H69 Creating a Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR) -Based Method for Studying Temporal DNA Degradation in Waterlogged Bone

Isis Thornton, Virginia Commonwealth University, Richmond, VA 23220; D'Arcy P. Mays III, Virginia Commonwealth University, Richmond, VA 23284-3083; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA 23284; Claire M. Cartozzo, MS, Richmond, VA 23220; Tal Simmons, PhD, Virginia Commonwealth University, Richmond, VA 23284*

Learning Overview: After attending this presentation, attendees will better understand the influences of time and temperature, type of bone, extraction method, and source of freshwater on DNA retrieval, variation in quantity, and variation of degradation in waterlogged porcine skeletal remains.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information concerning the ideal time interval in which DNA can be successfully obtained and amplified from submerged bone samples. Additionally, attendees will understand how extraction methods and skeletal element selection impact DNA extraction and amplification. Ultimately, laboratories will be able to evaluate whether submerged bone samples will provide viable DNA, thus saving time, labor, and money.

Human activities are often centered around the presence of water; thus, it is not surprising that there are many water-related human deaths, including those from mass disasters, murder, and/or clandestine body disposal. Scavenger activity, Accumulated Degree Days (ADD), and other aquatic variables may affect DNA retrieval from waterlogged bone. Organic and inorganic material in bone have an increased vulnerability, as water causes bone dissolution. This process can destroy the DNA contained in the osteocytes.[1] Calcium and collagen in bone can inhibit the PCR necessary to produce a Short Tandem Repeat (STR) profile; the current solution is a time-consuming organic extraction, reported to produce a 99% recovery rate.[2] While there are examples of research on DNA degradation in terrestrial bone over time, there has been little work done on submerged bone samples.[3] Although case studies are not controlled experiments, they are informative for research on human samples. One case report concerns skeletal and soft tissue remains of a murdered child who had been submerged in water for up to three years. DNA retained in the bone was still extractable DNA in this case.[4]

This research project was based on a long-term, controlled experiment that offers potential answers to the problem of DNA retrieval in waterlogged bone. The research also provides a shorter solid-phase protocol and a way to estimate over what length of time/temperature (in ADD) DNA may be extracted from skeletal remains, thus allowing a crime lab to determine if effort should be applied.

Fresh rib and scapulae from pigs (*Sus scrofa*) were submerged at Henleys Lake (November 2016–November 2017) and in the James River (November 2017–November 2018), with water temperature and quality measured every 500 ADD, using a 0°C as a base temperature for ADD calculation. The total sample size began with 200 ribs and 200 scapulae, though 46 samples were lost over time due to varying factors. In the lab, they were cut into pieces, placed into a mortar with liquid nitrogen, and ground into powder. Some of the bone powder was used in the ChargeSwitch® gDNA Plant Kit, following the Invitrogen™ CST Protocol for Extracting gDNA from Bone Samples (2009). The final elution volume was 100ul. The phenol-chloroform organic DNA extraction method followed Iyavoo et al., with a final elution volume of 150ul.[5] All samples were stored at -20°C until further analysis. These samples were amplified using PCR, and then subject to gel electrophoresis to determine the DNA quality. Those that failed to amplify (21.18% Chargeswitch, 22.32% organic) were all successfully cleaned up using the DNeasy® PowerClean Pro Clean Up kit. The cleaned samples were run through PCR and another gel was run to confirm quality. A TaqMan® qPCR approach was utilized to determine quality and quantity of extracted DNA. Two primer pair sets were chosen to amplify a short (62bp) and a large (147bp) fragment of porcine DNA extracted from submerged bone samples. For RT-qPCR, plates were run using the ABI® 7500 Real Time PCR Machine with analysis using SDS v1.x software. The statistics were run using fixed modeling on RStudio.

An Analysis of Variance (ANOVA) of the preliminary data indicates that extraction method (p=1.954e-05) and bone type (p=.04104) have a statistically significant (α=.05) effect on DNA quantity, while the location of the freshwater body did not. The overall results suggest that: (1) the simpler, time-saving ChargeSwitch® method is more effective than the current protocol of organic extraction; and (2) that one bone is better for extraction than another. Results for the DNA degradation index over the submersion interval are forthcoming.

Reference(s):

1. Latham, K.E. and Madonna, M.E. (2013). DNA Survivability in Skeletal Remains. In: J. Pokines, & S.A. Symes, *Manual of Forensic Taphonomy.* 403- 426. CRC Press.
2. Rucinski, C., Malaver, A.L., Yunis, E.J, and Yunis, J.J. (2012). Comparison of Two Methods for Isolating DNA from Human Skeletal Remains for STR Analysis. *Journal of Forensic Sciences*, 57(3), 706–712.
3. Mundorff, A.Z, Davoren, J., and Weitz, S. (2013). *Developing an Empirically Based Ranking Order for Bone Sampling: Examining the Differential DNA Yield Rates Between Human Skeletal Elements Over Increasing Post Mortem Intervals.* Available at: https://www.ncjrs.gov/pdffiles1/nij/grants/241868.pdf.
4. Crainic, K., Paraire, F., Leterreux, M., Durigon, M., and de Mazancourt, P. (2002). Skeletal remains presumed submerged in water for three years identified using PCR-STR analysis. *Journal of Forensic Sciences*, 47(5), 1025–1027.
5. Iyavoo, S., Hadi, S., and Goodwin, W. Evaluation of five DNA extraction systems for recovery of DNA from bone. *Forensic Sci. Int. Genet.* Suppl. Ser. 2013; 4: e174–e175.

Skeletal Remains, RT-qPCR, DNA Analysis

*Presenting Author