G84 DNA Persistence in Soft Tissues

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After attending this presentation, attendees will become familiar with the relationship between Accumulated Degree Days (ADD) and DNA degradation and will have developed an understanding of how this relationship can be used in order to estimate the time frame during which it is still possible to amplify a complete DNA profile in temperate climates.

This presentation will impact the forensic science community by delivering results from a controlled decomposition experiment on animal muscle tissue providing a stimulus for the refinement of the guidelines for soft tissue collections in Disaster Victim Identification (DVI) cases, as well as discussing the feasibility of using DNA degradation as an alternative means of assessing the postmortem interval (PMI) of decomposing remains.

DNA plays a vital role in the process of disaster victim identification; DNA analysis can provide the identification of an individual, aid the re-association of remains which are fragmented, and be of assistance in on-going legal or medical investigations. In many cases, the collections of samples for DNA based victim identification needs to be part of the first response plan. Depending on the condition of the body, Interpol has given recommendations on which samples are best to extract for DNA typing. Blood and buccal swabs are the preferred source for DNA from non-decomposed cadavers; however, where neither blood nor buccal cells are available other samples need to be collected. The Interpol recommended sample for decomposing remains is compact bone, whereas the use of muscle tissue is only proposed in cases of non-decomposed, mutilated remains. However, what is regarded as the onset of decomposition is not further specified. While it is undisputed that DNA in bone and teeth experiences greater protection against degrading environmental influences and hence exhibits superior preservation to DNA in soft tissues, the processing of hard tissues for DNA analysis can be quite labor intensive and time consuming. Depending on the rate of DNA degradation in soft tissues, it could be possible to utilize muscle tissue for DNA based identification in DVI cases for a longer time postmortem, which could significantly increase the sample throughput and speed up the identification process.

The decomposition of tissues is a process dependent on accumulated temperature rather than time itself. Accumulated Degree Days (ADD), the cumulative total of daily average temperatures, are successfully being used as a quantitative measure to estimate the PMI in the fields of forensic entomology and anthropology. Recent research has also suggested ADD to be a measure of predicting DNA degradation in soft tissues.

The present study monitored DNA degradation in muscle tissue of common rabbits (*Oryctolagus cuniculus*) and domestic pigs (*Sus scrofa*). This research was conducted at the Taphonomic Research in Anthropology Centre for Experimental Studies (TRACES) in the Northwest of England. A total of 120 tissue samples were cut into pieces weighing 5g and placed into open 50ml plastic tubes. They were suspended on a mesh plateau about 3cm from the bottom of the tube into which a hole was drilled. This allowed rain water to drain, preventing the samples from becoming submerged. Thirty samples of each species were left open to insect access and 30 were covered by a mesh preventing insect activity, accounting for four different experimental groups. Tissue samples were collected in triplicates per experimental group roughly every 50 ADD and frozen immediately after collection in order to stop any enzymatic or bacterial DNA degrading activities. DNA extractions were carried out using a standard blood and tissue Kit, according to the manufacturer's protocol. The extracted DNA was then visualized on a 1.5% (w/v) agarose gel and quantified fluometrically using a dsDNA assay kit. The samples were genetically profiled using a species specific PCR multiplex (4-plex) which simultaneously amplifies genomic DNA amplicons of 70 bp, 194 bp, 305 bp and 384 bp.

Results show that the total amount of extractable DNA of all samples decreases from death to around 200 ADD (this interval broadly equates to two weeks in a temperate environment, e.g., 14-15°C) and then exhibits a continuous increase. This can be accounted for by an increase in foreign bacterial DNA which coincides with the tissue samples losing their physical structure and integrity. There is no statistically significant difference between the different species ($p=0.33$) or samples to which insect access was enabled and those to which it was not ($p=0.93$).

Partial profiles can be obtained up to 200 ADD, which correlates with the minimum amount of extractable tissue DNA present. Increasing amounts of foreign DNA outcompete the tissue-specific DNA after this point. Studies at the University of Central Lancashire show that full profiles can be obtained up to 173 ADD for individual tissue samples.

The initial results of this study indicate that, depending on the climate, a full DNA profile can still be obtained from muscle tissue after several days. These results suggest, that the current guidelines for soft tissue sampling in DVI cases are very conservative, and the time for victims to be identified could potentially be significantly reduced by expanding the postmortem interval in which to sample muscle tissues.

DNA Persistence, Accumulated Degree Days (ADD), Disaster Victim Identification (DVI)

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