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DIFFERENTIATION OF HUMAN AND ANIMAL BONES

Summary

The paper presents the problem of differentiation of human and animal bones. Based on a review of the literature, the authors analyzed methods for differentiating bones, motivated by the need to systematize knowledge in the field of forensic veterinary medicine and the small number of publications addressing this issue. In this way, the current state of knowledge on this issue in the face of advances in modern forensic science and the achievements of archaeology were examined.

Keywords: forensic veterinary science, archaeology, forensic medicine, osteology, identification

Introduction

Visual inspection of the site where the bone material is revealed begins with an assessment of the bone alignment (anatomical or disordered sequence) and depth of embedment in the soil profile¹. The discovery of a complete skeleton facilitates recognition, and the distinct anatomical differences

¹ M. Kała, *Ekspertyza sądowa*, 3rd edition, Wolters Kluwer, Warsaw 2017, p. 94.

make it possible to assign it to a specific species². The problem is more complex when remains or bone fragments have been secured at the site, the material is significantly damaged; when more than one body has been found or is believed to be from several species³. Human bones are qualified based on “shape, contouring of muscle attachments and joint surfaces, degree of hardness and cross-sectional area”⁴. In the next stage, their age (based on tooth structures and cranial sutures), gender (pelvic and cranial bone features), and height (femur features) are determined. More sophisticated analyses are performed by forensic laboratories and commissioned to expert witnesses; an example of such tests is histomorphometry⁵. Some biological features become apparent with the use of various histological and immunohistochemical stains⁶. During microscopy, the cortical surface of the bone should be evaluated first⁷.

Morphological evaluation of skeletal remains

Compared to other vertebrate tissues, bones do not show a significant degree of differentiation. They are made up of bone tissue, and hematopoietic cells and adipocytes can also be located within them⁸. There is a distinction between coarse fibrous bone tissue (*textus osseus rudifibrosus*), typical of the prenatal stage, and the early postnatal period⁹. In an adult, it is present at tendon-bone attachment sites, alveolar processes, the vagus, sutures of the cranial bones, sites of damage to bony structures, and retrograde lesions¹⁰. Fine fibrous tissue (*textus osseus parallelifibrosus seu lamellosus*)

² D. France, *Human and Nonhuman Bone Identification. A Color Atlas*, CRC Press, Boca Raton 2009.

³ Ch. Briggs, *Is it human? Identifiers that distinguish animal skeletal remains from human*, “Pathology” 2010, vol. 42, p. 26.

⁴ D.H. Ubelaker et al., *The use of SEM/EDS analysis to distinguish dental and osseous tissue from other materials*, “Journal of Forensic Sciences” 2002, vol. 47(5), pp. 940–943.

⁵ R. Recker et al., *Issues in modern bone histomorphometry*, “Bone” 2011, vol. 49, pp. 955–964.

⁶ M.L. Hillier, L.S. Bell, *Differentiating human bone from animal bone: A review of histological methods*, “Journal of Forensic Sciences” 2007, no. 52(2), pp. 249–263.

⁷ A. Christensen et al., *Forensic fractography of bone. A new approach to skeletal trauma analysis*, “Forensic Anthropology” 2018, vol. 1(1), pp. 32–51; C. Crowder et al., *Bone histology as an integrated tool in the process of human identification*, in: K.E. Latham et al. (ed.), *New Perspectives in Forensic Human Skeletal Identification*, Elsevier, London–San Diego 2018, pp. 201–213.

⁸ C. Crowder, S. Stout (eds.), *Bone Histology: An Anthropological Perspective*, CRC Press. Taylor & Francis Group, Boca Raton 2012.

⁹ G. Bourne, *The Biochemistry and Physiology of Bone*, vol. 1, Academic Press, New York 1972, pp. 1–19.

¹⁰ M. Kulej et al., *Micromorphological assessment of bone tissue remodeling in various hip degeneration conditions*, “Advances in Clinical and Experimental Medicine” 2020, no. 29(1), pp.

is the mature building tissue of long and flat bones. The outer surface of the bone is covered by the periosteum, and the inner surface is covered by the intraosseous¹¹. Active bone growth is evidenced by epiphyseal plates, which connect the epiphysis to the shaft during adolescence¹². In the case of flat bones, these are the fontanelles, which are later replaced by osteosarcomas¹³.

In mammals, bones are divided into short, long, flat, and variously shaped¹⁴. Tubular bones are made of both types of bone substance, while flat bones are made of either compact or spongy substances¹⁵. In the axial skeleton of animals, there are differences in the number of vertebrae of the different parts of the spine¹⁶. This number can even vary between breeds or by performance type (as can be seen in lightweight, fighting, and draft horses)¹⁷. The identification process looks for species variation in the structures of the skull, cervical vertebrae, thoracic vertebrae, sacrum, ribs and sternum, scapula, and limb bones.

For the first *vertebra cervicalis I*, the differentiating features are the width of the dorsal and epigastric arch, the degree of protrusion of the dorsal cusp, indentations or wing holes, and the presence of a lateral vertebral opening. The *vertebra cervicalis II* is characterized by its length (it is short in pigs and humans), tooth shape, and spinous process. The initial evaluation of further vertebrae primarily takes into account their size. The vaulting of the arches, the presence of the dorsal crest, and the height of the spinous processes are analyzed¹⁸. In the case of the *sacrum (os sacrum)*, attention is paid to the characteristic shape, the number of vertebrae that build it, the articular processes, the sacral canal, the lobular surfaces, etc.¹⁹ In ruminants, it has

51–60.

¹¹ W. Sawicki, J. Malejczyk, *Histology*, 6th edition, Wydawnictwo Lekarskie PZWL, Warsaw 2012; H. Lippert, *Anatomie kompakt*, Springer-Lehrbuch 1994.

