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Appl Environ Microbiol. 2005 Nov; 71(11): 7107–7112.
doi: 10.1128/AEM.71.11.7107-7112.2005

PMCID: PMC1287693
PMID: 16269747

# Occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in Untreated Water in Northern Ireland

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Received 2005 Apr 15; Accepted 2005 Jul 20.

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## ABSTRACT

*Mycobacterium avium* subsp. *paratuberculosis* is the known cause of Johne's disease of both domestic and wild ruminants and has been implicated as a possible cause of Crohn's disease in humans. The organism is shed in the feces of infected animals and can survive for protracted periods in the environment and hence could be present in catchment areas receiving agricultural runoff. 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. *M. avium* subsp. *paratuberculosis* was successfully isolated from eight untreated water samples, three by BACTEC culture and five by culture on HEYM. Although the highest incidence of *M. avium* subsp. *paratuberculosis* was found in spring, overall, there was no statistically significant difference between the seasons. No significant correlation was found between numbers of coliforms or fecal coliforms and the presence of *M. avium* subsp. *paratuberculosis*. In general, a higher incidence of *M. avium* subsp. *paratuberculosis* was found in untreated water entering those WTWs that had a high mean water pH value over the sampling period. This work indicates the need to determine the efficacy of water treatment processes to either kill or remove *M. avium* subsp. *paratuberculosis* from untreated water and the possible risks posed by contact with recreational water sources.

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*Mycobacterium avium* subsp. *paratuberculosis* is the known cause of Johne's disease of both domestic and wild ruminants and their predators (2-4) and is excreted in the feces of both clinical and subclinical animals. It has also been implicated as a possible cause of Crohn's disease in humans (7). Crohn's disease is a chronic inflammatory bowel disorder that commonly affects the terminal ileum but can occur in any part of the gastrointestinal tract from mouth to anus. At present, there is no recognized cure, but sufferers can experience periods of remission. However, the quality of life of the sufferer and their immediate family is low (20). Although a role, if any, for *M. avium* subsp. *paratuberculosis* as a contributory factor in Crohn's disease is not proven, the evidence was sufficient for the United Kingdom government to advocate a precautionary approach and to adopt a strategy to minimize exposure of the public to this organism via milk (29).

*M. avium* subsp. *paratuberculosis* is known to survive for protracted periods in the environment and can survive engulfment by protozoa, which, it has been suggested, allows them to acquire a phenotype more pathogenic to humans (17). Survival studies of *M. avium* subsp. *paratuberculosis* have been performed in simulated surface waters. Lovell et al. (25) spiked sterilized pond water, tap water, and distilled water with *M. avium* subsp. *paratuberculosis* and were able to recover it at monthly intervals for up to 9 months. Larsen et al. (23), again using spiked samples of tap water, were able to recover *M. avium* subsp. *paratuberculosis* up to 17 months postinoculation. Water must therefore be considered a possible route of transmission of the organism to both humans and animals.

*M. avium* subsp. *paratuberculosis* is the slowest growing of the cultivable mycobacteria, taking up to 18 weeks to grow on primary culture. There is no recognized selective medium for *M. avium* subsp. *paratuberculosis*, so successful isolation usually requires a chemical decontamination procedure (16). Several chemical decontamination procedures have been shown to have an adverse effect on the viability of *M. avium* subsp. *paratuberculosis* as well as the non-acid-fast bacteria they are intended to inactivate (12). This could have an impact on the recovery of injured *M. avium* subsp. *paratuberculosis* cells that may arise as a result of protracted periods in the environment under adverse nutrient and temperature conditions such as those they might experience in agricultural runoff and groundwater. The logistical difficulties in isolating *M.avium* subsp. *paratuberculosis* have, in our opinion, severely impeded research into the incidence and persistence of this organism in veterinary, food/water, and human clinical studies. One of the main identifiers of *M. avium* subsp. *paratuberculosis* is the IS*900* insertion element which is present as multiple copies in the genome and which makes the organism amenable to detection by PCR assays. IS*900* PCR forms the basis of an immunomagnetic separation (IMS)-PCR assay that has been successfully used for rapid detection of the organism in milk (15).

