From: Harwood, Valerie <vharwood@cas.usf.edu>
Sent: Friday, January 23, 2009 12:53 PM
To: David Page <dpage@riggsabney.com>; Olsen, Roger <OlsenRL@cdm.com>; tamsen@gmail.com; Jennifer Weidhaas <jweidhaas@northwind-inc.com>
Subject: Manuscript rejected

Sorry folks, bad news on a Friday (see below).

Valerie J.(Jody) Harwood, Ph.D.
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-----Original Message-----
From: marylynn.yates@ucr.edu [mailto:marylynn.yates@ucr.edu]
Sent: Friday, January 23, 2009 12:38 PM
To: Harwood, Valerie
Subject: Decision on manuscript AEM02759-08 Version 1

Dr. Valerie Harwood
University of South Florida
Dept. of Biology
4202 East Fowler Ave.
Tampa, FL 33620-5550
United States

Re: Identification of a Poultry Litter-Specific Biomarker and Development of a 16S rRNA Based Quantitative PCR Assay (AEM02759-08 Version 1)

Dear Dr. Harwood:

Review comments on your manuscript have been received from three members of the editorial board or ad hoc reviewers. Two of the reviewers expressed serious concerns regarding your manuscript, as detailed in their comments. One of the most serious concerns is the potential for application of the method to other geographic regions, as other studies have shown that these biomarkers lose specificity when tests are conducted using samples from a broader geographic field regardless of the assurance made that these primers may have a broader application, Other concerns are over the lack of necessary controls and the lack of appropriate statistical analyses to support your conclusions. For these reasons, and the reasons in the attached reviews, I am unable to accept your manuscript for publication. As this is the second rejection of this manuscript for scientific reasons, per AEM policy, you may not re-submit the manuscript to any ASM journal. The reviewer comments are attached and I believe that they will be helpful to you. Your interest to publish in Applied and Environmental Microbiology is very much appreciated.

Sincerely,
Marylynn V. Yates
Editor, Applied and Environmental Microbiology (AEM)

Dept. of Environmental Sciences
University of California
Riverside, CA 92521-0424

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E-mail: marylynn.yates@ucr.edu

REVIEWER 1:
Comments:
The authors claim to have developed a sensitive and specific marker for the identification of poultry litter as a source of bacterial contamination in runoff and surface water. However, the manuscript is lacking in controls and proper statistical analysis as to make this claim unsupportable.

1. The authors developed and validated their biomarker on the basis of T-RFs derived ultimately from composite samples. They contend that their marker has a 100% sensitivity (over a presumably small, undefined geographic area). I am not convinced that their excellent specificity is not simply an artifact of using a composite sample. Consider the following example: 50 litter samples are collected and tested for the biomarker and 5 are positive. The sensitivity of the assay would then be 10%. If, however, I took the same 50 samples and by compositing them produced a total of 10 composite samples (5 samples/composites). If each of the original 5 positive samples was included in a different composite it is very possible that I would have 5/10 = 50% positive samples. Thus, I would be much more comfortable with the author's statements if they had used more individual samples.

2. The authors tested unused litter for their biomarker, however, they failed to test either unimpacted soil or runoff. Furthermore, the number of reference water samples they tested is quite small. Therefore, they do not present convincing data that the biomarker is not normally found in, at least some, soil and runoff without the presence of poultry litter.

3. Finally, the regression data presented in Figure 4 is meaningless. R2 is a guide to the "goodness-of-fit" of the data to the regression line. It does not indicate whether there is an association between the variables that is statistically significant. In order to demonstrate this, the authors should have performed an analysis of variance of the regression (a simple procedure given modern statistics packages).

4. As a side note, the paper is poorly written overall. Much of the material is redundant while in other sections critical information (e.g. the size of the watershed) is missing.

REVIEWER 2:
Comments:
This is a resubmission of a manuscript that I did not review previously. However, one of my major concerns is similar to one that was previously made by the other reviewers, the specificity of the primers has been only tested for a very limited geographic range using a relatively limited sample set despite the addition of more non-target fecal samples. The authors describe the determination of a poultry litter-specific 16S rRNA sequence for use in microbial source tracking (MST). They use a previously described T-RFLP approach to identify sequences unique to poultry litter. Other target sequences that have been identified using this approach have been shown to have limited

applicability once used in other geographic regions. Predictions about their long-term use can be made by a database search. A BLASTn search of the GenBank using the proposed primers suggests that their broad scale use is limited. Primer LA35F has one mismatch at the 3' end to Arthrobacter and not to any Brevibacterium except itself. The best matches to the reverse primer are not to 16S rRNA sequences but to some Eukaryotic DNA, which puts into some question the quality of data. Additionally, the best matches to the LA35 sequence deposited are to none avian sources of Brevibacterium despite the fact that the closest described species is B. avium. This leads to more questions about the specificity of these primers. This leads to more questions about the specificity of these primers. Then the question remaining for this reviewer becomes the scientific contribution of this work and the interest of this topic to AEM readers with its limited geographic range.

REVIEWER 3:
Comments:
the only criticism i have is the paragraph starting l341is a little overdone. Bit too defensive.