Curr Microbiol (2013) 67:725–731
DOI 10.1007/s00284-013-0427-1

The Tracing of Mycobacteria in Drinking Water Supply Systems by Culture, Conventional, and Real Time PCRs

Barbora Klanicova · Jaromir Seda · Iva Slana · Michal Slany · Ivo Pavlik

Received: 4 April 2013 / Accepted: 16 June 2013 / Published online: 31 July 2013

Abstract Mycobacteria are widely present in diverse aquatic habitats, where they can survive for months or years while some species can even proliferate. The resistance of different mycobacterial species to disinfection methods like chlorination or ozonation could result in their presence in the final tap water of consumers. In this study, the culture method, *Mycobacterium tuberculosis* complex conventional duplex PCR for detection of non-tuberculous mycobacteria (NTM) and quantitative real-time PCR (qPCR) to detect three subspecies of *M. avium* species (*M. a. avium*, *M. a. hominissuis*, and *M. a. paratuberculosis*) were used to trace their possible path of transmission from the watershed through the reservoir and drinking water plant to raw drinking water and finally to households. A total of 124 samples from four drinking water supply systems in the Czech Republic, 52 dam sediments, 34 water treatment plant sludge samples, and 38 tap water household sediments, were analyzed. NTM of 11 different species were isolated by culture from 42 (33.9 %) samples; the most prevalent were *M. gordonae* (16.7 %), *M. triplex* (14.3 %), *M. lentiflavum* (9.5 %), *M. a. avium* (7.1 %), *M. montefiorenase* (7.1 %), and *M. nonchromogenicum* (7.1 %). NTM DNA was detected in 92 (76.7 %) samples. By qPCR analysis a statistically significant decrease ($P < 0.01$) was observed along the route from the reservoir (dam sediments), through water treatment sludge and finally to household sediments. The concentrations ranged from 10^0 to 10^4 DNA cells/g. It was confirmed that drinking water supply systems (watershed–reservoir–drinking water treatment plant–household) might be a potential transmission route for mycobacteria.

Electronic supplementary material The online version of this article (doi:10.1007/s00284-013-0427-1) contains supplementary material, which is available to authorized users.

B. Klanicova (✉) · I. Slana · M. Slany · I. Pavlik (✉)
Veterinary Research Institute, Hudcova 70, 621 00 Brno, Czech Republic
e-mail: klanicova@vri.cz

I. Pavlik
e-mail: pavlik@vri.cz

J. Seda
Biology Centre of the Academy of Sciences of the Czech Republic, Institute of Hydrobiology, Na Sadkach 7, 370 05 Ceske Budejovice, Czech Republic

Introduction

For drinking purposes ground water is more suitable than treated surface water; however, the treatment of surface waters can meet the increasing demand of populations for potable water. The abstraction of raw water from rivers faces some problems such as variations in water quality as well as the risk of contamination by various pathogens including mycobacteria. Mycobacteria can survive in the environment for months or even years and are common in diverse aquatic habitats like natural waters, drinking water systems, biofilms, and aerosols. Environmental mycobacteria also called potentially pathogenic mycobacteria (PPM) and environmental saprophytic mycobacteria (ESM); they are often described as non-tuberculous mycobacteria (NTM) and include different opportunistic human and animal pathogens [17, 25].

The life cycles of mycobacteria seem to be very diverse and have not yet been completely elucidated [17]. Some are pathogens causing mycobacterial infections in animals and humans and live in soil, fens, swamps, bogs, or marshes outside the hosts. From these semi-aquatic habitats they are transported to rivers and subsequently also to reservoirs. The

problem of the occurrence of mycobacteria, and also other microbial organisms, in drinking water reservoirs is partially solved by the technology of abstraction of raw water. Because of self-purification processes and sedimentation effects, the water in deep water strata (hypolimnion) is more suitable for treatment for drinking purposes. As a result water companies abstract raw water from the deep strata when possible. Thus, the resulting quality of raw water in the reservoir depends primarily on nutrient load, the depth and retention time [28].

