What Is *Mycobacterium avium* subsp. *paratuberculosis*?

We read with interest the article by Beumer et al. (1) on the detection of *Mycobacterium avium* subsp. *paratuberculosis* in 81% of drinking water and biofilm samples by PCR. Although these findings could have a significant influence on our understanding and the potential public impact of *M. avium* subsp. *paratuberculosis* as an agent of disease, much of the information on *M. avium* subsp. *paratuberculosis* based on genomic sequence detection and the environment tends to raise more questions than answers and fuels an ever-increasing mass of conflicting data.

It must first be questioned whether the detection of a specific sequence in bulk-extracted DNA actually represents a microbe, an *in situ* microbe, and a member of the microbial community without establishing a direct link between the molecular sequence and an actual *in situ* active microbe. It may be a leap of faith to assume that it does. Perhaps it would be more accurate to express such studies as detection of a genomic sequence rather than detection of an actual microbial species?

Although IS900 has proved to be species specific and reliable for the detection and confirmation of *M. avium* subsp. *paratuberculosis* infection in animals with Johne's disease (ruminant paratuberculosis) (2, 4), it may again be a leap of faith to extrapolate that specificity to other biological systems such as the environment and human tissues. In today's era of gene sequencing and genomic databases, we tend to assign sequence specificity based on information contained within those databases without appreciating that they reflect only a small fraction of known bacteria and even a smaller percentage of the genomic diversity of wild-type strains. Within the human gut microbiome, for example, only 20% of ribosomal gene sequences align with known cultivable species of bacteria, suggesting that 80% of all bacteria within the human gut are unknown and/or uncultivable (7). We should expect similar findings in other ecosystems and microbial communities.

Beumer et al. (1) have recognized and shown that other mycobacterial species that contain IS900 and/or IS900-like sequences have been identified (although not in genomic databases) and that some strains of *M. avium* subsp. *paratuberculosis* may not contain IS900. We agree with the authors' premise that *bona fide* strains of *M. avium* subsp. *paratuberculosis* are IS900 and 251F positive, as documented in their specificity studies (1), those of others (8), and our own. But what do we make of those strains that are IS900 negative but 251F positive and those that are IS900 positive but 251F negative? Are they or are they not *M. avium* subsp. *paratuberculosis*?

Historically, the identification of *M. avium* subsp. *paratuberculosis* has not been stringent and has been based solely on acid fastness, slow growth, and mycobactin dependency (4). With the advent of liquid culture systems and molecular biological methods, identification has been diminished further to simply acid fastness and IS900 positivity (2). Combined with the knowledge that "species-specific" insertion sequences have been shown to cross species barriers (5), the sole reliance on IS900 as a means of identifying *M. avium* subsp. *paratuberculosis* would also seem to be a misguided leap of faith when applied to remote ecosystems.

Accepting that the detection of IS900 is *prima facie* evidence of *M. avium* subsp. *paratuberculosis* and that *M. avium* subsp. *paratuberculosis* is widely distributed in the environment, the

data still fail to hold water. If *M. avium* subsp. *paratuberculosis* was as widespread as suggested by Beumer et al. and others (6) and the primary biomass of *M. avium* subsp. *paratuberculosis* was the environment, why are not all domestic and wild ruminants infected? Was it not these precise inconsistencies that led to the realization that *M. avium* subsp. *avium* (the causative agent of avian tuberculosis) was not an environmental organism and to the designation of *M. avium* subsp. *hominissuis* as the only *bona fide* environmental *M. avium* strain (9)? Is the overreliance on IS900 as the sole basis for the identification of *M. avium* subsp. *paratuberculosis* going to result in a state of confusion similar to that created by strain 18 (3)?

