Identification of Unknown Mammalian Quadruped Bones by Histological Techniques And Bone Morphology

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Abstract
Skeletal and dental remains are the end products of all mammalian species after death and decomposition. The remains have significant importance to forensic anthropologists and osteologists in providing them with the evidence needed to determine the species, gender, age and cause of death of skeletal remains. This article describes the techniques and methods that are most commonly used to evaluate skeletal remains and applies those techniques to the skeletal remains of an unknown mammalian quadruped that was found in Manassas, Virginia in 2014. Using these techniques, the remains were successfully identified as those of a juvenile red fox, *Vulpes vulpes*.

In addition to identifying skeletal remains that are clearly human, forensic anthropologists are frequently called upon to separate human skeletal remains from non-human remains. Sometimes the remains are fragmented or badly damaged by natural or man-made disaster and they may include comingled bone fragments from humans and non-humans. When bone fragments are small, comingled and not accompanied by animal pelts or intact skull remains, species identification can be problematic. Increasingly, forensic anthropologists are turning to histological analysis as an aid to species identification in situations when standard identification techniques cannot be used.

The diagenetic altering of bone is the tendency of bone to change in response to the changing external and internal postmortem environment. This process is well understood (Hillier and Bell 2007). Change is initially triggered when bone is subjected to bacterial invasion as bacteria from the digestive system move along vasculature channels to other body organs. The presence of digestive bacteria in bone disrupts the microstructure of the bone and these changes can be seen during histological assessment. Exposure to marine environments introduces a variety of microorganisms that are not observed in bone that is found in a terrestrial environment, while diatoms and other unique microorganisms may populate bone exposed to a fresh water environment. Heating bone causes the mineral content to melt and recrystalize, which causes Haversian canals to shrink and blurs the formative lines of individual lamellae.

Freezing bone, however, has no apparent effect on its microscopic structure (Byers 2008, Hillier and Bell 2007). The realization that specific types of changes in the microstructure of bone could be useful in identifying the place of death, or the place where a body was taken after death, lead forensic investigators to hypothesize that bone histology might be useful in species identification of fragmented bone material.

Histological considerations for species identification
There is limited research on the microstructure of non-human mammalian bone but a few representative species have been investigated. A histological study of dog bone, for example, has revealed that the compact bone of the ribs and long bones of adult animals is composed of dense Haversian systems that are small in comparison to human Haversian systems and more variable in shape. The periosteal surface has remnants of plexiform bone; a type of bone tissue that is rarely seen in humans and primates and frequently seen in large mammals that grow quickly such as dogs, cows and pigs. Plexiform bone is characterized by the presence of large vascular plexuses in a lamellar bone configuration. The resulting bone is arranged in
distinctive symmetrical blocks resembling the repetitive patterning of a brick wall (Hillier and Bell 2007).

The compact bone of domestic cats is also composed of dense Haversian systems. The Haversian canals in cat bone are very small and Volkman’s canals are much more numerous compared to human bone or other cat-sized small mammals. Circumferential lamellae are usually present in a thin layer at the periosteal surface of the bone and a thicker, more organized layer of circumferential bone is typically found at the endosteal surface (Hillier and Bell 2007).

The compact bone of deer contains both plexiform and Haversian bone tissue. In immature deer, plexiform bone is concentrated near the periosteal surface while Haversian bone forms along the endosteal surface. The long bones of adult deer are composed primarily of dense Haversian systems that have replaced plexiform bone. Through out the body, a thin layer of circumferential lamellar bone covers the periosteal bone surface. In fetal and newborn animals, the compact bone of the long bones consists primarily of plexiform and reticular tissue with scattered avascular areas and acellular bone regions (Hillier and Bell 2007).

Organized Haversian systems are rare in the long bone of common brown rats and when present, they tend to be scattered near the endosteal surface. The compact bone of long bones is composed primarily of longitudinal bone tissue containing scattered areas of small avascular and acellular bone material (Hillier and Bell 2007).

A survey of just a few of these relatively common representative animals; dog, cat, deer and rat, gives evidence that it might be possible to assess species based on species specific Haversian and non-Haversian bone patterns. Unfortunately, prepared slides of non-human bone material are not yet readily available on the commercial market and individual schools may not have the equipment necessary to make histological slides of representative animal bone.