The most common NTM present in surface water are in alphabetical order *M. avium*, *M. chelonae*, *M. fortuitum*, *M. gordonae*, *M. nonchromogenicum*, *M. terrae*, and *M. triviale* [32]. *M. avium* species are divided into four subspecies: *M. a.* subsp. *avium*, *M. a. hominissuis*, *M. a. paratuberculosis*, and *M. a. silvaticum* [22, 29]. The first two subspecies *M. a. avium* and *M. a. hominissuis*, known zoonotic pathogens, cause various disseminated infections, tuberculosis-like illnesses, lymphadenitis, and osteomyelitis in animals and in immunocompromised humans [12, 16, 25]. In particular, *M. a. hominissuis* is able to grow outside of host organisms when trophic/temperature conditions are good [17]. On the other hand, *M. a. paratuberculosis* is suspected zoonotic pathogen, causing paratuberculosis (Johne's disease), chronic enteritis in ruminants, and *M. a. silvaticum* causing mycobacteriosis in birds require the growth stimulator Mycobactin [29]. Their growth outside of host organisms in water has yet to be confirmed [17].

The principal transport vehicles of mycobacteria are water and soil. They can be washed away from pastures furthermore contaminated with infected feces (the most common way of excretion from infected hosts) and from fertilized soil into rivers, and thus contaminate water for drinking purposes [17, 23, 34]. Some studies investigating the resuspension of reservoir sediments into water have been carried out, suggesting that sediments could act as a reservoir of PPM and constitute a potential health hazard [3].

Previously, in the laboratory-based microcosm study, has been demonstrated that bacteria can survive longer in sediment than in overlying water and similarly pointed to an increased risk of exposure because of the possible resuspension of pathogenic microorganisms [5].

Pickup et al. [23] detected *M. a. paratuberculosis* in sediment cores from valley reservoirs by quantitative real-time PCR (qPCR). They argued that this was due to extensive cattle and sheep farming with heavy rainfalls in that region. Subsequently the same team detected *M. a. paratuberculosis* in sediment from a domestic cold-water tank [24].

M. a. hominissuis and *M. a. avium* have already been detected in municipal drinking water distribution systems, hospital water systems, and in ice machines, swimming-pools and whirlpools [4, 6, 7, 14, 33]. There is increasing evidence that tap water is a medium for mycobacteria to colonize the human body [19].

Aboagye and Rowe [1] tested the presence of *M. a. paratuberculosis* in water treatment stations for potable water production. They found one *M. a. paratuberculosis* culture-positive sample in the final treated water and concluded that the public might be exposed through water supplies.

The abundance of the above mentioned subspecies in water could stem from their resistance to disinfection methods like chlorination and/or ozonation. Drinking water distribution systems might thus present possible means for the transfer of mycobacteria to immunocompromised humans and animals [2, 8, 30, 33, 35].

In this study, four drinking water supply systems were subjected to analysis for mycobacteria. The four systems treat deep water from four distinct reservoirs which differ in watershed area, land use, nutrient load, and the depth and retention time. From each system samples of reservoir sediment close to the reservoir dam, sludge from the water treatment plant, and finally the sediment from household tanks have been analyzed. We investigated (i) the frequency of recovery of mycobacteria from diverse locations by culture and (ii) the frequency of NTM using the conventional duplex PCR technique and (iii) the presence of the three most important *M. avium* subspecies *M. a. hominissuis*, *M. a. avium*, and *M. a. paratuberculosis* by qPCR analysis. Our hypothesis was that there would be an uneven distribution of mycobacteria, and that high human activity in the watershed of the drinking reservoir might influence the occurrence of mycobacteria.

Materials and Methods

Origin and Collection of Samples

The four studied water supply systems (designated as I, II, III, and IV) in the Czech Republic differ in their parameters, but all four abstract raw water from the deep strata. The reservoirs differ in the human activity in their watershed and three (I, III, and IV) out of four reservoirs are closed for public having a zone of no entry of 100–1,000 m from the shoreline (Table 1). Disinfection practices in all four water treatment plants of studied reservoirs do not differ and include chlorination and filtration.

