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August 11, 2008

Professor L. Nicholas Ornston
Editor-in-Chief, *Applied & Environmental Microbiology*
American Society for Microbiology
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RE: Manuscript submission by Harwood *et. al.* (J.L. Weidhass, T.W. Macbeth, R.L Olsen, V.J. Harwood) entitled *Identification and Validation of a Poultry Litter-Specific Biomarker and 1 Development of a 16S rRNA Based Quantitative PCR Assay*

Dear Professor Ornston:

I write regarding the above referenced manuscript, attached as Exhibit 1, currently under submission to *Applied & Environmental Microbiology*. As the authors acknowledge, the work discussed in that article was undertaken on behalf of one side of an ongoing court case. The authors of the article are paid experts retained by the plaintiffs in that litigation. I represent the Tyson Foods companies, who are some of the poultry-producer defendants in that case. As the defendants in the case have worked with various experts, including Drs. Mansour Samadpour and Samuel Myoda, those experts have pointed out a number of considerations that cause them to question the validity of plaintiffs' work and their proposed article. In particular, there are a number of inaccuracies, material omissions, and flawed conclusions that should be of substantial concern to an objective observer. Because plaintiffs have involved you in their work, we thought you would appreciate us submitting a summary of some of these problems to you.

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Background

The litigation in question, *Oklahoma v. Tyson Foods, et al.*, is presently pending in federal district court in Tulsa, Oklahoma. The plaintiffs allege that the use of poultry litter as a fertilizer causes natural resource damages throughout the Illinois River Watershed (IRW). While the named plaintiffs are Oklahoma officials, the State is not itself funding the litigation. Rather, the Oklahoma Attorney General has retained private plaintiffs' lawyers who are funding and conducting the litigation in the hope of themselves recovering a substantial portion of any verdict as a contingency fee. Accordingly, the case is not like the usual government litigation where the state's interest is solely in reaching a just result. Rather, the attorneys advancing theories on behalf of the state have a substantial personal interest in obtaining a large verdict.

The authors of the article have been selected, retained and compensated by these private contingency fee lawyers. Dr. Olsen and his firm have already been paid more than $6 million in the case, and have been tasked with identifying potential bases for linking poultry production to alleged environmental injuries. Professor Harwood likewise has been tasked to develop a new method of microbial source tracking to link bacteria to poultry production. The remaining authors of the proposed article, Jennifer Weidhass and Tamzen Macbeth, are employees of North Wind, Inc., a laboratory working as part of the plaintiffs' litigation expert team.

The authors purport to have identified a previously unknown species of *brevibacterium*, closely related to *b. avium*, which they associate with poultry feces. This bacterium, they assert, contains a unique genetic sequence, which, when found in the environment, can serve as a "biomarker" for poultry-derived fecal contamination. The manuscript presents the authors' conclusions in relatively couched terms, observing that while their "results indicate that the watershed is indeed being impacted by the application of poultry litter . . . the magnitude of the impact . . . cannot be quantified." However, the authors have previously given sworn testimony about their work. In that testimony, the authors told a different story to the court, asserting that this work proves that poultry are the "dominant contributors to the fecal indicator bacteria loads" in the IRW and constitute a "substantial, serious and immediate threat to human health." The authors' work, however, supports neither conclusion.

As noted above, the defendants have retained Dr. Mansour Samadpour and Dr. Samuel Myoda to advise us in connection with microbial source tracking questions and to respond to the testimony advanced by Drs. Harwood and Olson. Drs. Samadpour and Myoda previously submitted a declaration to the Court addressing this research. For your convenience, I am

attaching this report as Exhibit 2. Drs. Samadpour and Myoda are currently preparing an additional report for the court.[1]

Discussion

With that background, let me turn to a few of the substantive points that have been made in court and in related sworn testimony. First, we believe that a necessary first step in a study such as this is to ensure the integrity of the sampling regime from which data will be drawn. I am attaching as Exhibit 3 a report prepared by Jay Churchill of Conestoga-Rovers, Inc., previously submitted to the court, which documents a portion of the sampling program undertaken by the plaintiffs. As Mr. Churchill relates, his staff documented numerous instances of deficient sampling, including drawing water and soil samples in close proximity to cattle contamination, failing to discharge water sources to clear potential surface contamination, failing to segregate soil samples, and failing to clean instruments properly.

Please note that, while the manuscript that plaintiffs submitted to you asserts that litter samples were drawn "through the entire depth of the litter," Mr. Churchill's team observed litter being collected with a triangular-shaped spade, resulting in samples biased towards the top, where fresher layers of litter reside. Also, please note the manuscript asserts that indicator bacteria were enumerated "according to standard methods." In fact, the plaintiffs' sampling regime violated both hold time and geomean standards. Indeed, samples collected in Oklahoma and Arkansas were shipped to a laboratory in California under unknown conditions where they were enumerated days, and in some instances, weeks, later. Moreover, plaintiffs' samples disproportionately reflect high-flow and other non-representative conditions.

