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A qualitative histomorphological comparison of Human and Pig femoral cortical bone: implications for forensic species identification

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Abstract
Introduction: Accurate species identification is crucial in forensic and archaeological investigations, especially when skeletal remains are fragmented or lack clear anatomical features. Traditional macroscopic assessments may be inconclusive, necessitating microscopic methods like histological analysis. This study compares the femoral cortical bone of humans (*Homo sapiens*) and pigs (*Sus scrofa*).
Objective: This study compares the femoral cortical bone of humans (*Homo sapiens*) and pigs (*Sus scrofa*) to establish microstructural criteria for species differentiation.
Methods: Femoral samples were obtained from nine adult humans and nine sub-adult pigs. Ground sections were prepared using a modified Frost's method to preserve cortical architecture. Samples were analysed at ×100 magnification under a light microscope. Key histological features assessed included primary and secondary osteons, Haversian systems, plexiform structures, and Volkmann's canals.
Results: Human femora exhibited a dense Haversian system with abundant secondary osteons, osteon fragments, and Volkmann's canals—hallmarks of extensive bone remodelling. Pig femora showed a plexiform pattern dominated by regularly arranged primary osteons and limited secondary remodelling. These differences reflect adaptations to bipedal and quadrupedal locomotion, respectively.
Conclusion: The arrangement and type of osteons, especially the presence or absence of plexiform bone, are reliable indicators for distinguishing human from pig femora. Histological analysis proves essential when remains are too degraded for macroscopic or genetic evaluation. These findings also emphasize the limitations of using pigs as proxies in human forensic models and highlight the need for regional histological databases to improve forensic accuracy.

Keywords: forensic anthropology, bone histology, Haversian system, species differentiation, *Sus scrofa,* human femur

Introduction

A common problem faced by anthropologists is the need to invest substantial time and effort analysing bones brought to mortuaries, even when those remains turn out to be non-human [1]. Typically, human and non-human bone fragments are distinguished through gross morphological examination; however, when these macroscopic characteristics prove inconclusive, histological analysis provides an alternative approach [2].

Histology complements macroscopic analysis, especially when remains are fragmented or altered, helping differentiate human bones from animal bones [2]. In forensic contexts, this microscopic assessment is particularly valuable when traditional morphological markers are absent due to taphonomic processes or thermal alterations [3].

The histological analysis of bone microstructure is a vital tool in forensic anthropology, evolutionary biology, and comparative anatomy. By examining osteonal patterns, vascular canal orientation, and remodelling activity, histology helps researchers identify species, determine the age at death, and explore biomechanical adaptations, especially in fragmented skeletal remains [4].

Biomechanical forces specific to each species influence the microstructural organization of long limb bones. For instance, variations in locomotion and body mass shape these structures [5]. Since limb bones bear heavier loads, the body may respond by reducing the size of osteon canals and increasing the size of the osteons, making the bone stronger and less prone to weakness [6]. Such variation in cortical bone structure can reflect physical activity and is essential for understanding interspecies differences [7].

Therefore, histological analysis of femoral cortical bone provides valuable insights into forensic anthropology and comparative anatomy, offering robust species differentiation [8]. Enlow and Brown's pioneering work [9] in comparative histology across vertebrates revealed that humans typically exhibit dense Haversian tissue, while larger mammals tend to show a plexiform structure. This foundational study introduced the concept of histo-diversity, emphasizing the variability in bone microstructure across species.10 Histological features, such as bone tissue structures, are also crucial in species identification [11, 12].

In light of these concepts, the present study offers a qualitative histological comparison of femoral cortical bone from Homo sapiens and Sus scrofa. These species were selected due to their anatomical and forensic significance, with pigs often serving as human proxies in taphonomic and experimental research [13, 8]. qualitative differences in vascular canal patterns, noting that pigs exhibit primary vascular (plexiform) bone, while humans display Haversian systems on the femoral periosteal surface. Although not focused on histology, [14] observed significant differences in the decomposition rate and tissue breakdown between human and pig cadavers, concluding that pigs are inadequate proxies for human decomposition studies. Given the relationship between bone histology and decomposition, this reinforces the necessity of recognizing species-specific histomorphological differences before applying pig-based skeletal data in human forensic investigations. By examining key microstructural features such as secondary osteons, plexiform bone, and vascular canal organization, this study aims to enhance species differentiation through bone histology and contribute to the advancement of forensic identification techniques

Materials and Methods

This descriptive study was conducted at the Department of Anatomy, University of Port Harcourt, with a focus on the qualitative comparison of the histomorphological features of human and pig femoral cortical bone.

Sample Collection

Femoral bone samples were obtained from two sources: human adult cadavers (n = 9) and sub adult pigs (*Sus scrofa*) (n = 9). Human bones were sourced from the Department of Anatomy, while pig femora were obtained fresh from a local abattoir. All samples were free of trauma, pathological lesions, or visible deformities.