The reservoir sediment from the deepest point near the dam was sampled using a gravitational corer with an inner diameter of 50 mm [15]. The reservoir sediment is thought to be a cumulative picture of all the occurring and sinking seston, including bacteria. For our analyses the top 2 cm surface was removed and stored in the dark at 5 °C. Altogether 52 reservoir sediment samples were analyzed.

The 34 water treatment sludge samples from the water treatment stations were collected from rapid gravity sand

Table 1 Characteristics of the four drinking water reservoirs

Reservoir characteristics[a]	I	II	III	IV
Phosphorus content (μg/l)	9–12	13–17	19–23	35–40
Retention time (days)	604	89	86	166
Max depth (m)	53.6	43.5	47.5	65.6
Surface area (km^2)	14.0	2.1	1.9	2.1
Agriculture/forest/urban (%)	58/36/4	50/47/2	46/51/2	50/45/4
Closed for recreation (Y/N)	Y	Y	N	Y

[a] Data kindly provided by local authorities of water reservoirs

filters during the washing procedure and stored in the dark at 5 °C. In water treatment plants was as the primary coagulant used ferric sulfate. Water treatment sludge can also be thought of as a cumulative trap where organisms, including bacteria, are flocculated from a relatively big volume of raw water.

The 38 household sediment samples were collected from household drinking water tanks by plastic Pasteur pipettes and stored in the dark at 5 °C. No extra chlorine or other disinfectant is present in the water in the water tanks. The household sediments from tap water supplies represent cumulative samples of drinking water supplying households.

Culture Examination and Isolate Identification

All samples ($n = 124$) were decontaminated according to a procedure described previously with a slight modification [10]. One gram of each sample was homogenized in a stomacher, decontaminated in 1 M HCl and then neutralized with 2 M NaOH. 200 μl of the suspension were inoculated onto three solid Herrold's egg yolk media (HEYM) with and without 2 μg/ml Mycobactin J (Allied Monitor, Fayette, MO, USA), HEYM with antibiotics (penicillin 4 IU/ml, amphotericin B 50 μg/ml, chloramphenicol 50 μg/ml; Becton–Dickinson United Kingdom Ltd., Oxford, United Kingdom), and one medium according Lesslie (modified Stonebrink). These were then incubated in triplicate at 25, 30, and 37 °C. Media were checked for bacterial growth after 1, 2, 3, 5, 7, 9, 11, and 16 weeks of incubation.

Mycobacterial isolates were analyzed by conventional duplex and M4D PCR assays suitable for differentiating members of the *M. avium* complex, as described previously by Moravkova et al. [21]. All isolates recognized as non-members of the *M. avium* complex were further classified with consideration of growth characteristics and data from sequencing analysis of the *16S rRNA* gene [13].

DNA Isolation

DNA isolation from environmental samples (all 124 samples were analyzed by qPCR and only 120 samples were analyzed by MTC conventional duplex PCR) was achieved using the MoBio PowerSoil DNA Isolation kit (MoBio, Carlsbad, CA, USA) with slight modifications to the protocol, as previously described by Kaevska et al. [16]. Briefly, the provided PowerSoil Bead beating tubes were added to 0.25 g of sample to which 6.25 μg of carrier DNA (fish sperm DNA; Amresco, Solon, OH, USA) was additionally added. The samples were homogenized using a MagNA Lyser Instrument (Roche Molecular Diagnostics, Mannheim, Germany) at 6400 rpm for 60 s.

The subsequent steps were performed according to the manufacturer's instructions. DNA was eluted in 100 μl of pre-heated TE buffer (pH 8.0; Amresco, Solon, OH, USA), after 3 minutes of on-column incubation. The isolated DNA was used as a template for conventional PCR (MTC duplex) and qPCRs: IS*900* qPCR, IS*901*, and IS*1245* triplex qPCR described bellow.