¹² E. Hunziker, *Mechanism of longitudinal bone growth and its regulation by growth plate chondrocytes*, "Microscopy Research & Technique" 1994, no. 28(6), pp. 505–519.

¹³ M. Hrehorowicz, *Archeologia sądowa – już kryminalistyka czy jeszcze archeologia?*, National School of Judiciary and Public Prosecution 2018, no. 1(29).

¹⁴ M. Wake (ed.), *Hyman's Comparative Vertebrate Anatomy*, The University of Chicago Press, Chicago 1979, pp. 112–119.

¹⁵ H. Jaffe, *The structure of bone: With particular reference to its fibrillar nature and the relation of function to internal architecture*, "The Archives of Surgery" 1929, vol. 19(1), pp. 24–50.

¹⁶ K. Kardong, *Vertebrates: Comparative Anatomy, Function, Evolution*, McGraw-Hill Education, New York 1994.

¹⁷ A. Pluskowski et al., *Potential osteoarchaeological evidence for riding and the military use of horses at Malbork Castle, Poland*, "International Journal of Osteoarchaeology" 2009, no. 20(3), pp. 225–343.

¹⁸ P. Lów, *Atlas of Animal Anatomy and Histology*, Springer, Cham 2016.

¹⁹ D. Ubelaker, *The forensic evaluation of burned skeletal remains: A synthesis*, "Forensic Science International" 2009, vol. 183(1–3).

a median *sacral* ridge (*crista sacralis mediana*) of fused spinous processes²⁰. In draft horses, on the other hand, the high load on the spine promotes the formation of pathological adhesions²¹.

In the case of ribs, the species vary in their number, width, and bend, the sharp or blunt surface of the cranial and caudal edges²².

In the sternum, the degree of development and cross-section of the handle and the presence of the gladius process are assessed²³. In ruminants, the sternal segments are fused into a bony plate, while in horses there is a sternal crest (*crista sterni*)²⁴. In the case of the scapula, a species feature may be the shoulder process (*acromion*) typical of carnivores and ruminants²⁵.

The *humerus* at the distal end in carnivores is formed by the head and block of the *humerus*, while in other animals that lack the mobility of the forearm bone, only the block is present. Also absent from them is the beak pit²⁶. In cats, a species feature is the supracondylar hole²⁷.

Knowledge of the anatomical differences of the pelvic limb, including the bones of the thigh and shank, and topographical knowledge of the pits, tuberosity, and detailed structure of the vertebral bodies (such as the third vertebra in horses) is also essential in differentiation²⁸.

Birds have pneumatic bones that are filled with air and lined with mucous membranes²⁹. Long bones in these animals have variable cortical thickness and mechanical properties, including bending strength. The order of small and medium-sized birds of the family *Charadriiformes* is characterized by a diffe-

²⁰ K.D. Budras, R. Habel, *Bovine Anatomy*, Schlütersche, Hannover 2011.

²¹ C.J. Diedrich, *Pathologic historic mining horses from central Europe*, "Journal of Pathology and Disease Biology" 2017, no. 1 (special issue), pp. 1–28.

²² A. Pluskowski et al., op. cit. pp. 225–343; K. Dyce et al., *Veterinary Anatomy*, Elsevier, Wrocław 2019.

²³ S. Langley-Hobbs, M. Straw, *The feline humerus. An anatomical study with relevance to external skeleton fixator and intramedullary pin placement*, "Veterinary and Comparative Orthopaedics and Traumatology" 2005, no. 18(1), pp. 1–6; E. Schmid, *Atlas of Animal Bones*, Elsevier, Amsterdam–New York 1972.

²⁴ D. Ubelaker, op. cit.; K.K. Richter et al., *Fish 'n chips: ZooMS peptide mass fingerprinting in a 96 well plate format to identify fish bone fragments*, "Journal of Archaeological Science" 2011, vol. 38(7), pp. 1502–1510.

²⁵ K.K. Richter et al., op. cit., pp. 1502–1510; M. Buckley, *Zooarchaeology by mass spectrometry (ZooMS) collagen fingerprinting for the species identification of archaeological bone fragments*, in: Ch.M. Giovas, M.J. LeFebvre (eds.), *Zooarchaeology in Practice*, Springer, New York 2017, pp. 227–247; E. Tomaszewska et al., *The effect of tannic acid on the bone tissue of adult male Wistar rats exposed to cadmium and lead*, "Experimental and Toxicologic Pathology" 2017, vol. 69(3), pp. 131–141.

²⁶ D. Ubelaker, op. cit.; M. Buckley, op. cit.; E. Tomaszewska et al., op. cit.

²⁷ S. Langley-Hobbs, M. Straw, op. cit., pp. 1–6.

²⁸ K. Budras et al., *Anatomy of the Horse: An Illustrated Text*, Schlütersche, Hannover 2003.

²⁹ M. Carrano, P. O'Connor, *Bird's-eye view*, "Natural History" 2005, no. 114(4), pp. 42–47.

rent morphological differentiation, as they do not have pneumatic bones³⁰. The lack of pneumatization of the humerus has also been confirmed in penguins³¹.

In animals, there are additional bones not described in humans³². These include the heart bone in cattle (*os cordis*)³³, the penis bone in the dog (*os penis*)³⁴, the antlers³⁵, the ossikon in giraffes³⁶, the osteoderms present in reptiles³⁷ and amphibians³⁸, and the coracoid bone in birds³⁹. Also species-specific is the dentition or lack thereof (in birds, collared, anteaters, and whalebills). The same is true for the absence of some other bones, such as the clavicle in cats⁴⁰, as well as the presence of bones that are diagnosed in animals and described as non-physiological, such as the *clitoral* bone (*os clitoris*)⁴¹.