Bone Preparation Technique

Samples were collected from the midshaft of the femur. Ground sections were prepared using a modified Frost's manual method, which avoids decalcification to preserve microarchitectural integrity. Bone fragments were manually ground on P220 sandpaper fixed to a glass slab, lubricated with water, until thin and translucent.

Once sufficiently thin, the sections were cleaned in distilled water with mild detergent, mounted on glass slides with DPX mountant, and covered with cover slips. Slides were left to dry for 24 hours and then analysed under a Leica ICC 50E photomicroscope at ×100 magnification.

Qualitative Histological Examination

Photomicrographs were taken from representative fields on the periosteal surface of each sample. Key microstructural features assessed include: Primary osteons, Secondary osteons, Osteon fragments, Haversian systems, Plexiform bone structure, Lamellar arrangement, Volkmann's canals

Two independent observers selected and reviewed regions of highest osteon density, and representative images were documented for analysis and description.

Results

Photomicrographs revealed distinct histomorphological differences between human and pig femoral cortical bone.

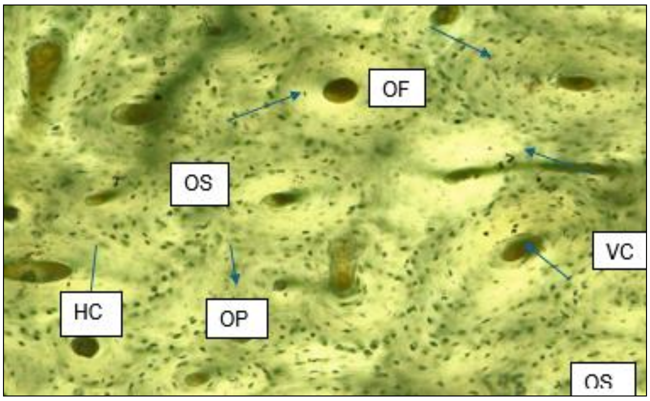

Fig 1: Micrographs of the human femur bone showing dense haversian pattern of bone tissue with profound secondary osteon (OS) and haversian canal (HC), osteon fragment (OF), primary osteon (OP) and Volkmann's canal (VC).

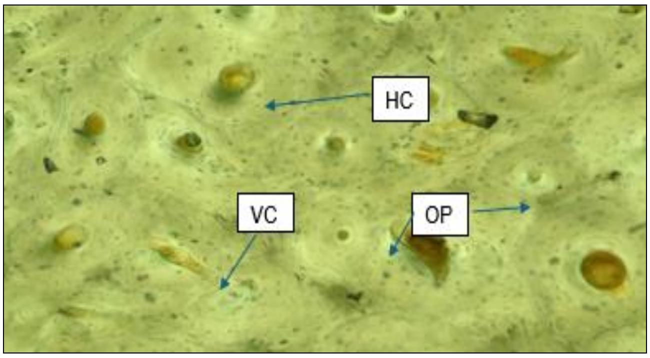

Fig 2: Micrograph of the cross section of human femur bone showing the Primary Osteons (OP), Haversian canals (HC) and Volkmann's canals (VC).

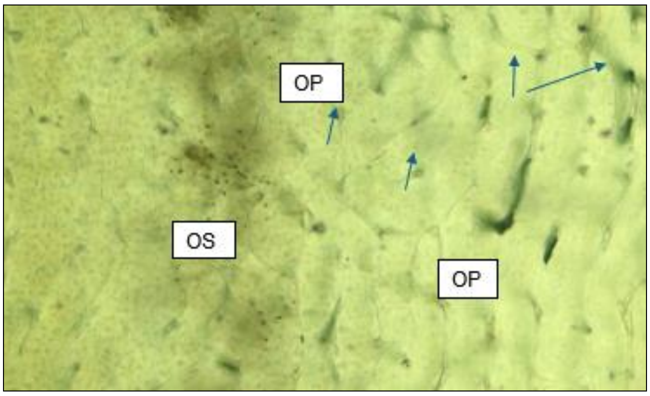

Fig 3: A photomicrograph Showing plexiform pattern of pig femoral cortical bone of plexuses and primary osteons (OP)

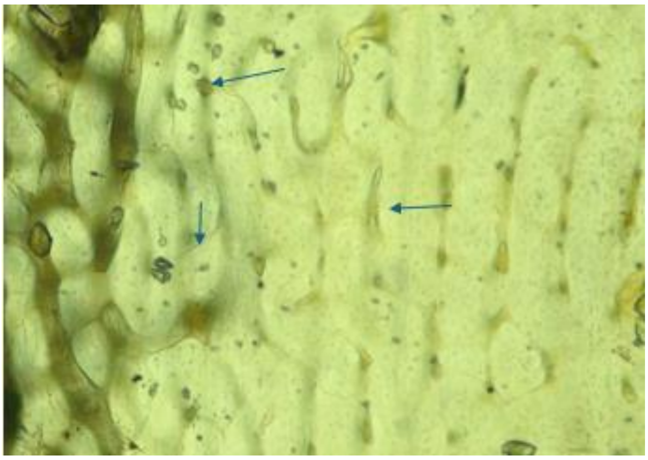

Fig 4: A photomicrograph showing plexiform bone of pig femur showing Primary Osteons (OP) and Secondary Osteons (OS). X100.