PCR Detection of Non-tuberculous Mycobacteria

Conventional broad range duplex PCR targeting the *rpoB* gene was used to detect mycobacteria in environmental samples. As published previously, the MTC duplex PCR assay is suitable for differentiation of *M. tuberculosis* complex members from other mycobacterial species [20].

qPCR Detection and Quantification of *M. avium* Subspecies

Duplex qPCR with an internal amplification control was used in all samples for the detection of the *M. a. paratuberculosis*-specific insertion sequence IS*900*, as previously described by Slana et al. [27]. Triplex qPCR with an IAC for the detection of *M. a. avium*-specific insertion sequences IS*901*, and IS*1245* specific for *M. a. hominissuis* and *M. a. avium* was used in all samples. All three targets were amplified in the same reaction based on a semi-competitive principle, as previously described by Slana et al. [26].

Statistical Analysis

For statistical analysis of data the programs Statistica 9.0 (StatSoft, Inc., Tulsa, OK, USA) and GraphPad Prism 5.04 (GraphPad, Inc., San Diego, CA, USA) were used. *P*-values lower than 0.05 were considered statistically significant.

Results and Discussion

NTM and particularly mycobacteria in the *M. avium* complex are highly adaptable to moist soil and aquatic ecosystems [34]. Due to the resistance of mycobacteria to

chlorination and ozonization, tap water might represent a means for the colonization and transfer of mycobacteria to immunocompromised animals and people through water supplies [2, 30, 33, 35].

Isolated and Indentified Mycobacteria

Mycobacteria of 11 different species were isolated from 42 (33.9 %) out of 124 samples. The most prevalent were *M. gordonae* (16.7 %), *M. triplex* (14.3 %), *M. lentiflavum* (9.5 %), *M. a. avium* (7.1 %), *M. montefiorenase* (7.1 %), and *M. nonchromogenicum* (7.1 %). The detailed isolate location and number of detected mycobacteria are listed in Table 2 in the supplementary file. From each sample only isolate of one species was cultured. *M. gordonae* (mainly detected in tap water household sediments), *M. triplex* (mainly detected in dam sediments), and *M. lentiflavum* (mainly detected in water treatment sludge) were the most frequently isolated mycobacterial species (Fig. 1). The highest incidence of mycobacteria was observed in dam sediments of reservoir No. IV, which correlates well with the fact that this reservoir has the highest value of phosphorus, i.e., the highest human activity in its watershed (Table 1). Culture analysis of sediment and sludge samples is rather difficult due to the necessary disinfection procedure which decreases concentration yields by up to three orders of magnitude in comparison with qPCR methods [18]. Although mycobacterial isolates could be cultured not only from dam sediments and water treatment sludge, of most concern are the mycobacteria isolated from tap water household sediment such as *M. absecessus/M. chelonae, M. gordonae, M. llatzarense*, *M. montefiorense*, *M. peregrinum/M. septicum*, and other *M.* species (Fig. 1). Bearing in mind that analyzed tap water household sediment came from water intended for direct consumption, primarily households with immunocompromised individuals should consider some establishment of preventative measures, e.g., microbiological filtration, boiling, or others [11]. The hypothesis that the highest occurrence of mycobacteria would be linked with human activities in the reservoir watersheds, i.e., also with nutrient load, was confirmed. In fact the highest numbers of culture isolates were in reservoir No. 4, which is the reservoir with highest phosphorus content. Nevertheless Torvinen et al. [31] showed that the incidence of *M. avium* in potable water biofilms is not increased by the addition of phosphorus because of the occurrence of other heterotrophic bacteria and competition between them. Torvinen et al. [31] also described temperature as an important factor in the survival of *M. avium* in drinking water biofilms (with increased temperature they observed an increase in the survival of MAA). We could not observe this trend due to insignificant differences in temperatures among the reservoirs.

