Seasonal variations in the occurrence of environmental mycobacteria in potable water

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A fluctuation in the prevalence of environmental mycobacteria in relation to nutritional conditions in nature has been repeatedly described in the literature. A seasonal difference in the potable water supply system has not yet been documented. Potable water samples from the supply systems of 16 identical localities were analyzed. Samples of running water and tap swabs or tap scrapings were collected twice a year, in the spring and in the autumn. The samples were processed as stipulated by the international standards. McNemar's test was used to analyze the difference in the occurrence of environmental mycobacteria between the vernal and the autumnal samples. A significant change in the presence of environmental mycobacteria in the potable water supply system was observed, the vernal samples yielding more positive results. This finding supports other observations respecting surface water. We suggest that this effect on the potable water supply system may be caused by the change in temperature. Contamination rates were similar, with no statistically significant differences between running water samples and those from swabs or scrapings. No time trend in the period 1984-1989 respecting the prevalence of mycobacteria was detected. Direct microscopy showed massive colonization with environmental mycobacteria of the potable water supply system. The public health consequences of these findings should be further evaluated, as colonization of water pipes can be associated with outbreaks of mycobacterial disease in immunocompromised patients. There has also been an increase in the incidence of mycobacterioses in the North Moravian region in recent years.

Key words: Environmental mycobacteria; potable water; seasonal variations.

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Many synonyms have been used to denote environmental mycobacteria (mycobacteria other than tuberculous, nontuberculous mycobacteria, anonymous, tuberculoid, atypical, potentially pathogenic mycobacteria). The first widely used microbiological description and identification scheme was suggested by Runyon in 1959 (10). The human response to environmental mycobacteria varies from hypersensitivity to tuberculin skin tests and innocuous colonization to disseminated disease (3, 6, 8, 11, 14), including a suspected association of environmental mycobacteria with Crohn's disease and sarcoidosis (3). The potable water supply system was suspected to have acted as a vehicle for the transmission of environmental mycobacteria in several nosocomial outbreaks of mycobacterioses, especially in immunocompromised hosts (1, 2, 11).

The seasonal fluctation in the occurrence of environmental mycobacteria in relation to nutritional conditions in nature has been repeat-
edly described in the literature, and was recently well documented by Kirschner et al. (6). The seasonal difference in the potable water supply system has - to our knowledge - not yet been documented.

MATERIALS AND METHODS

Sample collection
Potable water samples from the supply systems of 16 identical localities were collected within a county of about one million inhabitants. The original sources include both surface water, such as lakes and dams, in 10 localities, and ground water, such as wells and old mines, in the remaining 3 localities. In three localities the water sources are mixed. These sources supply eight hospitals, four waterworks, three households and one village shop, from where the samples were collected. The samples were obtained from tap water outlets (i.e., where hot and cold water are mixed). The samples of running water were taken after 10 min, and tap swabs or tap scrapings were collected twice a year, in the spring (regularly in each sampling period from 21st March to 21st June each year) and in the autumn (regularly in each sampling period from 23rd September to 21st December each year). These two seasons were chosen for logistic reasons. From 1984-1986 10 liter samples of water, processed by filtering through membrane filters (Synpor #7) with a pore diameter of 3 μm, were examined. Each filter was then put into 20 ml of sterile phosphate-buffered saline (PBS) and shaken for 20 min using a mechanical shaker (Chirana, Stara Tura). The PBS solution was subsequently processed by centrifugation. From 1987-1989 only 1 liter water samples were taken. These were successively centrifuged. There was no difference in the quality of samples or in the number of positive samples using these two methods (4).

Lauryl sulfate decontamination with 1% NaOH was applied to improve the efficacy of the samples. The resuspended samples were inoculated onto a Loewenstein-Jensen slant and incubated at 25°C and 37°C. Growth of mycobacteria was evaluated after one week. Mycobacteria were subcultured to purity. The species were identified by Bonicke's enzymatic rows and growing properties, i.e., by standard methods (7).

Apart from culture, all the processed and decontaminated samples were also examined by direct microscopy of Ziehl-Neelsen staining.

Statistical analysis
McNemar's test for analyzing the different occurrences of environmental mycobacteria in the vernal and the autumnal samples and differences in contamination was applied, using the STATGRAPHICS statistical package. Linear regression analysis was performed to assess the time trend over the follow-up period from 1984-1989.