Skull morphology differs markedly in humans and animals. Humans are characterized by a large brain volume relative to their overall body weight. Compared to animals, its head musculature is less pronounced, and its muscle attachments and associated structures are less developed. The cranial vault and expanded dipole (layer of spongy substance) are also characteristic. The distinguishing feature of the mandible in humans is its U-shaped profile, which differs from that found in animals (usually V-shaped)⁴².

³⁰ N. Adam Smith, J.A. Clarke, *Osteological histology of the Pan-Alcidae (Aves, Charadriiformes): Correlates of wing-propelled diving and flightlessness*, "The Anatomical Record" 2013, vol. 297(2), pp. 188–199; J. Cubo, A. Casinos, *Incidence and mechanical significance of pneumatization in the long bones of birds*, "Zoological Journal of the Linnean Society" 2008, vol. 130(4).

³¹ K.E. Slack et al., *Early penguin fossils, plus mitochondrial genomes, calibrate avian evolution*, "Molecular Biology and Evolution" 2006, vol. 23(6), pp. 1144–1155; W. Meister, *Histological structure of the long bones of penguins. The anatomical record*, "Advances in Integrative Anatomy and Evolutionary Biology" 1962, vol. 143(4).

³² A. Nasoori, *Formation, structure, and function of extra-skeletal bones in mammals*, "Biological Reviews" 2020, no. 95(4).

³³ T. James, *Anatomy of the sinus node, AV node and os cordis of the beef heart*, "The Anatomical Record" 1965, no. 153(4), pp. 361–371.

³⁴ N. Misk et al, *Os penis in dogs*, "Assiut Veterinary Medical Journal" 1996, vol. 35(69), pp. 115–122.

³⁵ S. Demarais, B. Strickland, *Antlers*, in: D.G. Hewitt (ed.), *Biology and Management of White-tailed Deer*, CRC Press, Boca Raton 2011, pp. 1–40.

³⁶ C. Spiiiage, *Horns and other bony structures of the skull of the giraffe, and their functional significance*, "African Journal of Ecology" 1968, vol. 6(1), pp. 53–61.

³⁷ C. Williams et al., *A review of the osteoderms of lizards (Reptilia: Squamata)*, "Biological Reviews" 2022, t. 97(1).

³⁸ F. Witzmann, R. Soler-Gijon, *The bone histology of osteoderms in temnospondyl amphibians and in the chroniosuchian Bystrowiella*, "Acta Zoologica" 2009, vol. 91(1), pp. 96–114.

³⁹ A. Charuta, J. Bartyzel, *Morfologia i morfometria kości obręczy i odcinka nasadowego kończyny piersiowej kaczki domowej*, "Veterinary Medicine" 2005, vol. 61, no. 7, pp. 811–813.

⁴⁰ E. Hazel, M. Taylor, *An Atlas of Cat Anatomy*, The University of Chicago Press, Chicago 1969.

⁴¹ S. Sumner et al, *Os clitoris in dogs: 17 cases (2009–2017)*, "The Canadian Veterinary Journal" 2018, vol. 59(6), pp. 606–610.

⁴² J. Watson, J. McClelland, *Distinguishing Human from Non-Human Animal Bone*, The University of Arizona, Arizona State Museum, Tucson 2018.

Jaw fragments can cause doubts in species determination. However, when one has a full upper or lower jaw, as well as a complete skull, identification of the species becomes more certain. According to Bruno Holyst, the human dentition most closely resembles that of the great apes. In humans, the teeth form a closed arch, maintaining contact with each other. In contrast, in great apes, there is a gap between the canine and first molar in the upper jaw, which is called the “monkey gap”. Differentiation is made much more difficult when the material is a tooth fragment. Comparative chemical analysis of dental proteins using precipitation or agar gel diffusion is then used⁴³. The study of a person’s dentition is crucial in the search for individual characteristics⁴⁴. In the osteological expertise of mass events, a number of odontological identification methods are used to analyze tooth and bone remains.

The structure of a person’s skeleton is correlated with upright posture, which has important consequences for his biomechanics and the way he moves. The vertebrae have dual axes of orientation, and all four limbs exhibit a large range of motion. Human ribs are more curved, and the bones of the limb rims may be relatively less robust and not exhibit as tight connections as those observed in animals⁴⁵. Human bones are evolutionarily adapted to grow quite fast and reach great body height despite maintaining a relatively lightweight skeleton. During bone growth in humans, however, there is a need to reduce the energy expended for this, leading to structural differences. Therefore, animal bones tend to be denser, while in humans they have a more porous cortical structure and marked shaft beams⁴⁶.

Many biological parameters of the remains are assessed histomorphometrically, including age at death⁴⁷. However, the presence of implants or biomaterials in the bone can be an obstacle, as freezing or heating the tissue is not recommended in this situation⁴⁸. They can also make it difficult to

⁴³ B. Holyst, *Kryminalistyka*, Wolters Kluwer, Warsaw 2018, pp. 471–476.

⁴⁴ J. Ata-Ali, F. Ata-Ali, *Forensic dentistry in human identification: A review of the literature*, “Journal of Clinical and Experimental Dentistry” 2014, vol. 6(2), pp. 162–167.

⁴⁵ B.A. Michael et al. *The effects of soil texture on the ability of human remains detection dogs to detect buried human remains*, “Journal of Forensic Sciences” 2016, vol. 63(3), pp. 649–655.

⁴⁶ J. Watson, J. McClelland, op. cit. pp. 1–9.