In the work reported here, the occurrence of *M. avium* subsp. *paratuberculosis* in untreated water destined for domestic use in Northern Ireland over a 1-year period was determined using a combination of the IMS-PCR assay and culture, the latter using BACTEC 12B medium and Herrold's egg yolk medium (HEYM), both supplemented with mycobactins, a specific growth requirement of this organism. Nine water treatment works (WTWs) were chosen to give a geographical spread and also different types of geophysical catchment areas in order to make the results generated as representative of Northern Ireland as possible. The survey was performed over a 1-year period to take possible seasonal effects into account.

## MATERIALS AND METHODS

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Sampling locations and catchments. The origins of the samples tested for *M. avium* subsp. *paratuberculosis* were unknown to the analyst to minimize operator bias, although they were not unknown to those performing coliform and pH determinations, the latter being performed by the Northern Ireland Water Service. The geographical locations of the WTWs are given in Fig. 1. Descriptions of the water catchment areas for the nine treatment works are given in Table 1.

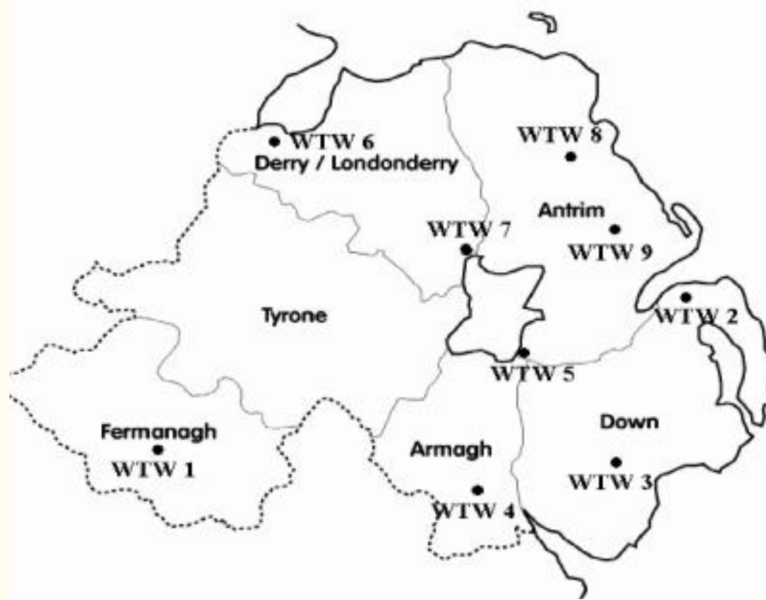


FIG. 1.

Location of WTWs within Northern Ireland at which untreated water was sampled.

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TABLE 1.

Description of catchment areas supplying WTWs

| WTW | Water source |
|---|---|
| 1 | Lowland |
| 2 | Lowland, agricultural |
| 3 | Upland impounded, sheep grazing |
| 4 | Lowland impounded reservoir, agricultural, forested |
| 5 | SE Lough Neagh[a] |
| 6 | Lowland river |
| 7 | Lowland river (Lough Neagh[a]), agricultural |
| 8 | Upland impounded reservoir, deforested |
| 9 | Upland impounded reservoir, forested |

[a]Lough Neagh is a comparatively large catchment area and has a dense surrounding population of ruminant animals, both domestic and otherwise. SE, southeast.

**Testing of water samples for *M. avium* subsp. *paratuberculosis* by IMS-PCR.** Each water sample (1 liter) was centrifuged at 2,500 × *g* for 20 min, and the pellet was resuspended in 10 ml of 0.01 M phosphate-buffered saline, pH 7.4 (Sigma, Poole, United Kingdom) supplemented with 0.4% (vol/vol) Tween 80(PBS-T; Sigma). This was again centrifuged at 2,500 × *g* for 20 min, and the pellet was resuspended in 1 ml PBS-T. The samples were transferred to Eppendorf tubes, and 10 µl of antibody-coated immunomagnetic beads was added and mixed for 30 min at room temperature. These polyclonal antibody-coated beads used were prepared “in-house” and have been previously shown to be effective for recovering *M. avium* subsp. *paratuberculosis* from milk (15). The IMS procedure was performed, and the beads were washed three times with PBS-T with magnetic separation for 2 min between washes. After the final wash, the beads were resuspended in 800 µl of lysis buffer (2 mM EDTA, 400 mM NaCl, 10 mM Tris-HCl, 0.6% sodium dodecyl sulfate, pH 8.0) containing 20 µg proteinase K and incubated overnight at 37°C. The incubated samples were transferred to Hybaid Ribolyser tubes (Anachem Ltd., Luton, United Kingdom) and ribolysed at 6.5 m/s for 45 s, after which they were placed on ice for 15 min to allow any foam to dissipate. The treated sample was taken to a separate location for DNA extraction and IS*900* PCR to minimize cross-contamination.