Moving to the data itself, we believe you should be aware that the manuscript bases its conclusions on a limited and biased dataset. In Table 4, the authors present the results of the application of their qPCR assay to 46 environmental samples – 10 litter samples; 10 soil samples; and 26 water samples – to detect the presence or absence of their purported "biomarker." While the manuscript discusses only these 46 samples, as part of the litigation the authors tested more than 200 environmental samples, the results of which are far more ambiguous than the manuscript leads the reader to believe. Table 4 suggests that nearly all samples were positive, and that a majority were quantifiable. But in fact, a plurality of the

[1] The declaration attached as Exhibit 2 was prepared in response to expert materials submitted to the Court in support of a motion by the plaintiffs' for a preliminary injunction. Our opposition to that motion was prepared on an extremely short timeframe, and with limited discovery into the plaintiffs' case. I have therefore attached a brief errata sheet to the declaration noting a few errors.

authors' tests resulted in non-detection, and fewer than a quarter were quantifiable. The full range of test results is set out below.

	Total Samples	Quantifiable	Present	Non-Detect
Litter	10	10	0	0
Soil	40	6	29	5
Water	187	35	53	99
Totals	**237**	**51**	**82**	**104**

When deposed on July 18, 2008, as part of this litigation, Professor Harwood excused the omission of this substantial body of contrary data by stating that the manuscript had been drafted a year ago and therefore not all of the relevant data could be included. But the authors' correspondence demonstrates that the qPCR assay described by the manuscript had not yet been fully developed as of July 2007, that the majority of the plaintiffs' testing was undertaken in November 2007 through April 2008, and that the article in fact was being edited until shortly before its submission in June 2008. A copy of Dr. Harwood's recent deposition, which discusses the manuscript and her work in this litigation more generally, is attached for your review as Exhibit 4.

Based upon this dataset, the authors purport to have identified a new species of *brevibacterium* that allegedly carries a unique "biomarker" that might be used to identify poultry-derived fecal contamination. This "biomarker" was developed based on the 16s rRNA sequence. The DNA used, however, was not recovered from poultry feces directly, but rather from "used" poultry litter. Poultry litter is made of bedding material and bird excrement. The plaintiffs never tested poultry feces to confirm that the *brevibacterium* is actually present. This is significant given the fact that, as you are doubtless aware, many *brevibacterium* species have been associated with rice or decaying wood. It is therefore an open question whether this unknown bacterium is even a component of poultry feces. Accordingly, the authors cannot determine whether the previously-unknown bacteria is associated with poultry feces or some other environmental condition.

To be of any practical use as a marker for poultry-associated fecal contamination, the PCR assay must be specific to poultry, and must not reproduce DNA from other sources. In order to confirm the specificity of their assay, the authors tested it against fecal samples from cattle, geese, ducks, swine, and humans. But these tests are insufficient to assure the assay's specificity.

The authors provide no explanation as to why they limited their tests to these five alternate sources when the IRW contains hundreds of species of wildlife, all of which shed fecal indicator bacteria. Professor Harwood has testified that the plaintiffs focused on the dominant sources of indicator bacteria, yet admitted that they never actually calculated fecal loading from most animals in the watershed. Nor are the levels of *brevibacteria* identified in environmental samples high enough to warrant discounting other sources. Indeed, despite the fact that almost all of the authors' water samples were taken close to poultry operations, a plurality of samples resulted in non-detects, and those few that resulted in a quantifiable reading identified only a few hundred to a few thousand *brevibacteria*.[2] These figures are too low to discount any potential source.

Even with regard to those species examined, the authors' testing does not effectively rule them out as possible sources. As Professor Harwood candidly admitted at her deposition, the authors made no attempt to ensure that their samples were sufficiently large as to characterize accurately the populations of those various animals in the IRW. Lacking statistical significance, it cannot be said that the assay is specific to poultry feces. Indeed, we know this not to be the case as the assay identified the same DNA sequence in duck and goose feces.

Lack of statistical significance is characteristic of plaintiffs' work more generally. As Professor Harwood admitted at her deposition, the plaintiffs' lawyers made no effort to ensure that their experts' samples were gathered so as to be representative of the watershed they are now used to characterize. Rather, sampling locations were selected based on their proximity to poultry operations and on the perceived likelihood that they would they would yield high bacterial counts. Plaintiffs' qPCR testing appears to have been similarly selective. The plaintiffs tested four sets of samples, beginning with poultry litter and soil where poultry litter had been applied, and then moving out towards surface waters. While they appear to have originally intended to test all 550 samples, they suspended testing after results turned increasingly negative for their theory that the newly discovered bacterium is associated with poultry. The only further testing was a narrow and highly selective set of bacteria-laden "dirty" samples, selected to maximize the chances of finding the "biomarker." The chart below sets out the results of plaintiffs' testing by set.

