gm of peat or potting soil 30 cm. Using the 6-Stage Andersen Cascade Sampler, we have found that the resulting aerosolized particles contain mycobacteria in substantial numbers. In addition, some of the particles associated with mycobacteria were within the size range that could enter human alveoli (Falkinham, unpublished).

3.4.3 Trauma

Trauma, resulting from either surface abrasions or injury or during surgical procedures, may also lead to infection by *M. avium* and other mycobacteria. *Mycobacterium marinum* granulomas on the hands have been found in individuals who handle fish and have skin abrasions (Wolinsky 1979). The presence of substantial numbers of *M. avium* in water may also result in human exposure. The disinfectant resistance of *M. avium* and other mycobacteria can lead to their persistence in solutions used in surgeries. A nosocomial *M. chelonae* infection was associated with the use of a gentian violet-containing skin-marking solution (Safranek *et al.* 1987).

3.4.4 Biofilms

Biofilms not only represent a mechanism for the persistence and growth of *M. avium* in tubes, but they also represent a reservoir for cells. For example, a persistent *M. avium* infection was shown to be associated with biofilm formation in a catheter (Schelonka *et al.* 1994). Not only are the biofilms sources for more cells, but *M. avium* and *M. intracellulare* cells in biofilms are more resistant to antibiotics (Steed & Falkinham, in preparation). Furthermore, *M. avium* and *M. intracellulare* cells grown in biofilms but exposed to antibiotics in suspension are more resistant to antibiotics (Steed & Falkinham, in preparation), suggesting that biofilm growth alone renders *M. avium* more resistant to antibiotics.

3.5 OVERLAP OF HUMAN AND *M. AVIUM* ENVIRONMENTS

There are a number of factors that suggest that the incidence of disease caused by *M. avium, M. intracellulare* and other mycobacteria will continue to increase. In part, this will be a consequence of the existence of overlaps of human and mycobacterial environments. For example, it is possible that widespread use of chlorine and other disinfectants to improve water quality have led to increases in mycobacterial numbers. Because *M. avium* is so resistant to disinfection, competing microorganisms are killed by disinfectants leaving mycobacteria free to proliferate in the absence of competition. Distribution systems and hot water systems provide environments for the growth of mycobacteria and thus increase numbers to which humans and animals are exposed. The use of potting soils rich in peat leads to exposure of gardeners (professional and amateur) to particles containing high numbers of *M. avium* and other mycobacteria. Thus some human activities may increase the risk of exposure of individuals to mycobacteria. Those overlaps, coupled with an ageing human population containing more individuals with immunodeficiency through therapy or infection, suggest that infection by *M. avium*, *M. intracellulare* and other mycobacteria may become more prevalent.

3.6 KEY RESEARCH ISSUES

In spite of the enormous progress in the understanding of *M. avium* epidemiology, ecology, and physiologic ecology, there are still important questions concerning this opportunistic pathogen. Some of the questions involve the methodology used to detect, isolate, and enumerate *M. avium* in environmental samples. Others involve questions of defining *M. avium* and its various types. The final issue of importance is the development of effective disinfection strategies for reduction of *M. avium* in the environment. Below is a list of methodological research issues.

- Improve recovery or detection of *M. avium* in environmental samples
- Define *M. avium* and its various types

- Identify markers for *M. avium* virulence
- Identify the dose-response to *M. avium* infection in different human hosts
- Develop effective *M. avium* disinfection strategies

Current methods for recovery of *M. avium* from environmental samples are limited by losses due to transfer, adherence, and decontamination. Another problem that impacts on recovery and enumeration of *M. avium* and other mycobacteria is the fact that colony counts are usually 10-fold lower than counts of cells, even in laboratory medium. This suggests current methods for enumeration of colonies underestimate numbers. Furthermore, recovery methods suffer from the need for relatively long term incubation. Although, PCR-based methods offer the promise of rapid and sensitive detection of *M. avium* and other mycobacteria, they are limited by difficulties in lysing mycobacterial cells and the lack of sensitivity of PCR-based detection compared to colony-formation based detection. Developing a quantitative PCR-based detection system is a further difficult step to achieve.

The current status of *M. avium* taxonomy is in a state of flux (Mijs *et al.* 2002). First, the species *M. avium* and *M. intracellulare* must be distinguished from one another. The relatives have different epidemiological and ecological patterns. *M. avium* predominates in AIDS patients and children with cervical lymphadenitis, whereas both are found at equal frequencies in non-AIDS patients with pulmonary disease (Drake *et al.* 1988; Guthertz *et al.* 1989; Colville 1993; Wolinsky 1995). Further, there has been no study comparing the utility of different typing methods (e.g., IS*901*, IS*1245*, PFGE) for discriminating between different *M. avium* isolates from patients and from epidemiologically matched environmental samples. Such a study might identify virulence markers of *M. avium*. Such knowledge would simplify and reduce the cost of efforts to identify sources of *M. avium* in humans and animals. Currently, every mycobacterium is recovered, identified, and enumerated.

It is important to develop alternative strategies for reduction of numbers of *M. avium, M. intracellulare*, and other mycobacteria in the environment. Current disinfection strategies for drinking water appear to select for mycobacteria and their growth. One strategy for reduction of *M. avium* is reduction of particulates (i.e., turbidity) in raw and treated water (Falkinham *et al.* 2001). Filtration can be used, but it is important to recall that *M. avium* and other mycobacteria can grow on filters and the filters can, in turn, serve as sources for mycobacteria by elution (Ridgway *et al.* 1984; Rodgers *et al.* 1999). Another approach would be to identify novel disinfectants that are active against *M. avium, M. intracellulare*, and other mycobacteria. Identification of factors leading to disinfectant-resistance of *M. avium* would contribute to this goal.