**DNA extraction.** A total of 500 µl of phenol (pH 8.0; Sigma) was added to each Ribolyser tube before vortexing for 1 min followed by centrifugation at 6,500 × *g* for 10 min. The resulting top aqueous layer was transferred to a new centrifuge tube, an approximately equal volume of chloroform-isoamylalcohol (24:1; Sigma) was added, and the mixture was vortexed for 30 s followed by centrifugation at 6,500 × *g* for 10 min. The upper layer was transferred to a fresh tube containing a 0.6 volume of isopropanol (400 µl; Sigma) to precipitate the DNA during a period of 30 min at −20°C. The DNA was recovered

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by centrifuging at 6,500 × *g* for 10 min and washed once with 70% (vol/vol) ethanol. After carefully decanting the ethanol, the resulting DNA was allowed to air dry briefly before being resuspended in 50 μl of Tris-EDTA buffer (10 mM, pH 8.0).

**IS*900* PCR assay.** The total reaction mixture volume was 50 μl and was comprised of the following: 31 μl sterile distilled water, 5 μl *Taq* buffer (10×; Invitrogen, Paisley, United Kingdom), 1.75 μl $MgCl_2$ (50 mM), 2 μl primer P90 (100 ng/ml), 2 μl primer P91 (100 ng/ml), 3 μl deoxynucleoside triphosphate mix, 0.25 μl Platinum *Taq* DNA polymerase (5 U/μl; Invitrogen), and 5 μl of template DNA. The primers used were P90 (GAAGGGTGTTCGGGGCCGTCGCTTAGG) and P91 (GGCGTTGAGGTCGATCGCCCACGTGAC) (26).

The thermal cycle used was an initial denaturation cycle of 94°C for 2 min, 62°C for 15 s, and 72°C for 1 min followed by 35 cycles of 94°C for 30 s, 62°C for 15 s, and 72°C for 1 min and a final extension cycle of 94°C for 30 s, 62°C for 15 s, and 72°C for 5 min. The amplicons were separated by gel electrophoresis and visualized by staining with ethidium bromide (1 mg/ml). A molecular weight marker, φX174 RF DNA HaeIII digest (Sigma), was used, and a positive and negative control were included in each run.

**Culture of *M. avium* subsp. *paratuberculosis* from water samples.** Water samples (1 liter) were shaken manually and centrifuged at 2,500 × *g* for 20 min. The pellets were resuspended in 10 ml of freshly prepared 0.75% (wt/vol) cetylpyridinium chloride (Sigma), and the mixtures were transferred to sterile centrifuge tubes (50 ml) and incubated at room temperature for 5 h with occasional shaking. The cultures were subsequently centrifuged at 2,500 × g for 20 min, and the pellets were resuspended in 1 ml PBS-T. For each sample, two slopes of HEYM supplemented with 2 μg/ml mycobactin J (Synbiotics Europe SAS, Lyon, France) were inoculated with 200 μl of resuspended pellet. In addition, one vial of BACTEC 12B medium (Becton Dickinson, Oxford, United Kingdom) supplemented with PANTA antibiotic supplement (as directed by the manufacturer, Becton Dickinson) was inoculated with 200 μl of resuspended pellet. The HEYM slopes were examined periodically for evidence of typical growth (small translucent entire colonies not visible before 3 to 4 weeks). The BACTEC vials were read weekly on a BACTEC 460 machine (Becton Dickinson). When growth was observed in either medium, the presence of acid-fast cells was confirmed by carrying out a Ziehl-Neelsen stain. Acid-fast isolates from HEYM or BACTEC cultures were also subjected to IS*900* PCR to confirm identification as *M. avium* subsp. *paratuberculosis*. Coliform counts were performed on all water samples, according to a British standard method (6), by the Northern Ireland Water Service.

