H96 The Application of Eukaryotic Community Succession on Porcine Remains for Postmortem Interval (PMI) Estimation

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Learning Overview: After attending this presentation, attendees will understand how changes in the structure of eukaryotic communities found on decomposing remains may aid in the estimation of PMI.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by highlighting a novel area of research that uses eukaryotic communities to provide a useful alternative to traditional PMI estimation techniques by employing next generation sequencing and statistical modeling. Furthermore, this study explores the use of eukaryotic communities for such estimations, an underresearched area.

Determining time since death is an essential component of forensic investigations to complete victim timelines, eliminate suspects, and corroborate the testimonies of those involved. Current techniques for estimating PMI involve observing the physical appearance of remains against known time-/temperature-related changes to calculate a time frame. Recent studies on porcine, murine, and human models have shown that the succession of the microbial community of remains can be used as an alternative method to estimate time since death.[1-5] However, these studies had insufficient replicates, were performed in a laboratory setting, or could not provide long-term estimations. The goal of this study was to combat these issues by performing a better-replicated (N=6) field study on a porcine model over an extended period (two months, or 1,703 Accumulated Degree Days (ADD)) and to characterize the eukaryote community of decomposing remains, a topic that is underresearched.

Skin microbial samples were collected from the torso of each set of remains every day during the first week, on alternate days during the second week, and once a week for the remainder of the 60-day period. The eukaryote community of each sample was determined using 18S recombinant DNA (rDNA) MiSeq® sequencing. Sequence data were analyzed in the mothur pipeline (v1.39.5) and subsequent statistical analyses were performed in R (v3.4.3). The relative abundance of eukaryote taxa across time points and an Analysis of Molecular Variance (AMOVA) indicated similarities between sequential ADD, but significant differences in the eukaryote communities as different stages of decay were reached. At Level 5 (Family), fresh remains (0–57 ADD) were characterized by the combined presence of *Trichostomatia* (12.1%), *Saccharomycetaceae* (5.6%), *Rhabditida* (18.9%), and *Trichosporonaceae* (11.0%). During bloat and active decay (87–209 ADD), *Diptera* (86.4%) was the most abundant family. At the advanced decay stage (267–448 ADD), *Rhabditida* (34.3%) was predominant, but *Coleoptera* (19.3%) also appeared toward the end of this stage. Dry/skeletal remains (734–1,703 ADD) were dominated by the combined presence of *Dipodascaceae* (33.3%), *Trichosporonaceae* (18.9%), *Debaryomycetaceae* (32.3%), and *Sporidiobolaceae* (8.8%). A random forest model generated using Level 5 taxa for the first 15 days of decomposition (0–448 ADD) explained 84.65% of the variability observed with a Root Mean Square Error (RMSE) of 58.6 ADD (approximately 2 days). Another model generated using Level 5 taxa from all collection times explained 89.51% of the variability observed with an RMSE of 178.1 ADD (approximately 6.6 days). Both models show improvement over those generated using only bacterial community succession data and highlight the importance of the microbial eukaryote community throughout the process of decomposition.

In conclusion, this study will impact the attendees by increasing their understanding of methodologies currently in place and their awareness of emerging forensic techniques for PMI estimation. It will also provide recommendations on how investigators may better approach crime scenes to preserve and collect evidence for use in necrobiome sequencing. Finally, the presentation will highlight how the application of next generation sequencing may change the way that postmortem interval is determined by providing a supplemental technique to traditional estimation methods.

Reference(s):

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3. Metcalf, J.L., Parfrey, L.W., Gonzalez, A., Lauber, C.L., Knights, D., Ackermann, G., Knight, R. (2013). A Microbial Clock Provides an Accurate Estimation of Postmortem Interval in a Mouse Model System. *ELife*, 2, e01104.
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