Non-tuberculous Mycobacterial DNA Detection by MTC Duplex PCR

MTC duplex PCR revealed the presence of NTM DNA in 92 (76.7 %) of all tested sediment and sludge samples. The prevalence of NTM DNA in dam sediments was 40 (80.0 %), which declined slightly en route. NTM DNA was detected in 29 (90.6 %) of samples from the water treatment sludge, while in consumer tap water household sediments NTM DNA was detected in 23 (60.5 %) of the analyzed samples. The detailed location of mycobacteria is listed in Table 3 in the supplementary file. The highest prevalence of NTM in dam sediments declined en route to the consumers in households (Fig. 2). However the number

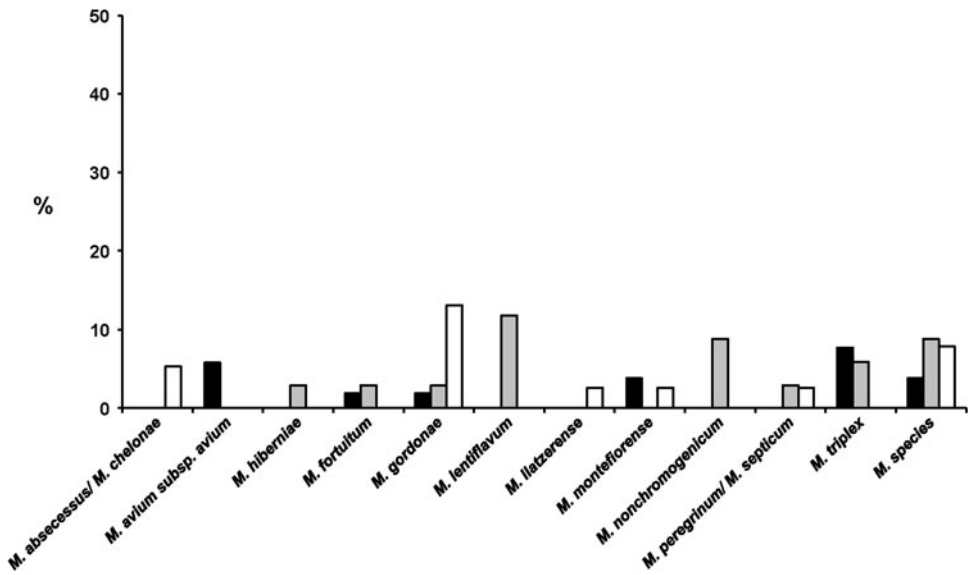

Fig. 1 Percentage of mycobacterial isolates detected in dam sediment (*black column*), water treatment sludge (*gray column*), and in household sediment from tap water supplies (*white column*)

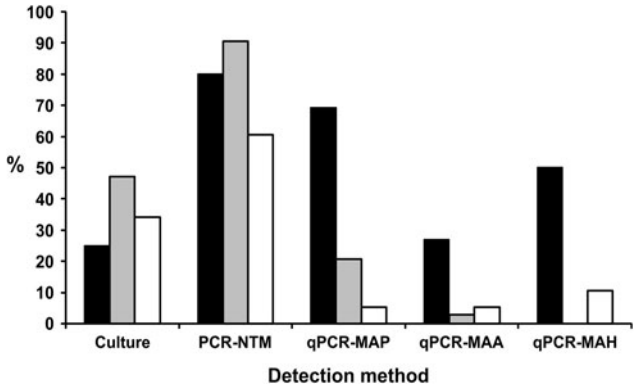

Fig. 2 Percentage of mycobacteria detected in dam sediment (*black column*), water treatment sludge (*gray column*), and in household sediment from tap water supplies (*white column*) using three detection methods. Culture for mycobacterial subspecies detection, Mycobacterium tuberculosis complex conventional duplex PCR for DNA detection of non-tuberculous mycobacteria (NTM) and quantitative real time PCR (qPCR) results for DNA detection of *M. a. paratuberculosis* (MAP), *M. a. avium* (MAA), and *M. a. hominissuis* (MAH)

of NTM detected in water treatment sludge and tap water household sediment did not differ significantly. Almost the same percentage of NTM-positive samples detected in household water systems was previously reported in the study of Falkinham III [9]. NTM are ubiquitous organisms living in water and biofilms and are resistant to chlorination and ozonization; thus, their complete eradication would seem to be impossible.