⁴⁷ B. Mnich, *Ocena wieku osobnika na podstawie badań histomorfometrycznych kości*, master’s thesis, Jagiellonian University Repository, 2014; J. Bednarek, *Methods of estimating age at death based on histomorphometry of the compact substance of bone tissue*, “Archives of Forensic Medicine and Criminology” 2008, no. 4(58), pp. 197–204.

⁴⁸ A. Scarano, G. Iezzi, A. Piattelli, *Common fixatives in hard-tissue histology*, in: Yuehuei H. An, K.L. Martin (eds.), *Handbook of Histology Methods for Bone and Cartilage*, Humana Press, Totowa, NJ 2003, pp. 159–165.

cut scraps⁴⁹. Standard histology of bone tissue is performed using various staining techniques, including hematoxylin and eosin (H&E), Masson-Goldner trichrome, Movat pentachrome, orcein, silver nitrate, and immunohistochemistry⁵⁰. Microscopy of the preparation highlights the specific organization of the Haversian system⁵¹. Osteons form lamellae in it, and these are arranged in multiple layers. In the center runs the Haversian canal, containing vessels and nerves. Perpendicular to these are the Volkman canals for contact between osteons⁵².

The organization of the Haversian system makes it possible to distinguish humans from other animal species on the so-called plexiform and according to the Haversian channel index⁵³. This image can change under the influence of physiological conditions, disease factors, and climate. The variation in the diameter of the middle canal is not only a reflection of differences between species but also characterizes individual bones. The process of bone reorganization during life is correlated with muscle specialization. The sites of their attachment are most often characterized by a multiplication of the central canals due to the greater number of microtraumas leading to locomotor strengthening of this area. In humans, bone tissue shows a Haversian system structure similar to that of primates and small mammals. In large mammals, thick-fiber support tissue is also present. Such tissue occurs in humans in fetal life, while in adults it occurs in periostitis or after trauma. Thus, if the bone tissue fragment examined is not of neonatal origin or trauma, it can be indicated that it is of animal origin. In some cases in large mammals post-mortem, coarse fibrous tissue may be degraded, so additional identification methods are needed⁵⁴.

⁴⁹ H. Yuehuei An, H.E. Gruber, *Introduction to experimental bone and cartilage histology*, in: Yuehuei H. An, K.L. Martin (eds.), *Handbook of Histology Methods for Bone and Cartilage*, Humana Press, Totowa, NJ 2003, pp. 3–31.

⁵⁰ C. Rentsch et al., *Comprehensive histological evaluation of bone implants*, “Biomatter” 2014, vol. 4, pp. 1–11.

⁵¹ G. Dragoi et al., *Histomorphometric evaluation of osteons inside the compacta of long bones diaphysis. Implication in pathology*, “Romania Journal of Legal Medicine” 2014, vol. XXII, no. 1, pp. 109–116.

⁵² L. Fontoura Costa L., M. Palhares Viana, M.E. Beletti, *Complex channel networks of bone structure*, “Applied Physics Letters” 2006, no. 88, pp. 1–3.

⁵³ G. Teresinski, *Medycyna sądowa*, vol. 1, Wydawnictwo Lekarskie PZWL, Warsaw 2019, p. 190.

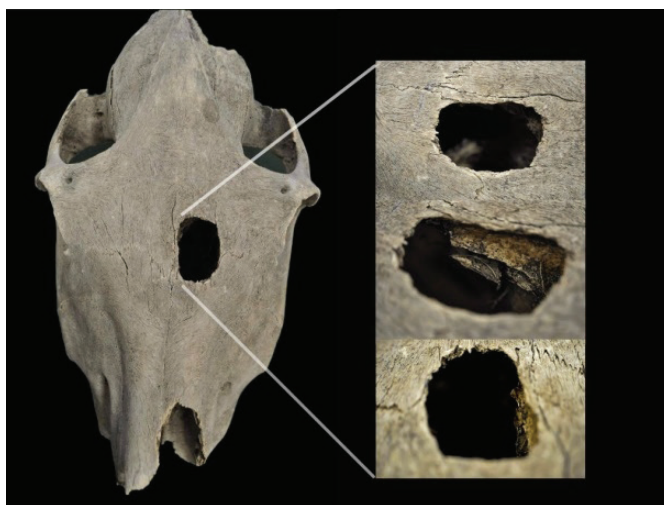
⁵⁴ H. Kobryń, F. Kobryńczuk, K. Krysiak, *Anatomia zwierząt*, t. 1, Wydawnictwo Naukowe PWN, Warsaw 2021, pp. 246–514; C.M. Bagi, E. Berryman, Ch. Moalli, *Comparative bone anatomy of commonly used laboratory animals: Implications for drug discovery*, “Comparative Medicine” 2011, vol. 61, no. 1, p. 76–85; S. Hillson, *Mammal Bones and Teeth. An Introductory Guide to Methods of Identification*, Routledge, New York 2016, s. 100–132.

Fig. 1. 3D reconstruction of a dog skull from the multicultural settlement of Polwica-Skrzypnik in Lower Silesia. Identification of a probable telangiectatic osteosarcoma



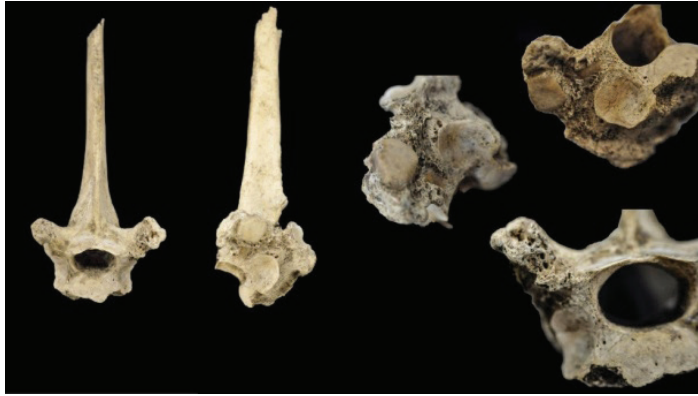
Source: Archaeozoology Laboratory and Model Museum of the Department of Animal Biostructure and Physiology at the Wrocław University of Life Sciences.

