Comment on "Environmental Occurrence of the Enterococcal Surface Protein (*esp*) Gene is an Unreliable Indicator of Human Fecal Contamination"

We appreciate the opportunity to respond to a recent article by Byappanahalli et al. "Environmental ocurrence of the enterococcal surface protein (*esp*) gene is an unreliable indicator of human fecal contamination" (*1*). This article followed a preceding paper on the same general subject by Whitman et al. (*2*).

With any molecular method that may be used in environmental pollution studies, the specificity and sensitivity of the method are critical. Polymerase chain reaction (PCR) methods are routinely being used in a presence or absence format but are quickly moving toward quantitative approaches. In addition, the ability to distinguish genetic variations of even one base pair requires great diligence in regard to description and testing of targets. The purpose of each method and its use in decision making are also important, and the interpretation of studies using molecular methods should be made in the framework of decision science and weight-of-evidence approaches rather than insisting that methods show 100% accuracy (which can be unrealistic when dealing with environmental samples).

The authors present work focusing on two recently designed PCR protocols for investigating the source of fecal pollution in surface waters: One detects the *Enterococcus faecium esp* gene, and the second detects a very similar DNA sequence found in *E. faecium* and *E. faecalis*. The authors have been working in the area of beach pollution for many years, and their work with *Cladophora* and the identification of reservoirs of fecal indicators in temperate climates is very exciting; however, the title and content of this particular work (as well as their previous publication on the same topic) is misleading. We believe that the misinterpretations that have been perpetuated now in two separate manuscripts (*1, 2*) regarding the use and utility of the *esp* gene of *E. faecium* for microbial source tracking should be addressed.

As mentioned above, two very similar yet distinguishable variants of the *esp* virulence marker exist in *E. faecium* and *E. faecalis*. The *E. faecium* variant gene was first explored for its possible utility in microbial source tracking in 2005 (*3*) and utilized a forward primer designed specifically to discriminate the *E. faecium* and *E. faecalis* variants. In their recent manuscript, Whitman's group (*2*) contrasted the sensitivity and specificity of this assay (esp_{fm}) with that of a previously published assay (*4*) designed to detect both variants ($esp_{fs/fm}$). Byappanahalli et al. (*1*) reported >95% specificity for the esp_{fm} marker (11/233) and slightly lower specificity for the $esp_{fs/fm}$ marker (93.3%). In spite of detecting the esp_{fm} marker in 93% of sewage influent samples, the authors concluded that the esp_{fm} gene was "not a consistent marker of human contamination" due to the much lower frequency of detection in pit toilet and septic system samples. A review of the literature will show that very few markers of fecal pollution source perform as well as esp_{fm} did in the Whitman et al. study (*2*). Environmental microbiology is performed in a mileu where an enormous variety of animals, plants, and microbes interact; thus, 100% accuracy is almost never a requirement for a useful method, even in a standard method such as those approved by the U.S. Environmental Protection Agency for enumeration of indicator bacteria.

Byappanahalli et al. (*1*) continues the comparison of the *esp* variants; however, this study does not compare the *esp* assays to any other markers of human sewage. The enterococci are cultured but there is no mention of the number of colony forming units per membrane, in spite of the fact that this parameter is known to influence the sensitivity of the assay (*3*). In addition, to our knowledge, the *esp* gene that can be detected in *E. faecalis* has never been suggested as a marker of human fecal pollution and in fact has been shown to be found in many animals (*5*).

We acknowledge that the esp_{fm} gene is not an "ideal" (perfect) microbial source tracking marker; however, such a marker has yet to be found. We have done extensive studies and routinely detect the gene in approximately 1% of the culturable enterococci population that grows on mEI agar in untreated sewage. We have also developed large volume methods for detection in treated wastewater effluent. As pointed out by the authors, the marker is sometimes entirely absent from septic tanks (single family samples) and pit toilets; however, it is consistently present in septage haulers. These limitations, however, do not make it an "unreliable" indicator of human fecal pollution as suggested by Byappanahalli et al. (*1*)and Whitman et al. (*2*).

In fact, the *esp* marker has exhibited value when used in conjunction with additional DNA human markers such as the *Bacteroides* human marker originally developed by Bernhard and Field (*6*) and corroborated by McQuaig et al. (*7*). It has been proposed that the *esp* method may be valuable in distinguishing between treated (disinfected) and nontreated sewage as it is a cultivation-based method. Thus, in areas where treated sewage is discharged but the wastewater is being disinfected to meet bacterial standards, the *E. faecium* esp gene should be found in very low frequency or not at all. Others now are also finding the esp_{fm} marker to be a useful marker of human fecal pollution (*8*).

The conclusion of the 2008 article (*1*)suggests that differential occurrence of *esp* gene variants in the environment is due to lack of human specificity. This conclusion is a non sequitur that is not supported by the data. Occurrence of the variants is related, and many factors no doubt contribute to differential distribution, of which one may be that esp_{fm} is a more accurate marker of human fecal pollution than $esp_{fs/fm}$ The authors state that "the majority of stream water samples ... were acquired from Dunes Creek, which is not known to have any significant point-source inputs." The esp_{fm} gene was not detected in Dunes Creek samples, yet the $esp_{fs/fm}$ gene was detected in 60% of postrain samples. The simplest interpretation of that observation is that $esp_{fs/fm}$ is a less specific detector of human fecal contamination than esp_{fm}. The authors also find that esp_{fm} does not correlate with indicator bacteria levels or F+ coliphage, all of which are nonspecific indicators of fecal contamination. It appears that the absence of expected correlations with nonspecific indicators is driving the authors' conclusions, when in fact they may support the specificity of esp_{fm} and its utility in detecting human fecal pollution.

We appreciate the opportunity to respond to our concerns with this publication, and we point out that we and others have found that when applied with adequate quality control and appropriate quality assurance, the *esp* gene of *Enterococcus faecium* is a valuable and useful tool for identifying sources of human fecal pollution in the environment and for use in the decision-making process as it pertains to environmental and public health.

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