**Soil analyses.** The predominant soil types of each catchment area feeding the respective WTWs were determined by the method described previously by Cruickshank (8).

**Statistical analysis.** The differences in detection rates and the effects of factors such as seasonal variation, type of water catchment area, and microbial status of water sample were determined using chi-square analysis with a $P$ value of <0.05 as the threshold of significance.

## RESULTS

An *M. avium* subsp. *paratuberculosis*-positive result from one or more of the three methods (IMS-PCR, HEYM culture, and BACTEC culture) was obtained for 15 of the 192 untreated water samples tested (Table 2). Two of the water samples tested positive by both IMS-PCR and culture (i.e., 17 positive results from 15 water samples). In these cases, it was assumed that the responses were due to the same strain of *M. avium* subsp. *paratuberculosis*. Eight *M. avium* subsp. *paratuberculosis* isolates

were obtained during the study; five were cultured from HEYM only and three were cultured from BACTEC only. These were confirmed to be viable *M. avium* subsp. *paratuberculosis* isolates on the basis that colonies were acid fast, slow growing with typical colony morphology, IS*900* PCR positive and mycobactin dependent (i.e., unable to grow in the absence of mycobactin J). *M. avium* subsp. *paratuberculosis* was detected from eight out of the nine WTWs surveyed with no isolates being obtained from WTW3.

TABLE 2.

Comparison of the rates of detection of *M. avium* subsp. *paratuberculosis* in untreated water at nine WTWs in Northern Ireland by the three methods employed

| WTW (no. of samples) | No. of IMS-PCR-positive samples (%) | No. of BACTEC culture-positive samples (%) | No. of HEYM culture-positive samples (%) | No. of water samples positive by any method (%) | Mean water pH for WTW over period of study |
|---|---|---|---|---|---|
| 1 (27) | 1 (3.7) | 0 | 0 | 1 | 7.4 |
| 2 (41) | 3[a] (7.3) | 1 (2.4) | 2[a] (4.9) | 5 | 8.1 |
| 3 (7) | 0 | 0 | 0 | 0 | 6.7 |
| 4 (7) | 1 (14.3) | 0 | 0 | 1 | 7.5 |
| 5 (7) | 2 (28.8) | 0 | 0 | 2 | 8.2 |
| 6 (25) | 1 (4) | 0 | 0 | 1 | 7.3 |
| 7 (26) | 0 | 1 (3.9) | 1 (3.9) | 2 | 7.7 |
| 8 (27) | 0 | 0 | 1 (3.7) | 1 | 7.4 |
| 9 (25) | 1[a] (4) | 1 (4) | 1[a] (4) | 2 | 7.5 |
| Total (192) | 9 (4.7) | 3 (1.6) | 5 (2.6) | 15 (7.8) | |

[a]One HEYM culture-positive result and one IMS-PCR-positive result were obtained from the same water sample.

A chi-square test was carried out on numbers of positive samples in order to determine whether there was any significant difference in detection rates between the nine WTWs. The chi-square value ($\chi^2$ = 15.507; 8 degrees of freedom) exceeds the calculated value ($\chi^2$ = 8.076) at the 5% level, indicating that there was no significant difference in detection rates of *M. avium* subsp. *paratuberculosis* from the nine WTWs surveyed.

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Using an analysis of proportions, taking account of the standard error and confidence limits if another 192 untreated water samples were tested, the number of positive *M. avium* subsp. *paratuberculosis* samples would range between 4.0 and 11.6 (95% confidence interval).

The highest detection rates, taking primary test results into account, were reported during the spring months (March to May) (Fig. 2), when 11 out of 83 samples tested positive. The next-highest detection rate was in the summer months (June to August) (Fig. 2), when 3 out of 28 samples were positive. During autumn (September to November) (Fig. 2), only 1 out of 36 samples were positive, while during winter, (December to February), none out of 36 was positive. Overall, however, using the chi-square test, there was no significant difference (5% level) in detection rates by month or season with a calculated $\chi^2$ value of 7.07, which was less than the table value ($\chi^2$ = 7.82; 3 degrees of freedom). Chi-square analysis showed no significant difference (5% level) between the contrasting catchment features, i.e., upland versus lowland, impounded versus nonimpounded, and forested versus nonforested (Tables 1 and 2).