REFERENCES

1. Beumer, A., et al. 2010. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in drinking water and biofilms by quantitative PCR. *Appl. Environ. Microbiol.* **76**:7367–7370.
2. Bower, K. L., D. J. Begg, and R. J. Whittington. 2010. Culture of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from blood and extra-intestinal tissues in experimentally infected sheep. *Vet. Microbiol.* doi:10.1016/j.vetmic.2010.06.016.
3. Chiodini, R. J. 1993. Abolish *Mycobacterium paratuberculosis* strain 18. *J. Clin. Microbiol.* **31**:1956–1958.
4. Chiodini, R. J., H. J. Van Kruiningen, and R. S. Merkal. 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. *Cornell Vet.* **74**:218–262.
5. da Silva Rabello, M. C., R. S. de Oliveira, R. M. Silva, and S. C. Leao. 2010. Natural occurrence of horizontal transfer of *Mycobacterium avium*-specific insertion sequence IS1245 to *Mycobacterium kansasii*. *J. Clin. Microbiol.* **48**:2257–2259.
6. National Advisory Committee on Microbiological Criteria for Foods. 2010. Assessment of food as a source of exposure to *Mycobacterium avium* subspecies *paratuberculosis* (MAP). *J. Food Prot.* **73**:1357–1397.
7. Qin, J., et al. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**:59–65.
8. Rajeev, S., Y. Zhang, S. Sreevatsan, A. S. Motiwala, and B. Byrum. 2005. Evaluation of multiple genomic targets for identification and confirmation of *Mycobacterium avium* subsp. *paratuberculosis* isolates using real-time PCR. *Vet. Microbiol.* **105**:215–221.
9. Turenne, C. Y., R. Wallace, Jr., and M. A. Behr. 2007. *Mycobacterium avium* in the postgenomic era. *Clin. Microbiol. Rev.* **20**:205–229.

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Author's Reply

We appreciate Chiodini and Chamberlin's comments on and interest in our work. The Safe Drinking Water Act of 1974 requires the U.S. Environmental Protection Agency to prioritize research on emerging chemical and microbial contaminants in drinking water and specifically contaminants thought to occur in drinking water but for which data are lacking to conduct a comprehensive human-health based risk assessment. *Mycobacterium avium* is such a contaminant. The goal of our study (1) was to gather occurrence data on *M. avium* subsp. *paratuberculosis*, a subspecies of *M. avium* associated with Crohn's disease, in drinking water. At the time, no occurrence

data existed for *M. avium* subsp. *paratuberculosis* in drinking water in the United States.

Both culture and molecular-based methods introduce their own biases and limitations. Due to the extreme difficulty of culturing *M. avium* subsp. *paratuberculosis* from environmental samples, we chose the rapid and sensitive quantitative PCR technique and required detection of two *M. avium* subsp. *paratuberculosis*-specific targets (IS900 and target 251) to reduce the probability of nonspecific detection. We did not report results as the number of *M. avium* subsp. *paratuberculosis* cells but rather as the estimated number of target gene copies per volume of water. We agree with Chiodini and Chamberlin that our method is not perfect. The ideal method for detecting these organisms in environmental samples and drinking water specifically has yet to be developed. Should we wait until the ideal method exists to begin collecting occurrence data on *M. avium* subsp. *paratuberculosis*? Rather, this first occurrence study justifies the allocation of resources to develop better methods for definitive detection and quantification of *M. avium* subsp. *paratuberculosis* in drinking water and to conduct additional surveys.

Chiodini and Chamberlin state that our results demonstrate that *M. avium* subsp. *paratuberculosis* is widespread in the environment. Actually, our results suggest the converse. We detected two *M. avium* subsp. *paratuberculosis*-specific DNA sequences in approximately 18% of the total drinking water samples (56 of 304 samples). Furthermore, all positive samples were obtained during a 7-month period from a small geographic area. Two years later, all positive taps were negative for *M. avium* subsp. *paratuberculosis*-specific targets. In addition, more than 200 water samples from diverse geographical areas in the United States were negative for *M. avium* subsp. *paratuberculosis*-specific targets. Our results suggest that the occurrence of *M. avium* subsp. *paratuberculosis* may be more episodic than what has been observed for other *M. avium* strains.

