A60 Oh Deer! Detecting Non-Human Skeletal Remains Using Bone Collagen Fingerprinting

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Learning Overview: The goal of this presentation is to demonstrate through case example the utility of mass spectrometry bone collagen fingerprinting for species identification to distinguish between human and non-human skeletal remains.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information on species identification of unknown bone using proteomic tandem mass spectrometry methods during medicolegal investigations.

A human skeletal case recovered in December 2018 in Parker County, TX, was complicated by the finding of a rib fragment containing 18 Sharp Force Trauma (SFT) kerfs. The remains were partially recovered and commingled with multiple non-human bones, primarily identified, by morphology, as deer (*Odocoileus virginianus*). During the analysis of the skeleton, it was determined that the remains belonged to a middle-aged Hispanic female. Aside from the single rib fragment with SFT, no additional information on the cause of death was observed on the skeleton. As the skeleton was incompletely recovered, it was not possible to immediately rule out the rib fragment as a possible rib of the unidentified female. Observation by the anthropologist suggested that the morphology was not human; however, the pathologist requested additional testing to determine the taxonomic origin of the bone fragment.

To distinguish skeletal remains as human or non-human, professionals in the forensic community use a variety of macroscopic, microscopic, and chemical methods.[1-5] Comparative anatomy differentiates human from non-human bone by examining gross morphological traits, a reflection of skeletal function. Ribs can be problematic to distinguish between humans and non-human mammals of a similar size.[1] Gross morphology can have limited utility when presented with fragmented remains. Histological analysis can reliably distinguish human from non-human bone by observing bone tissue type, primary (plexiform) or secondary (Haversian), as well as other microscopic features of osteon size, Haversian canal size, and density.[2-4] Disadvantages to histomorphometric methods include a need for undecalcified sections, a skill set frequently not available in a medical examiner setting. Additionally, fragmentary or burned remains may not include the exterior layer of bone where plexiform bone, considered diagnostic of non-human bone, is found.[2,3] Chemically, bone collagen is a genetically informative biomolecule. Even after taphonomic processes occur, small amounts of remaining collagen can be obtained and species identified.[5]

The identification of the rib fragment in this case was accomplished by the New York City Office of Chief Medical Examiner (OCME) using a method known as proteomic tandem mass spectrometry to analyze the amino acid sequences of proteins found in bone, soft tissue, and body fluids. Typically, very small bone samples (50mg) are required. In the case of the rib fragment, approximately 5mg of medullary bone was scraped from the rib, and the protein was extracted using 10 volumes of 8 M urea for 72 hours at 4°C and quantified using a bicinchoninic acid protein assay with bovine serum albumin as standard. Twenty µg of sample protein was reduced, alkylated, and digested overnight with trypsin. Peptides were separated by high-performance liquid chromatography using a 30 minute 5%–40% acetonitrile gradient and analyzed by tandem mass spectrometry on a SCIEX™ 6600 TripleTOF. Data were searched against an all-vertebrate National Center for Biotechnology Information (NCBI) database downloaded in April 2018 using SCIEX™ ProteinPilot™ 5 software. Forty-seven proteins were detected with a confidence score ≥95% per a personal communication with Dr. Donald Siegel. The sample submitted to Dr. Siegel at the OCME had a 122 peptide match identifying the rib fragment as *Odocoileus virginianus texanus*, commonly known as a Texas subspecies of the Virginia whitetail deer. The proper identification of this rib fragment with SFT as a deer bon, allowed for the medicolegal investigation to attribute the trauma/modification to an irrelevant behavior.

This case study shows that crossing borders between the fields of forensic anthropology, zooarchaeology, and forensic toxicology has allowed accurate differentiation of non-human skeletal remains of a critical component in a medicolegal investigation. Sharing information and technology across fields of study benefits all, even when each discipline uses the results to answer differing questions.

Reference(s):

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