Fig. 2. Skull injury to a horse



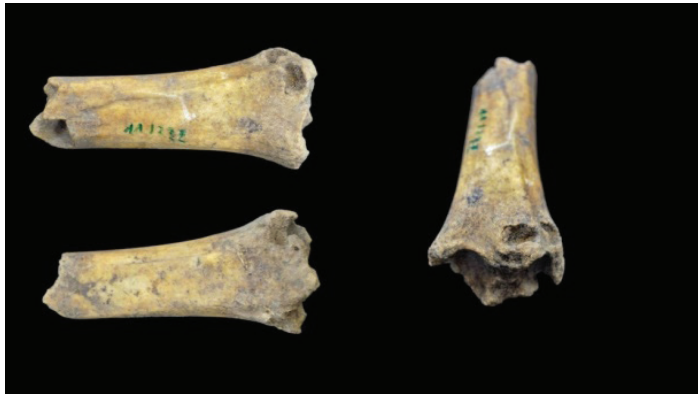
Source: exhibit courtesy of Prof. Dr. Hab. Daniel Makowiecki.

Fig. 3. Tuberculous lesions of the vertebrae



Source: Archaeozoology Laboratory and Model Museum of the Department of Animal Biostructure and Physiology at the Wrocław University of Life Sciences.

Fig. 4. Bones subjected to zooarchaeological analysis



Source: Archaeozoology Laboratory and Model Museum of the Department of Animal Biostructure and Physiology at the Wrocław University of Life Sciences.

Fig. 5. Trace of mandibular separation indicating that the dog has been subjected to consumption



Source: Archaeozoology Laboratory and Model Museum of the Department of Animal Biostructure and Physiology at the Wrocław University of Life Sciences.

Advanced diagnostic techniques and technologies

Radiological diagnostics⁵⁵ and advanced techniques⁵⁶, such as micro-computed tomography, are commonly used in osteology. It evaluates structural parameters of cortical and spongy bones and bone mineral density⁵⁷. This method is used to study the skeletons of laboratory animals (such as mice, rats, rabbits, dogs, and primates, in addition to humans), focusing on the femoral head region, lumbar vertebrae, and mandible. The study characterizes the number of beads, thickness, and separation of bone plates. However, the greatest development in techniques for identifying remains is due to genetics⁵⁸.

Some animal remains indicate that the bones were processed and used as tools, and therefore man-made objects. X-ray spectroscopy (EDX) and scanning electron microscopy (SEM)⁵⁹ are applicable in distinguishing

⁵⁵ T. Kahana, J. Hiss, *Identification of human remains: forensic radiology*, "Journal of Clinical Forensic Medicine" 1997, no. 4(1), pp. 7–15.

⁵⁶ M.L. Hillier, L.S. Bell, *op. cit.*, pp. 249–263.

⁵⁷ K.L. Colman et al., *The geometrical precision of virtual bone models derived from clinical computed tomography data for forensic anthropology*, "International Journal of Legal Medicine" 2017, no. 131(4), pp. 1155–1163.

⁵⁸ B. Budowle, A. van Daal, *Extracting evidence from forensic DNA analyses: Future molecular biology directions*, "BioTechniques" 2018, no. 46(5), pp. 339–347.

⁵⁹ S. Ellingham, T.J.U. Thompson, M. Islam, *Scanning Electron Microscopy-Energy-Dispersive X-Ray (SEM/EDX): A rapid diagnostic tool to aid the identification of burnt bone and contested remains*, "Journal of Forensic Sciences" 2017, vol. 63(2), pp. 504–510.

bone from other materials. An indicator of bone is calcium, and its level can indicate the origin of the tissue (e.g., from ivory or coral and minerals, which are common items of contraband)⁶⁰.

Special identification methods are required for burned bone samples. They are subjected to grinding and microscopic examination using polarized light. This uses chemical analysis of the absorption bands of hydroxyapatite (for the wave numbers of PO_4^{-3} , CO_3^{-2} , $-\text{CO}-\text{NH}^-$ and OH^- ions), which define the species and age of the bone. In addition, the origin of bones can be determined by DNA polymorphism, which is usually applied to teeth and their remains. DNA testing is the “gold standard” for identifying victims of mass events in the Interpol protocol. They make it possible to identify even century-old remains. The scope of the study includes STR (*short tandem repeats*) and mitochondrial DNA (mtDNA) analysis using PCR (*polymerase chain reaction*)⁶¹. The gastrointestinal contents of arthropods living in the presence of old bones or tissues with a strong degree of decomposition can also be a sample for such tests. For this purpose, it is recommended to use the so-called DNA barcoding – DNA strip tests according to Herbert⁶². The molecular data have low intra-species variability and high interspecies variability; key in assessing the species are fragments of CO1 (the first subunit of cytochrome oxidase, 650 nucleotides long). They are cataloged in biobanks, and the whole process is referred to as “species tagging”.

The interdisciplinarity of forensic osteology methods on the pillars of archaeozoology

Archaeological research allows the discovery of traces created throughout history, providing a lot of information about human life, its rituals, and animal life⁶³. Hence, in anthropology, the streams of physical and cultural anthropo-

⁶⁰ J.B. Ledoux et al., *Molecular forensics into the sea: How can molecular markers help to fight against poaching and illegal trade in precious corals?*, “The Cnidaria, Past, Present and Future” 2016, no. 45, pp. 729–745; D.H. Ubelaker et al., op. cit., pp. 940–943.

