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Short Communication

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The major public health issue - Presence of Mycobacterium avium paratuberculosis in drinking and unfit water of Porto geographical area detected by quantitative PCR

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Abstract

Mycobacterium avium subsp. paratuberculosis (MAP) has been implicated in the development of inflammatory bowel disease (IBD) and colorectal cancer. Portugal has a high prevalence of IBD and Porto is one of the Portuguese districts with higher IBD prevalence. Our goal was to assess MAP contamination in drinking and domestic untreated water sources in Porto geographical area, since water may be an important contaminating source of MAP for humans. One liter of public drinking water and/or a domestic untreated water source were collected at different locations in Porto area in early Autumn. Biofilms were also collected by swabbing tap inner surfaces with a sterile cotton swab. A second collection of domestic untreated water was performed after winter rains, in early February. Water samples were filtered and DNA was extracted from both water filters and biofilm suspensions using specific commercial kits. MAP contamination was evaluated through a IS900-based nested PCR assay. Drinking water samples showed higher MAP contamination than domestic water collected in Autumn. In February, MAP detection significantly increased in domestic water. MAP DNA was detected at a higher frequency in tap biofilms than in the corresponding water collected. Drinking and domestic water may be important sources of MAP contamination in Porto area. The increased MAP detection observed after winter rains in domestic water may reflect soil leaching. Since MAP can resist to water treatment procedures and persist in biofilms, increased microbial surveillance and development of new water treatment methods are most needed to avoid human exposure to this resilient pathobiont.

Drinking water samples (n = 238) were collected from January to December 2009 from taps in homes or commercial buildings at 41 sites in 25 states, one district, and one U.S. territory. A spectrum of source waters (surface and ground water), system sizes (large and small utilities, private wells), and disinfection types (chlorine, chloramine, and chlorine dioxide) was included. Twenty-nine samples were analyzed using TaqMan universal master mix, and 209 samples were analyzed using TaqMan environmental master mix 2.0. All reaction mixtures included the TaqMan exogenous internal positive-control reagent to detect PCR inhibition. A standard curve using the new reagents was and was not significantly different from the curve obtained with the original reagents ($\alpha = 0.05$; $P > 0.31$). Due to the potential for waterborne transmission of mycobacteria and the association of M. avium subsp. paratuberculosis with human illness, the focus of this study was to estimate the organism's occurrence in drinking water in the United States using quantitative PCR (qPCR).

A comprehensive method was developed for detection of M. avium subsp. paratuberculosis in drinking water and biofilms that includes the concentration of microorganisms from samples using membrane filtration, total DNA extraction and purification, and detection of two targets unique to this bacterium: IS900 and target 251. IS900 is a common target used to identify M. avium subsp. paratuberculosis, and the average number of copies per genome is 14 to 18. Target 251 qPCR analysis, which corresponds to the M. avium subsp. paratuberculosis gene 2765c was developed by Rajeev et al. Samples positive for both targets are considered positive for M. avium subsp. paratuberculosis.

A master standard curve was generated from six series of 10-fold dilutions of genomic DNA from M. avium subsp. paratuberculosis strain 49164 for quantification of IS900 target copies. Each dilution series contained eight standards run in triplicate for a total of 18 threshold cycle (CT) measurements per standard. A linear regression was performed on CT versus log IS900 copy number and R2 was 0.997. The standard error of y was used to create two equations to estimate the upper and lower concentration, or range, of M. avium subsp. paratuberculosis IS900 copy number.

The specificities of the IS900 and target 251 primer/probe sets were evaluated by Rajeev et al. on 211 M. avium subsp. paratuberculosis and 38 non-M. avium subsp. paratuberculosis isolates, and each assay was 100% specific for M. avium subsp. paratuberculosis. We further evaluated specificity using 22 M. avium subsp. paratuberculosis isolates from animals and 10 non-M. avium subsp. paratuberculosis ATCC reference strains (see Table S1 in the supplemental material).

The sensitivity of the method for detection of M. avium subsp. paratuberculosis in different drinking water matrices was evaluated by spiking serial dilutions of strain 1112 cells, ranging from 104 cells to no addition of cells, into 1-liter tap water samples obtained from five locations in the United States. The number of M. avium subsp. paratuberculosis cell equivalents was estimated by dividing the IS900 copy number obtained from the master standard curve by 18 (mean, 18 IS900 copies/M. avium subsp. paratuberculosis genome). The method provided consistent detection (5/5 samples) in a spiked sample of 100 cells/liter. In a spiked sample of 10 cells/litre, the IS900 target was detected 40% (2/5 samples) of the time, and at 1 cell/litre we did not detect the target in any spiked sample. Percentage recovery was variable and decreased as the number of spiked cells decreased. At a spike level of 1 × 104 cells/litre, the average percentage recovery was 64%; this decreased to 9.2% at 1 × 102 cells/litre. Cell surface hydrophobicity, a property of mycobacteria, may have influenced clumping of the spiked sample or partitioning of M. avium subsp. paratuberculosis onto the sample bottle or filtration unit, affecting recovery of the bacterium.

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