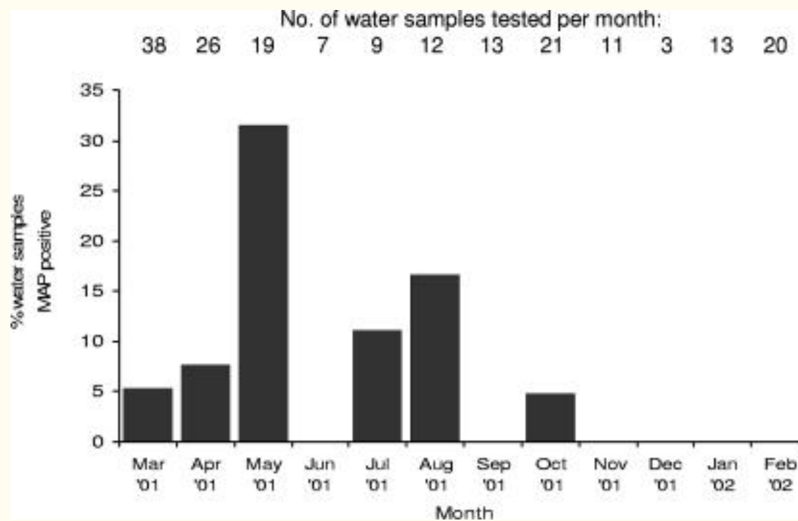


FIG. 2.

Detection rates of *M. avium* subsp. *paratuberculosis* (MAP) in untreated waters in Northern Ireland by month, March 2001 to February 2002. WTWs testing positive for *M. avium* subsp. *paratuberculosis* were as follows: March, WTW4 and WTW5; April, WTW2, WTW5, and WTW8; May, WTW1, WTW2, WTW6, WTW7, WTW8, and WTW9; July, WTW2; August, WTW2 (twice); October, WTW9.

An attempt was made to correlate the incidence of *M. avium* subsp. *paratuberculosis* and coliform counts (data not presented). There appeared to be no correlation between the incidence of *M. avium* subsp. *paratuberculosis* and high coliform or fecal coliform counts. With regard to water pH, there appeared a tendency for *M. avium* subsp. *paratuberculosis* detection when the mean pH of water in the WTW was comparatively high (Table 2). It is interesting that the mean pH of water in WTW3, from which no *M. avium* subsp. *paratuberculosis* was isolated, was the lowest mean pH value of all the WTWs surveyed.

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The effect of soil type was investigated. We gained access to a database of soil types for the province generated as a result of a recent survey. The prevailing soil types in the province, for the purposes of this survey, can generally be divided into two types, viz., peaty and nonpeaty. Nine and eight positive test results were obtained from WTWs where the predominant soil type of the catchment areas could be classified as peaty and nonpeaty, respectively.

## DISCUSSION

Of the 192 untreated water samples tested during this study, 15 tested positive for *M. avium* subsp. *paratuberculosis* by one or more of the three primary detection methods employed. This provides evidence that *M. avium* subsp. *paratuberculosis* can survive sufficiently in the environment to make agricultural runoff a possible route of exposure to the public. It consequently places added responsibility on those organizations responsible for the treatment and distribution of water to ensure that the treatment regimens employed effectively kill or remove *M. avium* subsp. *paratuberculosis*. It also has implications for the public, who may come into contact with agricultural runoff through recreational activities.