**Macroscopic considerations for species identification**

The best and easiest way to identify an animal is with an intact pelt (Travini et al. 2000). In the absence of an intact pelt or histological preparations of representative bone, or in addition to these if they are available, non-human bone can be distinguished from human bone on the basis of bone morphology if the bones are relatively intact. The pelvic bones of humans are broad and shallow and can be easily distinguished from the long, narrow pelvic bones of non-human quadrupeds. The human femur is the longest bone in the body and it has only one linea aspera. In quadrupeds, the femur is short in relation to the overall body size. If an unknown femur appears to be approximately the same length as an adult human femur, a non-human bone may be twice the thickness of the human bone and it is likely to have a double or plateau formed linea aspera. The tibia and fibula are two distinct bones in the human while they are likely to be fused in non-human quadrupeds. In non-human quadrupeds, the tibial tuberosity is very large with respect to the rest of the tibia. The compact bone of the long bones of the body is proportionally thicker in non-human mammals that it is in human bone. Typically, the compact bone of a human diaphysis accounts for about a quarter of the cross-sectional diameter of the bone. Any bone exceeding this cross-sectional diameter of compact bone is more likely to be non-human (Byers 2008).

The human scapula is triangular in shape, a clavicle is present, the humerus, radius and ulna are gracile and a thumb is present. In other mammals, the scapula is rectangular, the clavicle is usually absent, the humerus, continued on next page
radius and ulna are likely to be weight bearing bones and therefore more robust, and the thumb is small when present (Byers 2008).

Human remains are generally easier to identify than animal remains. The human bones most likely to be wrongly classified as non-human bones are bones of infants and small children. The most common areas of confusion include the presence of unfused epiphyses, which changes the bone count of the skeleton, and the fact that the bones of the hands, feet and clavicle may not be fused. The separated, unfused epiphyses may not appear to be human to the untrained observer. The long bones of infants are thin in cross section and the cranial bones may be separate and more uniform in thickness than they are in the adult. These immature human bones are most likely to be confused with the bones of small mammals such as rabbits, cats, raccoons and the bones of larger birds (Byers 2008).

When the animal pelt is not present with the remains, the skull is the best way to identify unknown animal species (Mengak and Moore 2012). In cases where the skull is absent, DNA analysis from mitochondrial DNA, genomic DNA or ribosomal RNA can be used to aid in the species identification (Melton and Holland 2007). DNA analysis, while very accurate, is more time and labor intensive than morphological or histological analysis (Crocker et al. 2009). DNA analysis includes but is not limited to: immunological analysis, protein radioimmunoassay and polymerase chain reaction (PCR). Each species has unique DNA fragment lengths, different numbers of genes, and unique genes. Problems with this technique arise when trying to sequence an unknown sample or to work with DNA from badly degraded bone fragments (Crocker et al. 2009). Currently investigators seek to establish more accurate and elaborate reference points for use with control samples.

Identification of the species must precede any attempt to determine the age of an animal at death. Animals all have specific growth rates that vary depending on genetics, food and water supplies, and the availability of habitat. The most common methods employed to determine the age at death include analysis of suture and epiphyseal closers and analysis of tooth structure. The canine tooth sectioning method examines the presence of annuli in the cementum and dentine of the tooth. Using this method the age of a mammal can be narrowed down by counting the number of layers of cementum and adding a year of age for each layer (Jensen and Nielsen 1968). Dentine layers can also be counted in longitudinally sanded canine roots. This process avoids having to prepare histological samples and it is reliable in determining the age of red fox specimens that are more than a year old (Roulichova and Milos 2007).

The presence or absence of suture closers can be used for most mammals to determine the age of skeletal remains in animals up to two years of age (Johnston and Beauregard 1969). Sutures are scored as being open, closing or closed. Using red fox skulls, Markina (1962) found that most of the basioccipital-basisphenoid (BOBS) sutures closed between six months and a year of age. The presphenoid-basisphenoid (PSBS) suture takes much longer to close and is prone to more variations. It beings closing after 7 months and can take up to two years to fully close (Harris 1978). Another widely used aging method is examination of epiphyseal closure. Smith and Allock (1960) reported finding great similarity in the ossification of epiphyseal cartilage in the canine family. In the red fox the method can be used on individuals older than 3 weeks (Smith and Allock 1960). Ossification of the bones of the foot takes place at 22 weeks of age, the vertebral column ossifies at 27 weeks, and the limb bones ossify at 30 weeks. Following these ossifications, the scapula, ilium and ischium gradually ossify and the symphysis pubis takes shape (Harris 1978). To separate juveniles from adults, the formation of the tibial tuberosity (apophysis fusion) on the diaphysis of the tibia is the most useful bone marking. This fusion is usually complete by the time the fox is a year old. The distal epiphysis of the radius and ulna ossify at 8 to 9 months and the proximal epiphysis of the humerus remains unfused for about 9 months. Bone ossification and epiphyseal closers happen relatively rapidly in the fox population in the United States (Harris 1978).
Sex Determination

A human skeleton has many markers that can be used to identify the probable sex of remains, including the angle of the pubic arch, the robusticity of the brow arches and rugosity of the mastoid process. Other mammals do not have skeletal markers such as these or the skeletal markers have not yet been clearly identified. Differentiating female from male is much harder in fox species and in the canine family in general (Travini et al. 2000).