⁶¹ D. Higgins, J.J. Austin, *Teeth as a source of DNA for forensic identification of human remains: A review*, “Science & Justice” 2013, vol. 53(4), pp. 433–441; M.E. Newman et al., *Identification of archaeological animal bone by PCR/DNA analysis*, “Journal of Archaeological Science” 2002, vol. 29(1), pp. 77–84; S. Lutz, H.-J. Weisser, J. Heizmann, S. Pollak, *mtDNA as a tool for identification of human remains*, “International Journal of Legal Medicine” 1996, no. 109, pp. 205–209.

⁶² K. Siemienkiewicz, *Entomologia sądowa*, in: B.A. Nowak, M. Maciąg (eds.), *Przegląd badań z zakresu kryminalistyki i medycyny sądowej*, Tygiel Scientific Publishing House, Lublin 2017, pp. 79–98.

⁶³ R.L. Schuyler, *Archaeological remains, documents, and anthropology: A call for a new culture history*, “Historical Archaeology” 1988, vol. 22, pp. 36–42; M. Mullin, *Animals and anthropology*, “Society & Animals” 2002, no. 10(4), pp. 388–391.

logy emerged; ethnography and ethnology⁶⁴, and in zoology, paleontology, and archaeozoology⁶⁵. Within their framework, several specific branches can be distinguished: the study of traces on animal bones – tafonomy, and the study of pathological changes in bone tissues – paleopathology. Depending on the species, they focus on analyzing the remains of fish, mollusks, amphibians, mammals, birds, and animal materials in everyday objects and art objects. The entire study of animal remains at archaeological sites is referred to as faunal analysis⁶⁶. The research methods used to study ancient traces are an outgrowth of the direction of many disciplines and serve the present. They support each other and include techniques such as organic residue chromatography and metallographic studies. Forensic science, including forensic medicine and forensic veterinary medicine, has developed significantly with the achievements of forensic science. In addition, many bone identification techniques are derived from archaeology⁶⁷. Modern forensic science is inferior even to older archaeology in terms of revealing traces, hence the methodological adaptations that have arisen within its framework⁶⁸, which can be observed in the study of human and faunal remains as early as the visual inspection stage. In a broader context, archaeological analysis of the past influences the understanding of the present and future of species distribution and the subsequent extinction process.

Forensic archaeology provides physical evidence for trial purposes⁶⁹. The archaeological site is first located and then mapped. The proceeding takes place in three main stages: reconnaissance, excavation, and detailed research⁷⁰. The main goal is to describe the material, its classification (including the study of post-mortem changes, and bone traumatization) – analysis of sex, age, race, and height⁷¹. Discovered remains are delivered to forensic science, anatomy, biostructure, and physiology departments or genetic laboratories.

⁶⁴ M. Mead, *Visual anthropology in a discipline of words*, in: P. Hockings (ed.), *Principles of Visual Anthropology*, Mouton de Gruyter, Berlin–New York 2003, pp. 32–33.

⁶⁵ S. Wolverton, R. Lee Lyman, *Introduction to applied zooarchaeology*, w: eidem (red.), *Conservation Biology and Applied Zooarchaeology*, University of Arizona Press, Tucson 2012, s. 1–17.

⁶⁶ P.E. McGovern et al., *Science in archaeology: A review*, “American Journal of Archaeology” 1995, vol. 99, no. 1, pp. 79–142.

⁶⁷ B. Sigler-Eisenberg, *Forensic research: Expanding the concept of applied archaeology*, “American Antiquity” 1985, vol. 50(3), pp. 650–655; M. Hrehorowicz, op. cit.

⁶⁸ P. Bahn (ed.), *History of Archaeology*, Arkady Publishing House, Warsaw 2019, pp. 520–530.

⁶⁹ M. Obledo, *Forensic archaeology in criminal and civil cases*, “Forensic Magazine” 2009, vol. 6(4), pp. 31–34.

⁷⁰ B. Sigler-Eisenberg, op. cit., pp. 650–655.

⁷¹ M. Richards, B. Sykes, *Authenticating DNA extracted from ancient skeletal remains*, “Journal of Archaeological Science” 1995, vol. 22(2), pp. 291–299.

The entire proceeding may require the drawing of specialized knowledge from several fields, the work of a team of investigators, or the expertise of experts. The need to prepare several analyses in separate laboratories can guarantee the authenticity of the result. This applies to molecular studies of centuries-old samples dating back to ancient times. This avoids the impact of laboratory contaminants on the overall DNA result. The control sample contains the genetic material of animals, which allows you to test for contaminating human DNA⁷². It is very easy for such samples to become contaminated, which is why units that conducted such tests implemented complicated protocols and even built new laboratories specifically for this purpose. A great deal of interdisciplinarity is also required to study incinerated remains. They recover incineration residues; and perform DNA testing and *thermogravimetric* analysis (*TGA*)⁷³.

Reproducing information from bone samples without insight into their morphological structure is possible with the ZooMS “collagen fingerprint”⁷⁴. It is a molecular “bar code” defined by the structure of collagen. This method offers a high level of accuracy in identifying the species⁷⁵.