Chiodini and Chamberlin discuss the hypothesis that *M. avium* subsp. *hominissuis* is the only subspecies of *M. avium* that predominates in the environment (8). The hypothesis arises in part from culture-based data that revealed a lower isolation prevalence of *M. avium* subsp. *avium* and a higher isolation prevalence of *M. avium* subsp. *hominissuis* from humans, suggesting either host specificity or the possibility that humans are not frequently exposed to environments containing *M. avium* subsp. *avium*. We are not aware of any study that has identified a large number of environmental *M. avium* isolates to subspecies level in order to determine if they are mostly subspecies *hominissuis*. We are aware that most culture-based methods for the isolation of environmental mycobacteria have not been evaluated for the ability to recover all subspecies of *M. avium*, which could alter one's conclusions about the prevalence of any *M. avium* subspecies in the environment. How many laboratories that isolate environmental mycobacteria routinely incorporate mycobactin in the culture medium and incubate for 16 to 23 weeks, which is necessary to recover *M. avium* subsp. *paratuberculosis*? There are reports of isolation of viable *M. avium* subsp. *paratuberculosis* from environmental samples, including surface water and soils (4, 5, 6, 9, 10).

Furthermore, it has been demonstrated that *M. avium* subsp. *avium* is not transmitted from bird to bird and is thought to be acquired from the environment (2, 7).

Finally, we recognize that any human health-based microbial risk assessment of drinking water would be based in the knowledge that exposure to opportunistic pathogens is different from infection and that infection is different from disease. Crohn's disease is considered multifactorial, involving many unknown host factors, and is not caused by mere exposure to a microorganism (3). This is why humans drinking from taps where *M. avium* subsp. *paratuberculosis* genetic targets were detected would not necessarily be expected to develop Crohn's disease. Humans routinely encounter microorganisms and chemicals through daily environmental exposures but do not develop illness or disease. We did not suggest illness in our conclusions. We acknowledge that our study poses more questions than it answers and extend an invitation to all interested laboratories to join us in the quest to develop a better understanding of the occurrence of environmental mycobacteria and their impact on human health.

REFERENCES

1. Beumer, A., et al. 2010. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in drinking water and biofilms by quantitative PCR. *Appl. Environ. Microbiol.* **76**:7367–7370.
2. Dvorska, L., et al. 2007. Avian tuberculosis in naturally infected captive water birds of the Ardeidae and Threskiornithidae families studied by serotyping, IS901 RFLP typing, and virulence for poultry. *Vet. Microbiol.* **119**:366–374.
3. Hugot, J. P., H. Zouali, S. Lesage, and G. Thomas. 1999. Etiology of the inflammatory bowel diseases. *Int. J. Colorectal Dis.* **14**:2–9.
4. Pavlik, I., A. Horvathova, L. Bartosova, V. Babak, and M. Moravkova. 2010. IS900 RFLP types of *Mycobacterium avium* subsp. *paratuberculosis* in faeces and environmental samples on four dairy cattle farms. *Vet. Med. (Praha)* **55**:1–9.
5. Pickup, R. W., et al. 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.
6. Pickup, R. W., et al. 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.
7. Schrenzel, M., et al. 2008. Molecular epidemiology of *Mycobacterium avium* subsp. *avium* and *Mycobacterium intracellulare* in captive birds. *Vet. Microbiol.* **126**:122–131.
8. Turenne, C. Y., R. J. Wallace, Jr., and M. A. Behr. 2007. *Mycobacterium avium* in the postgenomic era. *Clin. Microbiol. Rev.* **20**:205–229.
9. Whan, L., I. R. Grant, and M. T. Rowe. 2006. Interaction between *Mycobacterium avium* subsp. *paratuberculosis* and environmental protozoa. *BMC Microbiol.* **6**:63.
10. Whittington, R. J., et al. 2003. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from environmental samples collected from farms before and after destocking sheep with paratuberculosis. *Aust. Vet. J.* **81**:559–563.

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