Determination of the Presence and Quantity of *M. avium* Subspecies *M. a. hominissuis*, *M. a. avium*, and *M. a. paratuberculosis* by qPCR Analysis

Samples of dam sediments, water treatment sludge and tap water household sediment were collected to follow the incidence of mycobacteria. Some of the tested samples of dam sediments and water treatment sludge were positive for two or all three subspecies of *M. avium* subspecies, and thus the number of positive samples for mycobacterial DNA is higher than the number of tested samples. Altogether 92 (74.2 %) of the analyzed samples were found to be qPCR-positive. The mostly frequently detected DNA was that of *M. a. paratuberculosis* which was identified in 45 (36.3 %) of all tested samples. While *M. a. avium* DNA was identified in 17 (13.7 %) of the tested samples, *M. a. hominissuis* DNA was recovered from 30 (24.2 %) of analyzed samples. The detailed location and quantification of mycobacteria are listed in Table 3 in the supplementary file. The rate of positive dam sediment samples were found to be statistically significantly higher than the rate of positive water treatment sludge and household sediments (*P*-values for Fisher's exact tests were <0.01 or <0.05). Overall, a statistically significant declining trend of *M. avium* subspecies-positive samples in the route leading from dam–water treatment station–households (*P* value for χ^2 test for trend was <0.01) was revealed. This overall significantly decreasing trend in detected mycobacterial DNA was driven mainly by the drinking water system of reservoir No. III (the most positive) and reservoir No. IV ($P < 0.05$). Different trend was observed in the results from qPCR analysis in contrast to the occurrence of NTM (Fig. 2). While most DNA was detected in dam sediments ($P < 0.01$), we could observe a significant decrease in water treatment sludge and tap water household sediment samples. The effectiveness of drinking water plant purification seems to be comparable, probably reaching similar levels of water purity, as the declining trend of mycobacterial DNA in drinking water supplying reservoirs Nos. III and IV shows. The raw water (and also the mud sediment) originating from these reservoirs are probably more contaminated by mycobacteria than that of reservoirs Nos. I and II. This phenomenon was previously observed in the study of Whittington et al. [34], who showed that *M. a. paratuberculosis* can survive for longer in sediment than in the water column in the troughs. From the analyzed samples, the most frequently isolated was *M. a. paratuberculosis* DNA. This is in agreement with the study of Pickup et al. [23] and Aboagye and Rowe [1], who also detected *M. a. paratuberculosis* in reservoir sediments, sludge (schmutzdecke) from a water treatment plant and also in the final drinking water. Although Pickup et al. [23] reported a higher incidence in the water column due to extensive farming and heavy rainfalls; in our study we did not confirm a connection between the occurrence of *M. a. paratuberculosis* and the rate of agriculture because this indicator is very similar in all four valley reservoirs (Table 1). *M. a. paratuberculosis* was frequently recovered from the dam sediments of all four drinking water valley reservoirs, while its prevalence was less often confirmed in water treatment sludge. We were also able to detect *M. a. paratuberculosis* DNA in two samples of tap water household sediment from two different locations. The overall decline of *M. a. paratuberculosis* through the water distribution system was statistically significant. However, the concentrations detected by qPCR were under the detection limit of the culture method which is 10^3 copies/g (data not shown) [18], so the culture of live *M. a. paratuberculosis* cells was not successful. Nevertheless Aboagye and Rowe [1] obtained one culture-positive sample from final treated water and concluded that *M. a. paratuberculosis* can most likely in very low amounts enter the water distribution system.

M. a. hominissuis DNA was mainly isolated from dam sediments, while its presence was not revealed in any water treatment sludge. This disparity may be partly explained by

the fact that *M. a. hominissuis* is a common inhabitant of soil [17]. The fact that we identified some *M. a. hominissuis* DNA in tap water household sediment shows that it can probably travel after the sediment resuspension in water through the water distribution system reaching households, as in the case of *M. a. paratuberculosis* in the study of Aboagye and Rowe [1]. We could observe a similar phenomenon with *M. a. avium* DNA. *M. a. avium* DNA-positive samples originated chiefly from dam sediments, while only one *M. a. avium* DNA-positive sludge sample was detected. *M. a. avium* DNA was then also detected in tap water household sediment samples (Fig. 2).