Some doubt has been cast over the specificity of the IS*900* insertion element for *M. avium* subsp. *paratuberculosis* (5). Two strains, viz. *Mycobacterium cookii* and *Mycobacterium* sp. strain IMVS B76676, were found to yield IS*900*-like products upon PCR. In relation to the work reported here, since DNA sequencing of the PCR products was not carried out to verify *M. avium* subsp. *paratuberculosis*, the presence of false positives among the nine samples that were positive by IMS-PCR performed directly on the water sample cannot be ruled out. However, the eight culture-positive samples (including the two that were positive by more than one method) could not have been due to the presence of these non-*M. avium* subsp. *paratuberculosis* species since they are incapable of growing at 37°C (21). Colony morphology and mycobactin dependency, a characteristic regarded as unique to *M. avium* subsp. *paratuberculosis*, also distinguished *M. avium* subsp. *paratuberculosis* isolates from the other *Mycobacterium* spp. mentioned above. It is recognized that the PCR assay, except when performed on a colony, does not indicate viability, since a positive response could be obtained from dead or viable but nonculturable *M. avium* subsp. *paratuberculosis* cells or even extraneous DNA. To our knowledge, this study is the first to report the presence of viable *M. avium* subsp. *paratuberculosis* in untreated water.

No previously published water survey has isolated *M. avium* subsp. *paratuberculosis*, so the findings of this study will be compared with those of other studies concerned with the isolation of mycobacteria from water more generally. Although 192 untreated water samples representative of the province were tested over a period of a year, only 9 IMS-PCR-positive and 8 *M. avium* subsp. *paratuberculosis* culture-positive water samples were obtained, and hence, any conclusions must be regarded as tentative. Hunter et al. (18) undertook a comparable survey for *Mycobacterium avium* complex in drinking water treatment and distribution systems in the United Kingdom. *Mycobacterium* spp. were isolated from 19 of 170 water samples (28 untreated water samples, 84 final water samples, and 58 samples from distribution) overall. *M. avium* complex organisms were confirmed in three samples; however, no *M. avium* subsp. *paratuberculosis* was isolated from any water sample tested. Although the same decontaminant (0.75% cetylpyridinium chloride) was used as in this study, the decontamination time was only 30 min, compared to 5 h (18). An additional difference in methodology was the fact that Hunter et al. (18) used membrane filtration to concentrate *M. avium* subsp. *paratuberculosis* from water, whereas in this study, centrifugation was used. We have previously

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experienced difficulty in removing *M. avium* subsp. *paratuberculosis* from filters (32), and this may have been one factor contributing to the nonrecovery of *M. avium* subsp. *paratuberculosis* by Hunter et al. (18). Another factor may have been the lower volume of water tested for *M. avium* subsp. *paratuberculosis* (175 ml compared to 1 liter in this study).

Many research groups previously reported that organisms of the *M. avium* complex are ubiquitous and readily recoverable from natural water and drinking water systems worldwide (1, 9, 11, 13, 14, 19, 27). It should be noted, however, that in all of these previously published studies, mycobactin J was not added to the isolation medium and extended incubation periods were not employed, so *M. avium* subsp. *paratuberculosis* would not have been recovered even if it had been present. It is perhaps not surprising, therefore, that when appropriate culture conditions were provided during this study, viable *M. avium* subsp. *paratuberculosis* was isolated from 4.2% (8 of 192) of untreated water samples. It should be emphasized that this study concentrated exclusively on untreated water entering the water treatment works and not treated water. To our knowledge, there is no published information on the efficacy of water treatments, other than chlorination (31), for removing or killing *M. avium* subsp. *paratuberculosis*. It is recognized that in the limited survey reported here, the experimental design would have been improved if the biofilms present in the water supply pipes had also been sampled, since some mycobacteria exhibit a preference for that niche. It may also have been useful to measure the turbidity of untreated water samples since a correlation between the presence of *M. avium* in water and turbidity has been previously observed (13).

In the present study, although no significant difference was found in detection rates between months or seasons, the greatest incidence was found during the period of March to May (Fig. 2), corresponding to spring and early summer in Northern Ireland. This is in contrast to a number of studies on the isolation of mycobacteria that, in general, found a greater incidence during the summer and autumn months (10, 22). With respect to this survey, the incidence of *M. avium* subsp. *paratuberculosis* may be explained by the fact that in Northern Ireland, cattle are usually put to pasture in the spring and summer and housed during the autumn and winter, with the slurry during that time largely being retained in pits.

