H49 An Investigation on the Relationship of Postmortem Interval to the Microbial Biomass of Bone

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After attending this presentation, attendees will be informed about current research and development of methods for providing estimates of postmortem interval (PMI). The methods being evaluated are based on microbial biomass extracted from bone of varying postmortem intervals.

This presentation will impact the forensic science community by demonstrating how multidisciplinary approaches, which fall under the heading of forensic taphonomy, may be used to estimate time-since-death. The testing and analysis in this project are based on changes in the microbial community found in bone over a four-year PMI.

Bacteria and fungi contribute to the decomposition of a corpse and their taphonomic effects on the body and bone are well recognized and presented in the literature. This research investigates the temporal trends in bacterial and fungal community composition isolated from bone. The null hypothesis is that as the quality of the resource (i.e., the corpse) decreases with advancing time-since-death, the bacterial load will also decrease, and the relative abundance of fungi will increase. If the hypothesis holds true, then the ratio of bacteria to fungi will decrease over time as well.

To test this hypothesis, a cross-sectional sampling of lower ribs from 14 bodies in different stages of decomposition was performed. The stage of decomposition varied from active decay with skeletal exposure at 10 days postmortem to dry remains at 48 months postmortem. Each recovered rib was thin sectioned, mounted undecalcified, and analyzed for signs of microbial bioerosion. Subsequent to histological preparation and analysis, 200mg of bone powder was pulverized and DNA was extracted. Extracted DNA was purified using the Qiagen MinElute PCR Purification Kit following the manufacture's procedures.

A quantitative real-time PCR for the evaluation of bacteria and fungi from each bone sample was performed. Standard curves, inhibition tests, and primer efficiencies were evaluated through a series of SYBR Green assays using PCR products of the 16s rRNA gene from *E. coli* and an Internal Transcribed Spacer region (ITS), which is a highly conserved fungal rRNA gene, from *Fusarium solani*. Five serial dilutions of known concentration of PCR products were generated and run in triplicate for a single standard curve and used to estimate DNA concentration from unknown samples. Amplification of test specimens were completed using previously reported universal bacterial primers targeting a 200 base pair fragment of the bacterial 16S rRNA gene and fungi were amplified using the ITS primer sets.

Bacterial universal primers were efficient for detecting bacteria in mixed samples according to the obtained qPCR efficiency of 99-100%, while ITS primer sets gave a 10% lower efficiency than the bacterial primer set. Inhibition issues occurred in the early amplification step, but were resolved partially by double purification using the Qiagen purification kit. Given the analytical conditions provided, the amount of bacterial and fungal DNA found in the samples varied from 0.2 to 17.0pg/uL for bacterial DNA and 0.0 to 3.0pg/uL for fungal DNA. Based on the results, bacterial concentrations were highest in the samples of 9, 11, and 12 months postmortem, while the highest concentration of fungi were found in samples with a PMI of 12, 18, and 21 months. The relative abundance of bacteria to fungi, determined by the ratio of total bacteria to total fungi concentrations suggested an overall increase in the ratio over the four-year postmortem interval. This finding is contrary to our hypothesis, suggesting instead an early abundance of fungi relative to bacteria, which changed to a greater relative abundance of bacteria to fungi during the 12 to 18 month time period.

Quantitative PCR is an efficient tool to study a variety of microbial communities in different types of samples. However, primer specificity and inhibition are the most common issues that encounter PCR-based analysis methods. Further research is required that is directed at using primer sets with more specificity for bacterial and fungal groups, rather than using universal sets.

This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.

Forensic Taphonomy, Postmortem Interval, Microbial Biomass

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