[2] The authors present their findings in terms of numbers of gene copies of the 16S rRNA sequence identified through their process. *Brevibacteira sp.* have been found to carry 4-5 copies of the 16S gene. Because the authors have never cultured their newly-identified bacteria, it cannot be said for certain how many copies it carries. For simplicity's sake, we assume 5 gene copies.

Set	Date Reported	Total	Quantifiable	Present	Non-Detect
1	11/3/2007	67	26	28	13
2	12/4/2007	60	15	11	34
3	1/21/2008	77	8	19	50
4	4/24/2008	29	2	23	4

Finally, in order to serve as an effective indicator of poultry fecal contamination and of any associated health risk, a "biomarker" must share fate and transport characteristics with fecal indicator bacteria and/or pathogens derived from poultry feces. Yet, the authors made no effort to document any such relationship. The authors have also never cultured this newly discovered bacterium to discover its characteristics. *Brevibacterium* is a relatively easy organism to culture, and culturing allows the researcher to confirm the nature and properties of the organism. Failure to do so here is significant.

Having not actually cultured the *brevibacterium*, the authors have not studied its fate and transport characteristics, nor, as Professor Harwood admits, have they studied these characteristics of any other organism in the IRW. Moreover, no epidemiological work has been undertaken to demonstrate any health impact, and indeed the plaintiffs' efforts to identify pathogens such as *campylobacter* and *salmonella* in the IRW have largely come up empty.

Nevertheless, the authors present correlations between their *brevibacterium* and indicator organisms *enterococci* and *e. coli* in poultry litter and in water, and assert that these relationships demonstrate that the one indicates the presence of the other. This methodology suffers several substantial flaws.

First, the correlations themselves are based on far too few data points to be considered serious statistical work. The litter correlation, presented in Figure 3, is drawn from ten litter samples which, as discussed above, hardly characterize poultry litter across the million-acre watershed, and which were sampled in a biased manner. The water correlations, presented in Figure 4, appear to rely on only five of the 187 water samples actually taken.

Second, even if these correlations were individually valid, in order for the "biomarker" to be an effective indicator of poultry fecal contamination, a correlation must be demonstrated not only at individual locations, but rather consistently along the asserted "pathway". The fact that these organisms exhibit a correlation in poultry litter says nothing about their relationship in surface water miles away. The authors have not demonstrated any such correlation. Quite the contrary, the correlations they present are quite disparate. In the litter, the biomarker is said to

correlate to *enterococci* with an R^2 value of .75, and to *e. coli* with an R^2 of only .28. Yet in surface water, the correlations presented are .89 and .85 respectively. This substantial change in relationship from litter to water suggests that the respective bacteria have substantially different fate and transport characteristics, undermining the utility of the *brevibacteria* as an indicator organism. Moreover, as Professor Harwood admits, the authors took no steps to account for alternate sources of *enterococci* and *e. coli* in surface waters, rather using total counts in their correlation.

Third, as noted, the authors never tested poultry feces to confirm the presence of the *brevibacteria*. Table 4 reports remarkably high levels of *brevibacteria* in poultry litter, with readings ranging from 0.5 to 2.5 billion bacteria per gram of litter, eclipsing by several orders of magnitude the 1,200 *e. coli*/g and 52,000 *enterococci*/g measured in those same litter samples. It would be remarkable if a bacteria so ubiquitous in poultry feces has in fact gone unnoticed for so long by the many researchers who have studied poultry. Yet, if poultry feces do not contain this *brevibacteria*, and at levels correlating to the authors' sampling results, then the authors' thesis that the "biomarker" is an effective marker for poultry fecal contamination cannot hold.

Conclusion

The use of scientific evidence in litigation has not been without its critics. Indeed, federal courts are increasingly skeptical of science prepared at the request and under the direction of attorneys. This case, unfortunately, gives life to many of these concerns. Documents uncovered during the course of the litigation indicate that the plaintiffs' attorneys determined and outlined what their experts' scientific conclusions would be before the relevant experts were even hired or lab work begun, and decided that work which generated answers that did not support the attorneys' theories would be discontinued.

As noted, this article was created to be used as evidence in litigation. Accordingly, my client has a substantial interest in how the article is presented to others and reviewed. We would therefore be interested in providing you with more detailed information about the case and the expert work undertaken by the plaintiffs. Also, we respectfully request that you preserve any materials relating to the article, including any correspondence, records, data, or documents, relating to the same, as those may become evidence in the litigation.

I look forward to talking with you about this matter.

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Best regards,

Jay T. Jorgensen

encls.
cc w/ encls.: *Applied & Environmental Microbiology* Editors
Plaintiffs' Counsel