The treatment works WTW2, which gave the greatest number of *M. avium* subsp. *paratuberculosis*-positive samples (Table 2), is fed from eight separate catchment areas (609 ha in total) where the land use is dedicated to sheep and cattle as well as natural vegetation. The high number of isolates may, however, be more a reflection of the higher number of samples tested at that particular WTW compared to the others. As shown in Table 2, the WTW which gave the greatest isolation rate (WTW5, with two positive out of seven sampled [28.6%]) is situated on the shores of Lough Neagh, which is surrounded by a dense population of domestic animals and is fed by the Upper Bann River, which has significant pollution problems due to both agricultural practices and sewage. No *M. avium* subsp. *paratuberculosis* was isolated from WTW3, which is perhaps not surprising since the catchment feeding this WTW is situated upland from the Silent Valley reservoir, where domestic animals are excluded because of the danger of *Cryptosporidium parvum* contamination. The water here is considered pristine, and chlorination is the only treatment applied prior to distribution. The organism was no more frequently isolated from lowland river catchments than upland impounded reservoirs (Tables 1 and 2), which was unexpected since in a comparable survey, Hunter et al. (18) reported more *Mycobacterium* spp. from lowland rivers than other sources.

No significant correlation was found between either coliform or fecal coliform counts on water samples and the incidence of *M. avium* subsp. *paratuberculosis*. This was a little surprising since both *M. avium* subsp. *paratuberculosis* and coliforms are usually of fecal origin, and it would be expected that

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contamination of untreated water would be chiefly by that route. This casts doubt on the value of fecal indicators as predictors of the presence of *M. avium* subsp. *paratuberculosis*. Perhaps fecal streptococcus counts may have been a more useful test, since it is thought that their source is animal rather than human feces. However, fecal streptococcus counts are not routinely performed on untreated waters in Northern Ireland, and hence, these data are not available to present here. It should also be noted that survival of *M. avium* subsp. *paratuberculosis* in the environment is likely to be longer than that of any of the fecal indicators, with the exception of clostridia, and this may have affected recovery of *M. avium* subsp. *paratuberculosis* relative to coliforms.

In general, those WTWs that had a high mean water pH value over the sampling period showed an higher incidence of *M. avium* subsp. *paratuberculosis* (Table 2). No *M. avium* subsp. *paratuberculosis* was isolated from WTW3, which had the lowest mean water pH value (6.7) (Table 2) of all the WTWs. This is in contrast to the findings of other workers, who described a negative correlation between the incidence of mycobacteria and high water pH values (19, 30), and the fact that the incidence of Johne's disease is associated with acidic soil types. Perhaps if more samples had yielded *M. avium* subsp. *paratuberculosis* in the present survey, the observed trend may have been reversed. Iivanainen et al. (19) found that the occurrence of mycobacteria correlated positively ($P < 0.001$) with the presence of peatlands, which is the predominant soil type in Northern Ireland.

Although the current detection methods, both culture and molecular, are far from perfect, *M. avium* subsp. *paratuberculosis* was successfully isolated from water during this study, which, to our knowledge, has not been reported previously. It must be stressed that only untreated waters were sampled during this study. The waters would have subsequently gone through varied water treatment regimens before reaching consumers. Slow sand filtration, a process widely used for water treatment worldwide (24), has previously been shown to effectively remove mycobacteria (9). The propensity of *M. avium* subsp. *paratuberculosis* to form clumps (28) may make it particularly amenable to removal by this process, for example. It is clear that if the evidence for a causal relationship between *M. avium* subsp. *paratuberculosis* and Crohn's disease strengthens, the efficacy of water treatment generally will need to be revisited to more accurately assess risk and priority of resources to reduce exposure of the public to this organism. In addition, since mycobacteria such as *M. avium-M. intracellulare* complex have a propensity to colonize biofilms in water distribution systems, it would be of value to determine the extent to which *M. avium* subsp. *paratuberculosis* also fills this ecological niche.

## ACKNOWLEDGMENTS

We thank Randal Scott of the Drinking Water Inspectorate of Northern Ireland, T. Adamson of the Department of Environment Water Service of Northern Ireland, and D. J. Kilpatrick, Biometrics Division, Department of Agriculture and Rural Development for Northern Ireland, for the statistical analysis.

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