Identification of the sex of an animal is easiest when the animal is intact, or the genital tissue is present. Unfortunately in cases where the animal remains are in the later stages of decomposition, differentiating sex is much harder and often impossible. The teeth and skull are generally the most useful skeletal items in determining the sex of carnivore remains however, there is very little difference between female and male skeletal remains in the canine family. Travini et al. (2000) reported that male skulls are about 5% larger in the American culpeo fox, which is similar in size to the red fox. In many species of mammals the female is larger than the male due to the maternal duties of the female. The female is more invested in bearing and raising the young and therefore a larger size may increase survival of offspring (Ralls 1976, Travini et al. 2000, Travini and Delibes 1995).

Cause of Death

The cause of death is usually determined by an autopsy or necropsy but when the remains are skeletonized, examiners must use other techniques to determine the cause of death. All skeletal evidence must be collected, including any tissue that might lend itself to molecular examination. If the remains were removed from more than one site, DNA testing is required to make sure that they are from the same individual. Connective tissue is removed by boiling, washing or dermestid activity, and the skeletal components are displayed in anatomical position. The remains can then be examined for any abnormalities. Examiners look for signs of fracture, missing bones, bone fusions, bone lesions and bone fragmentation (Byers 2008).

Different types of fractures occur under different circumstances. A fracture is simply a break in the bone caused by an abnormal external force that is characterized by direction and focus (Ortner and Putschar 1981). The direction of the force depends on tension, compression, torsion, bending and/or shearing while the focus is categorized as narrow or wide, describing the impact surface area. A longitudinal fracture follows the long axis of the bone and runs parallel to the bone. A transverse fracture, also known as a stress fracture, is a break that goes straight across the bone usually as a result of a direct impact to the bone at the fracture site. Radiating fractures originate from a point of stress and extend out as the force dissipates through the bone. An oblique fracture is the result of a blow that comes in at an angle to the bone causing a break to occur at an angle across the bone. A twisting force that causes an oblique fracture through and across the bone is called a spiral fracture. Comminuted fractures occur when the bone has been splintered or crushed into more than three pieces. Incomplete fractures, or greenstick fractures, are fractures that occur in softer bones of juveniles and do not extend all the way through the bone (Ortner and Putschar 1981, Ortner 2003).

Examiners must further classify bone fractures as ante-mortem, perimortem or post-mortem. Antemortem injuries occurred prior to the time of death and may be in the process of healing. Abnormal bone growth or shape, callus formation, necrotic tissue or signs of infection are all indicative of past injuries (Ortner and Putschar 1981). If there are signs of bone remodeling, it takes about 1 to 3 weeks for the jagged edges of a fracture to become rounded, and at 6 weeks a bony callus begins to form (Sauer 1998). Perimortem fractures occur around the time of death and the bone may still be encased in fur, skin and muscle. Unlike ante-mortem fractures, perimortem skeletal injuries show no signs of healing. Postmortem fractures occur during the decomposition process. Depending on what decomposition stage the bone is in, the bone may be dry and the fractured bone ends may appear straight and have sharp edges. Postmortem radiating fractures are very rare because of the dry condition of the bone (Ortner and Putschar 1981).

Discussion: Investigation of the remains of an unknown mammalian quadruped

The remains of an unknown mammalian quadruped were found in the vicinity of Manassas, Virginia in the spring of 2014. There were no organs present, and most of the soft tissue was gone. The remains consisted of a collection of partially articulated bones, several teeth, claws, assorted ligaments, and a little fur. Forensic investigative techniques were used to determine the species, age and probable sex of these remains.

The remains were in the early skeletonization stage of decomposition when they were discovered. The bones were buried under a foot of mulch for the month of August 2014, to facilitate the decomposition of the remaining soft tissue and separation of the bones from the residual fur. Upon removal from the mulch, the bones were subjected to three ammonia washes to get rid of any remaining fat. Following the ammonia treatment, the remains were thoroughly rinsed in plain water prior to being dried and assembled in anatomical position. Missing bones, fractures, and abnormalities were carefully recorded at this time.