The traditional archaeological methods are open pit and surface surveys (combined in practice). However, the radiocarbon method played a major role in dating finds and artifacts. Isotopic analysis of human and animal remains requires specialized knowledge. Some of the animals’ remains are extremely small, so they are extracted by paraffin flotation, and then evaluated microscopically to separate them from artifacts. The road to knowledge was opened in this regard by the development of analytical chemistry. Isotopic testing provides a lot of information, such as the correlation between the test material and living descendants (both in humans and animals). The stability of individual isotopes of elements in the environment provides insight into how organisms feed. Based on strontium isotopes from teeth and bones, population migrations can be traced. Indeed, they were marked in the clothing of Stone Age people – bear fur and goat, deer and calf skins. Leather parts perform particularly well in swampy soils. The acidic pH of peatlands and the increased content of tannic acid (a natural tannin) induce

⁷² W. Parson et al., *Species identification by means of the cytochrome b gene*, “International Journal of Legal Medicine” 2000, no. 114, pp. 23–28.

⁷³ D. Ubelaker, op. cit., pp. 1–5.

⁷⁴ F. Welker et al., *Using ZooMS to identify fragmentary bone from the Late Middle/Early Upper Palaeolithic sequence of Les Cottés, France*, “Journal of Archaeological Science” 2015, vol. 54, pp. 279–286.

⁷⁵ K.K. Richter et al., op. cit., pp. 1502–1510; M. Buckley, op. cit., pp. 227–247.

the gradual elimination of calcium and nitrogen fixation in tissues while inhibiting the proliferation of bacteria⁷⁶.

Archaeology provides great insight into the history of animal life (Fig. 1-5). Domestication traits of some species are subject to succession, as evident in morphological adaptations. During the domestication process, the skeleton can decrease (as observed in cattle, pigs, goats, and sheep) or increase (in chickens, geese, ducks, and horses). With this awareness, the metric features of the aurochs were correctly reconstructed. The length of the bone and girth of the humerus were measured, and coefficients were developed to give a statistical reconstruction of the height at the withers of other animals of this habit. A corollary of this is the ability to identify gender, age, and utility⁷⁷.

Animal bones allow us to study historical facts about places, human and interspecies relations⁷⁸, and cultural and religious aspects⁷⁹. Thanks to them, it is possible to trace the history of zoonotic epidemics, reveal the various ways in which animals are killed and used, even abused, the techniques of taming and capturing, and the use of traps that leave visible marks on limb bones and jaws⁸⁰. The pathomorphology of retrograde lesions makes it possible to assess their vital or post-mortem nature; and to adjudicate whether they arose in young, adult, or old age⁸¹.

Compared to rapidly decomposing soft tissues, bones retain their structure long after death, providing great longevity of traces⁸². Thanks to this, it is known, among other things, that depositing a whole animal carcass in a tomb may have had ritual significance. Burial similar to that afforded to humans was provided to horses, and less frequently to dogs. Most of the animal remains recovered from archaeological sites are food scraps stacked far from where food was prepared and consumed by humans. Meat consumption is evidenced by traces of cutting and processing of carcasses

⁷⁶ E. Tomaszewska et al., op. cit., pp. 131–141; B.B. Dent, S.L. Forbes, B.H. Stuart, *Review of human decomposition process in soil*, “Environmental Geology” 2004, no. 45, pp. 576–585.

⁷⁷ A. Lasota-Moskalewska, *Zwierzęta udomowione w dziejach ludzkości*, Warsaw University Publishers, Warsaw 2005, pp. 32–33.

⁷⁸ J. Stojak, *Kości z potencjałem – co możemy wyczytać ze szczątków zwierząt?*, “Archaeology” 2018, no. 10.

⁷⁹ W.L. Kemp, *Postmortem change and its effect on evaluation of fractures*, “Academic Forensic Pathology” 2016, no. 6(1), pp. 28–44.

⁸⁰ L. Bartosiewicz, E. Gál, *Shuffling Nags, Lame Ducks: The Archaeology of Animal Disease*, Oxbow Books, Oxford 2013, pp. 19–31.

⁸¹ *Ibid.*, pp. 1–17.

⁸² A.A. Vass, *Beyond the grave – understanding human decomposition*, “Microbiology Today” 2001, vol. 28, pp. 190–192.

with characteristic indentations that are a consequence of the use of sharp tools and the tearing off of meat. Some similar changes may occur due to the presence of bacteria, insects, and the growth of plant root systems in the material⁸³. The excavation methodology of forensic veterinary medicine was well described during the work on Trench III F in Ostrów Tumski in Wrocław. The site should first determine TNF (*total number of fragments*) – the global number of remains, date individual specimens, and make species and anatomical identification – NISP (*number of identified species*). This is followed by osteometric testing according to Angela von den Driesch's methodology. The data presented are compared with the available literature. The metric measurements taken already at this stage can make it possible to determine gender. For cattle and dogs, this is calculated based on the length of the long bones (metacarpals and metatarsals, fetlock bones) using the method of Boessneck, Całkin, and Koudelka; the height at the withers of horses is determined by the length of the metapodium. In pigs, Teichert's calculus is used. Determining the height at withers of sheep and goats, on the other hand, is possible thanks to the Haak, Schramm, and Koudelka coefficients. Assessment of the age of animals at the time of death is made based on the degree of closure of the epiphyseal cartilages of long and sundry bones and the characteristics of the dentition. All this data can already suggest the utility type of the animal. In addition to livestock and companion animals, numerous remains of wildlife and fish have been found in the urban area⁸⁴. Animal remains were the main focus at other sites as well, such as Çatalhöyük in central Turkey⁸⁵.

Differentiating the species of origin of biological material is equally important in art expertise. It is sometimes commissioned by restorers or connoisseurs wishing to confirm the authenticity of a work of art, or by law enforcement agencies to evaluate evidence of a crime. In Dobrzeń Wielki, for example, the keyboards of the manuals in the church organ

⁸³ M. Schultz, *Paleopathology of bone: A new approach to the study of ancient diseases*, "Yearbook of Physical Anthropology" 2001, vol. 44, pp. 106–147.