The results of this monitoring study of sediments and sludge from four drinking water reservoirs and water treatment stations and households testifies to the presence of mycobacterial isolates, NTM and specific *M. avium* subspecies in these locations. In this study we confirmed that even though the mycobacterial counts decrease with increasing distance from the reservoirs, on the basis of our culture results, the drinking water distribution systems represent a potential risk for the transmission of mycobacterial diseases from dam to the consumer, and thus some safety measures should be adopted with regard to immunocompromised humans and animals.

Acknowledgments This study was supported by Grants Nos. MZE0002716202, QH91240 from the Ministry of Agriculture of the Czech Republic and by the Ministry of Education, Youth and Sports of the Czech Republic (AdmireVet, CZ 1.05/2.1.00/01.0006/ED0006/01/01). The authors would like to thank MVDr. Vojtech Mrlik, CSc. and Mgr. Krystyna Kantorova for their help with collection and culture of the samples, and also RNDr. Vladimir Babak for the statistical analysis of the data.

References

1. Aboagye G, Rowe MT (2011) Occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in raw water and water treatment operations for the production of potable water. Water Res 45:3271–3278
2. Aronson T (1999) Comparison of large restriction fragments of *Mycobacterium avium* isolates recovered from AIDS and non-AIDS patients with those of isolates from potable water. J Clin Microbiol 37:1008
3. Chandran A, Varghese S, Kandeler E, Thomas A, Hatha M, Mazumder A (2011) An assessment of potential public health risk associated with the extended survival of indicator and pathogenic bacteria in freshwater lake sediments. Int J Hyg Environ Health 214:258–264
4. Covert TC, Rodgers MR, Reyes AL, Stelma GN (1999) Occurrence of nontuberculous mycobacteria in environmental samples. Appl Environ Microbiol 65:2492–2496
5. Craig DL, Fallowfield HJ, Cromar NJ (2004) Use of macrocosms to determine persistence of *Escherichia coli* in recreational coastal water and sediment and validation with in situ measurements. J Appl Microbiol 96:922–930
6. Du Moulin GC, Stottmeier KD, Pelletier PA, Tsang AY, Hedley-Whyte J (1988) Concentration of *Mycobacterium avium* by hospital hot water systems. JAMA 260:1599–1601
7. Falkinham JO III, Norton CD, Lechavallier MW (2001) Factors influencing numbers of *Mycobacterium avium*, *Mycobacterium intracellulare*, and other mycobacteria in drinking water distribution systems. Appl Environ Microbiol 67:1225–1231
8. Falkinham JO III (2009) Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. J Appl Microbiol 107:356–367
9. Falkinham JO III (2011) Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. Emerg Infect Dis 17:419–424
10. Fischer O, Matlova L, Dvorska L, Svastova P, Bartl J, Melicharek I, Weston RT, Pavlik I (2001) Diptera as vectors of mycobacterial infections in cattle and pigs. Med Vet Entomol 15:208–211
11. Ford T, Hermon-Taylor J, Nichols G, Cangelosi G, Bartram J (2004) Approaches to risk management in priority settings. In: Pedley S, Bartram J, Rees G, Dufour A, Cotruvo JA (eds) Pathogenic mycobacteria in water: A guide to public health consequences, monitoring and management. IWA, London
12. 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. Am J Respir Crit Care Med 175:367–416
13. Harmsen D, Dostal S, Roth A, Niemann S, Rothganger J, Sammeth M, Albert J, Frosch M, Richter E (2003) RIDOM: comprehensive and public sequence database for identification of *Mycobacterium* species. BMC Infect Dis 3:26
14. Havelaar AH, Berwald LG, Groothius DG, Baas JG (1985) Mycobacteria in semi-public swimming-pools and whirlpools. Int J Med Microbiol 180:505–514
15. Hruska V (1986) A simple self-releasing core sampler. Limnologica 17:259–261
16. 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 the tracing of infection in the environment by culture and triplex quantitative real time PCR. J Clin Microbiol 49:167–172
17. Kazda J, Pavlik I, Falkinham JO III, Hruska K (2009) The Ecology of Mycobacteria: Impact on Animal's and Human's Health. Springer, Heidelberg
18. Kralik P, Beran V, Pavlik I (2012) Enumeration of *Mycobacterium avium* subsp. *paratuberculosis* by quantitative real time PCR, culture on solid media and optical densitometry. BMC Res Notes 5:114
19. Le Dantec C, Duguet J-P, Montiel A, Dumoutier N, Dubrou S, Vincent V (2002) Occurrence of mycobacteria in water treatment lines and water distribution systems. Appl Environ Microbiol 68:5318–5325
20. Mokkadas E, Ahmad S (2007) Development and evaluation of a multiplex PCR for rapid detection and differentiation of *Mycobacterium tuberculosis* complex members from non-tuberculous mycobacteria. Jpn J Infect Dis 60:140–144
21. Moravkova M, Hlozek P, Beran V, Pavlik I, Preziuso S, Cuteri V, Bartos M (2008) Strategy for the detection and differentiation of *Mycobacterium avium* species in isolates and heavily infected tissues. Res Vet Sci 85:257–264
22. Mijs W, de Haas P, Rossau R, Van der Laan T, Rigouts L, Portaels F, van Soolingen D (2002) Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp. *avium* for bird-type isolates and '*M. avium* subsp. *hominissuis*' for the human/porcine type of *M. avium*. Int J Syst Evol Microbiol 52:1505–1518