The skull was found fully intact and all the teeth were present except for two in the anterior mandible. Based on the size of the remains and the narrow morphology
of the skull the species possibilities were narrowed to either that of a fox or a dog. Animals of comparable size, such as cats and raccoons, have a more rounded skull. The northeast region of the United States is home to both the gray fox and the red fox but the red fox population is greater in this region. The two species of fox are very similar in their morphology except for the skull, which in the red fox bears a clear “V” shaped indentation and in the grey fox bears a “U” indentation (Mengak and Moore 2012). (Figure 1)

As in humans, the age of a fox is determined using the degree of cranial suture closure, epiphyseal plate ossification, or tooth cemenyum/dentine morphology. Examination of the fox remains revealed that the basioccipital-basisphenoid (BOBS), which starts to close at six months (Harris 1978), was in the process of closing. The presphenoid-basisphenoid (PSBS) suture, which takes longer to close, had not yet begun to close in the fox skull. All of the foot bones recovered had signs of epiphyseal ossification, indicating that the fox remains were older than 22 weeks. The vertebral column completes ossification at about 27 weeks and the vertebrae in this animal were not all ossified. The coccyx and sacrum had not yet ossified, but some of the lumbar vertebrae had completed ossification. The limb bones, which complete epiphyseal ossification at 30 weeks (Harris 1978), were not yet ossified. (Figure 2) This puts the red fox remains at under six months of age. Tooth samples were not examined because of the availability of the excellent suture closure and epiphyseal ossification data that were gathered.

The skeletal remains found were in the skeletonization stage of decomposition; there was no pelt or genital tissue to compare and the remains of this animal had been subjected to animal scavenging which resulted in the removal of the pelvic bones. It was judged to be too difficult to determine the sex of the remains with any degree of accuracy. The remains could be either a young male or a young female fox.

Determining the cause of death of the fox included examining the remains for any fractures and abnormalities. During the re-assembling process each bone was examined, a list of missing bones was made, and the bones were arranged in anatomical position. It was clear during the re-assembly process that scavenging had taken place. The pelvic bones, atlas and axis, coccyx, upper appendages, the right scapula, and several rib bones were missing. Refer to Table 1 for a complete inventory of the missing bones. Many of the ribs evidenced oblique and transverse simple fractures. (Figure 3) The positioning of the rib fractures led to the hypothesis that the lower thorax and abdomen had suffered a crushing injury. The remaining scapula also had an oblique fracture about 3 mm in length located in the infraspinous fossa. The scapula fracture did not support or disprove the theory that the lower thoracic and abdominal area had been crushed.

While the injuries sustained suggested that the lower thorax and abdomen of the fox had been crushed it was clear that this injury did not immediately kill the fox. The fox had moved away from the site of the injury to die beside a little stream. By applying osteological techniques to unknown skeletonized remains it was possible to identify the exact species of the animal, determine the age of the skeletal remains and develop a scenario for the probable cause of death. The remains of the red fox, *Vulpes vulpes*, described in this article belonged to a juvenile less than six months of age that likely died as a result of a crushing injury secondary to an encounter with a predator or with a moving vehicle of some type. The gender of the fox could not be determined.

*Editors note: The red fox is found throughout Virginia except for a few small areas in the extreme southeast corner of the state. It prefers less populated areas and gravitates towards farmland. Red foxes are omnivorous with a primary diet made up of rabbits and mice. The average red fox is the size of a small dog. The total length of the animal, including its tail, is from 39 to 41 inches and body weight ranges from nine to twelve pounds. The animal is characterized by the presence of large erect ears, a sharply pointed nose, and a
long, voluminous tail with a white tip. The fur is typically long and soft with the upper part colored reddish-yellow and the fur of the shoulders tipped with black. The breeding season is from December to February, with a peak in late January. A litter of 4-7 pups is born in a den in late March or early April. Red foxes are typically nocturnal and non-migratory and they usually live their whole life and die in the same geographic area. The average life span is about 5 years (Virginia Department of Game and Inland Fisheries 2015).

Photo credit: istock photo by Getty Images

**Literature cited**


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**About the Authors**

Olena has always been interested in anatomy, physiology and problem solving. She believes that working with unknown animal remains enables her to use both her skill and her passion. She hopes to find a job in the field of forensics where she can puzzle and piece together the truth using biological techniques of investigation.

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