⁸⁴ U. Albarella, "The mystery of husbandry": *Medieval animals and the problem of integrating historical and archaeological evidence*, "Antiquity" 2015, vol. 73(282), pp. 867–875; A. Chrószcz et al., *Analiza archeozoologiczna szczątków kostnych z wykopu III F przy ul. św. Idziego na Ostrowie Tumskim we Wrocławiu*, in: A. Limisiewicz, A. Pankiewicz (eds.), *In Pago Silensi. Wrocław Early Medieval Studies*, vol. 1: *The Shaping of a Castle on Wrocław's Ostrów Tumski. Research at Św. Idziego Street*, University of Wrocław, Wrocław 2015, pp. 421–452.

⁸⁵ N. Russell, S. Meece, *Animal representations and animal remains at Çatalhöyük*, in: I. Hodder (ed.), *Çatalhöyük Perspectives: Reports From The 1995–99 Seasons*, vol. 6, British Institute at Ankara, Cambridge–London 2005, pp. 209–230.

were reproduced using a particular species of ox bone⁸⁶. Zoonotic products are found in art in the form of dyes, leather, urine, silk threads, wax, glue, egg whites, and others⁸⁷. Human remains were also used to produce some “works of art” Ilse Koch (a German SS supervisor) answered before the Bavarian state tribunal for, among other things, ordering the killing of Buchenwald camp inmates and obtaining their tattooed skin for processing into lampshades and handbags⁸⁸. American psychopath Ed Gein, inspired by literature describing experiments conducted in Nazi death camps, dug up corpses from cemeteries and used the obtained human skin to upholster furniture and sew vests from it⁸⁹. The evaluation of such traces can be the basis for proceedings in cannibalism cases⁹⁰, desecration of corpses⁹¹, or the exhumation process⁹².

Another interesting aspect is the use of the animals themselves for research. Trained dogs can specialize in detecting human remains (*HRD – Human Remains Detection dogs*). Based on olfactory patterns, they can pinpoint their location and distinguish animal remains from human remains⁹³.

Summary

Differentiating human and animal bones is a complex process that can require specialized methodology, especially when the material is significantly damaged, fragmented, or ashed. The process begins with a visual inspection at the site where the bone material is found, where the location and depth of bone embedment in the ground are assessed. Although bone tissue may have some distinctive species characteristics, overall there is little morphological variation. It has the advantage of a slow degradation process

⁸⁶ E. Molak et al. (eds.), *Opolski informator konserwatorski*, Wojewódzki Urząd Ochrony Zabytków in Opole, Opole 2012.

⁸⁷ M.P. Colombini, F. Modugno, *Organic materials in art and archaeology*, in: eidem (ed.), *Organic Mass Spectrometry in Art and Archaeology*, Wiley, Chichester 2009, pp. 4–35.

⁸⁸ P. Pindel, *Zniewolenie osadzonych w obozie koncentracyjnym Buchenwald – wykorzystywanie ludzi na własny użytek przez Ilse Koch*, “Student Yearbooks of the Academy of Land Forces” 2017, R. 1.

⁸⁹ A. Gawliński, *Necrophilia as an interdisciplinary problem*, “Legal Studies” 2016, no. 34, pp. 25–28.

⁹⁰ W. Hurley, *A retrospective on the Four Corners Archaeological Program*, “CRM” 2000, no. 1.

⁹¹ D. Bouquin, J.P. Beauthier, G. Depierre, *The dead do not dress: Contribution of forensic anthropology experiments to burial practices analysis*, HAL SHS Science Humaines et Sociales, 2013, pp. 1–16.

⁹² K.C. Crouch, *Dealing with the dead: Understanding professional relations between archaeologists and human remains*, University of Manchester. School of Arts, Languages and Cultures, PhD thesis, 2017, pp. 22–84.

⁹³ B.A. Michael et al., op. cit.

and the availability of a lot of information resulting from the analysis of macro- and micro-anatomical and biochemical structures.

In the process of differentiating bones into species, it is crucial to know comparative anatomy, taking into account all the bones of the skeletal system and their possible adaptations. Equally important is the consideration of possible pathological changes and knowledge of the clinical applications of new tissue engineering, which indicates the usefulness of working with a medical specialist during identification. Human bones usually have a more porous cortical structure and marked shaft beams. Characteristic features of humans include a larger skull than in animals, an elaborate dipole of cranial bones, a U-shaped mandible, and a closed dental arch. The axial spine is characterized by the presence of vertebrae with double orientation axes, and the ribs show a pronounced curvature. The bones of animals are characterized by considerable variation, which can become apparent even within breeds.

A variety of diagnostic techniques are used for identification, such as radiography and CT scans, which do not induce loss of examination material, as well as those that may require wear and tear of a bone fragment, including histological studies and biochemical and genetic analyses. The molecular data have high interspecies variability and are included in the “gold standard” for the study of remains of high historical value and cremated bones.

Archaeology is a source of valuable methodology, contributing to a better understanding of both the present and the past. Analysis of historical traces of human and animal activity, based on studies of skeletal remains, has provided evidence of various aspects, including abuse or intentional neglect of animals. As laws on human-animal relations evolve, there is a growing need to synthesize this knowledge. Therefore, especially in the field of forensic veterinary medicine, there is a need to increase interest in this area. Despite significant advances in species identification techniques, such as X-ray spectroscopy, electron microscopy, and PCR analysis, publications on the identification of remains are still rare. It is also worth noting the growing need for biobanks of bone tissue obtained from different animal species for comparative purposes.

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