23. 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, and its potential relationship to clustering of Crohn's disease cases in the city of Cardiff. Appl Environ Microbiol 71:2130–2139
24. 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. Appl Environ Microbiol 72:4067–4077
25. Primm TP, Lucero CA, Falkinham JO III (2004) Health impacts of environmental mycobacteria. Clin Microbiol Rev 17:98–106
26. Slana I, Kaevska M, Kralik P, Horvathova A, Pavlik I (2010) Distribution of *Mycobacterium avium* subsp. *avium* and *M. a. hominissuis* in artificially infected pigs studied by culture and IS*901* and IS*1245* quantitative real time PCR. Vet Microbiol 144:437–443
27. Slana I, Kralik P, Kralova A, Pavlik I (2008) On-farm spread of *Mycobacterium avium* subsp. *paratuberculosis* in raw milk studied by IS*900* and *F57* competitive real time quantitative PCR and culture examination. Int J Food Microbiol 128:250–257
28. Straskraba M, Tundisi JG, Duncan A (1993) Comparative reservoir limnology and water quality management. Kluwer Academy, Hague
29. Thorel MF, Krichevsky M, Levy-Frebault VV (1990) Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. nov., *Mycobacterium avium* subsp. *paratuberculosis* subsp. nov., and *Mycobacterium avium* subsp. *silvaticum* subsp. nov. Int J Syst Microbiol 40:254–260
30. Tobin-D'Angelo MJ, Blass MA, del Rio C, Halvosa JS, Blumberg HM, Horsburgh CR (2004) Hospital water as a source of Mycobacterium avium complex isolates in respiratory specimens. J Infect Dis 189:98–104
31. 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. Appl Environ Microbiol 73:6201–6207
32. Tuffley RE, Holbeche JD (1980) Isolation of the *Mycobacterium avium-M. intracellulare-M. scrofulaceum* complex from tank water in Queensland, Australia. Appl Environ Microbiol 39:48–53
33. Von Reyn CF, Maslow JN, Barber TW, Falkinham JO III, Arbeit RD (1994) Persistent colonization of potable water as a source of *Mycobacterium avium* infection in AIDS. J Lancet 343:1137–1141
34. Whittington RJ, Marsch IB, Reddacliff LA (2005) Survival of *Mycobacterium avium* subsp. *paratuberculosis* in dam water and sediment. Appl Environ Microbiol 71:5304–5308
35. Wolinsky E (1992) Mycobacterial diseases other than tuberculosis. Clin Infect Dis 15:1–10