A118 Postmortem DNA Persistence in Soft Muscle Tissues in Relation to Accumulated Degree- Days (ADD)

Muhammad S. Nazir, MSc, University of Central Lancashire, School of Forensic & Investigative Sciences, Preston, PR1 2HE, UNITED KINGDOM*

After attending this presentation, attendees will have a better understanding of the likelihood of successfully retrieving DNA from postmortem muscle tissue in relation to accumulated degree-days (ADD). Attendees will also receive information about the rate of DNA degradation in whole carcasses, fragmented muscles, and suspended

muscle tissue in relation to ADD when all these tissues put in direct contact with the ground subjected to natural environmental conditions.

This presentation will impact the forensic science community by providing a new insight to access the patterns of DNA degradation using animal model based on both time and temperature. In mass disaster situations, scientists will be in a better position to have an idea about DNA persistence in soft muscle tissues in decomposing bodies at some specific time point under particular environmental conditions; otherwise they can collect hard tissues such as bones and hairs instead of soft muscle for mass disaster victim identification.

After the death of an organism, as the cells breakdown nucleases cause DNA degradation; the action of microorganisms also contributes to the DNA degradation. As the postmortem interval (PMI) increases, DNA continues to degrade until no high molecular weight DNA (HMW-DNA) remains. There is an inverse relationship between DNA yield and PMI with degradation accelerated by increases in temperature. Accumulated degree-days (ADD) provide a measure of time and temperature and have been used to assess DNA persistence in soft muscle tissues. The conclusions, however, are based on a very limited number of observations.

A series of experiments were conducted using 66 rabbit carcasses. Thirty-six rabbit carcasses, in three replicates sets, were placed in direct contact with ground and covered by a wire cage to prevent scavenger access. Thirty-six rear legs were cut from 18 rabbits and put alongside the whole carcasses. Muscle tissues were collected from the remaining six rabbits, were suspended inside 36 50-ml polypropylene tubes and left alongside the carcasses. The environmental temperature and humidity were recorded every hour using a data-logger. Muscle tissue samples were collected in triplicate starting from day zero until no soft tissue remained. Samples were stored at -20°C before processing.

DNA extraction was carried out using a blood and tissue kit according to the manufacturer's instructions. Two nuclear genes (Connexin 43 and RAG-1) were aligned to identify conserved regions for primer design to amplify 194 base pairs (bp), 305 bp, 384 bp, and 500 bp amplicons from pig, rabbit and human. Following DNA extraction PCR analysis was performed using a multiplex that has been developed to simultaneously amplify genomic DNA amplicons of 194 bp, 305 bp, 384 bp, and 500 bp.

500 bp to 122.75 ADD in whole carcasses and fragmented muscle tissues were amplified; however, the drop-out in amplification of 194 bp and 500 bp was found at 55.75 ADD and 122.75 ADD. A complete failure in amplification success of DNA for whole carcasses and fragmented muscle tissues occurred at 161 ADD and one of the replicates at ADD

122.75. In case of suspended muscle tissue samples, successful amplication of PCR multiplex was obtained up to 161 ADD. Further experiments are ongoing to assess how variable this value is; repeating the experiments with pigs and rabbits at different times of the year.

The future work will focus on analyzing quantitative DNA degradation in relation to ADD by quantitative real-time PCR using both pigs and rabbits and examine the relationship between decomposition and ADD at different times of the year.

Persistence, Accumulated Degree-Days, Postmortem Interval

 ** Presenting Author*