RESULTS

In total, 686 samples were obtained: 340 from running water and 346 from tap swabs or scrapings. Half the 686 samples were cultured at 25°C and half at 37°C. Of the samples, 29 (4.2%) were contaminated in spite of the decontamination procedure. These contaminated samples were omitted from further analysis. A total of 657 samples were analyzed, and the culture was negative in 381 (58%) and positive in 276 (42.0%). The prevalence of the different species was as follows: *M. gordonae* in 134 (20.4%) samples, *M. flavescens* in 91 (13.8%), IV group of mycobacteria in 33 (5.0%), and *M. aureum* in 10 (1.5%); *M. scrofulaceum*, *M. fortuitum* and *M. terrae* were found occasionally.

Analysis of contingency tables according to the season, spring versus autumn, was carried out for the type of sample and culture temperature, using McNemar's test. There was a significant difference (p-value < 0.05) between the vernal and autumnal samples at the 25°C culture temperature irrespective of the sample type. In the culture at 37°C the trend did not reach statistical significance. Table 1 presents a basic synopsis of results.

The total number of contaminated samples was 29 (4.2%). The number of contaminated samples in running water was 19 (5.6%), and for swabs and scrapings 10 (2.9%). The contamination was analyzed for the type of sample, using contingency table analysis and McNemar's test. No statistically significant difference was detected.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Culture temperature (°C)</th>
<th>Spring</th>
<th>Autumn</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Running water</td>
<td>25</td>
<td>46</td>
<td>28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>28</td>
<td>22</td>
<td>ns</td>
</tr>
<tr>
<td>Swab</td>
<td>25</td>
<td>54</td>
<td>33</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>30</td>
<td>20</td>
<td>ns</td>
</tr>
</tbody>
</table>
No significant time trend for the prevalence of environmental mycobacteria over the period 1984–1989 was found by ANOVA and linear regression analysis of the samples (p-value > 0.05). Fig. 1 shows the time trend.

**DISCUSSION**

A change in the presence of environmental mycobacteria in the potable water supply system was observed, the vernal samples giving more positive results. This finding supports other observations on surface water (5), acid brown-water swamps (6) and sputum (13). We suggest that the effect on the potable water supply system may be caused by a change in a general environmental factor, namely temperature.

Occurrence of environmental mycobacteria in potable water samples is documented both from public systems and from hospital water taps (1–4, 12). Heat susceptibility of mycobacteria and the way potable water pipes are colonized have been discussed elsewhere (2, 4, 6), leading to the conclusion that the optimal temperature for the growth of mycobacteria under these conditions varies round 37°C. The prevalence of environmental mycobacteria in the potable water supply system is important due to the fact that mycobacteria may occasionally cause disseminated mycobacterial disease which is resistant to common antituberculous drugs, especially in hospitalized immunocompromised hosts (1, 2, 11). Our study did not quantify the mycobacterial load, but if we indirectly estimate this from cultivation and microscopy, we can count a concentration of $10^3$ to $10^4$ microorganisms per milliliter. Recently an increase in the incidence of mycobacterioses has been reported in the northern Moravian region (4), mainly due to *M. kansasii*, *M. avium intracellulare* and *M. xenopi*.

Environmental mycobacteria are ubiquitous and can be found in soils, in both surface and underground water, and in dust and aerosols (1, 2, 5, 6, 10, 12). Our study, which focused on the potable water supply system, did not measure the changes in water pH, temperature, dissolved oxygen, and other environmental and nutritional conditions, such as humic and fulvic acid concentrations, presence of sphagnum, zinc and heavy metal concentrations, or other possible changes in the natural environment due to hygienic or agricultural interventions.

There is a constant fluctuation between the positive culture identified as *M. flavescens* and *M. gordonae* in our findings. There is no sound evidence in the literature as to the intrageneric relationship between these two species (9). Our routine identification, based on obsolete biochemical characteristics according to Bonicke's enzymatic reactions and growth properties, does not allow a more precise classification as achieved using modern methods.

**CONCLUSION**

There was a statistically significant difference between the numbers of positive cultures in vernal compared to autumnal samples. We infer that this effect on the potable water supply system may be caused by a change in a general environmental factor, namely temperature. Contamination rates were similar, with no statistically significant difference between running water samples and those from swabs or scrapings. The prevalence rates of environmental mycobacteria showed no time trend over the period 1984–1989. Further investigations are necessary in order to elucidate the fluctuation between *M.*
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gordonae and M. flavescens. Direct microscopy showed massive colonization of the potable water supply system, especially from scraping samples. The public health importance of the findings is discussed.

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REFERENCES