The human femur displayed a dense Haversian system composed predominantly of well-formed secondary osteons with well-defined concentric lamellae and cement lines (Fig. 1 and Fig. 2).

Osteon fragments were commonly observed, indicating a high degree of remodelling activity.

The Haversian canals were variably sized but generally centrally located and often interconnected by Volkmann's canals.

The osteons were scattered and irregularly spaced, consistent with typical human cortical bone architecture. The overall microstructure was compact and showed evidence of extensive internal remodelling.

The pig femur was characterized by the presence of plexiform bone, identifiable by its brick-wall-like arrangement of vascular canals. Primary osteons were the dominant feature, arranged in regular longitudinal and radial rows.
Secondary osteons were fewer, and osteon fragments were rarely observed. The bone exhibited osteon banding, where osteons align in parallel rows, a feature uncommon in humans. The concentric lamellae were either absent or poorly defined around many of the canals.
Fig. 3. – Fig. 4.o depict the pig femur's plexiform pattern and longitudinal canal arrangement typical of large quadrupeds.

Discussion

This study provides a qualitative histological comparison of human and pig femoral cortices, emphasizing distinct micro architectural features that can serve as practical forensic markers for species identification.
The human femur consistently displayed a dense and structured Haversian system, with abundant secondary osteons, osteon fragments, and occasional Volkmann's canals, indicating a high degree of internal bone remodelling. In contrast, the pig femur exhibited characteristic plexiform bone, made up predominantly of primary osteons arranged in linear, brick-wall-like patterns with minimal remodelling activity.
These microstructural differences reflect fundamental evolutionary and biomechanical adaptations. As bipeds, humans experience vertical load-bearing stresses that require continual bone remodelling to maintain mechanical competence. This results in a dense network of secondary osteons, an established hallmark of mature human bone.[4] Conversely, pigs—being quadrupeds—develop cortical structures suited for horizontal weight distribution, leading to the formation of plexiform bone with limited secondary remodelling. This observation is consistent with reports by Martiniaková [15] who identified similar differences in species like sheep, another quadruped with plexiform dominance.
The Haversian pattern in humans reflects slow, adaptive growth in response to sustained mechanical stress, while the plexiform pattern in pigs indicates rapid growth and early skeletal maturation, as noted by Clarke [16] and Crowder & Dominguez [17]. These differences are not only anatomically relevant but also forensically significant, especially in situations where skeletal remains are fragmented, charred, or stripped of distinguishing macroscopic features [18].
Our findings agree with Hillier *et al*., [4] who emphasized that secondary osteons and random osteon spacing are typical of human bone, while regular, parallel arrangements of primary osteons and the presence of plexiform architecture are suggestive of non-human origin. Although this study focuses on pigs, the findings also corroborate earlier works on other ungulates such as sheep [15, 8], reinforcing the utility of these features in forensic differentiation.
Notably, while Brits *et al* [8]. described ungulate bone as exhibiting "longitudinal" vascular patterns and this study identifies it as "plexiform," the variation may be due to differences in breeds, sample age, or analytic techniques. Regardless of terminology, the absence of disorganized secondary osteons in pig bone remains consistent and distinguishes it from the remodelled architecture of human bone.
Our observations also support the framework proposed by Mulhern & Ubelaker [19] which holds that the lack of plexiform structure is a near-universal trait in healthy human cortical bone, while its presence strongly suggests non-human origin—particularly among large domestic mammals. Furthermore, the contrast in osteon arrangements (disordered/concentric vs. parallel/plexiform) presents a reliable microscopy-based diagnostic tool for forensic anthropologists.
The practical implications of these findings are especially relevant in forensic contexts involving burned, degraded, or incomplete remains. As Labuschagne [20] emphasized, histological features remain discernible even in thermally altered bone, allowing continued identification of species through microstructural patterns. This is particularly useful when DNA recovery is compromised or unavailable.
Although some overlap in histological features may exist between human and certain large mammals, [21] the combined presence of irregularly arranged secondary osteons, osteon fragments, and Volkmann's canals remains largely exclusive to human bone. Therefore, the development of a regional histological reference database, particularly in tropical regions like Nigeria, is crucial. Such a resource would enhance the accuracy and speed of forensic assessments, especially in environments where advanced technologies may be limited [22].
In conclusion, this study affirms that qualitative histomorphology, particularly the presence or absence of plexiform bone, osteon type, and arrangement, provides a reliable basis for distinguishing human from pig bone. These microstructural features, when interpreted with contextual knowledge, can significantly aid forensic and archaeological investigations involving skeletal fragments.
This study was limited by a small sample size and age differences between human and pig specimens, which may affect generalizability. Despite these limitations, the study highlights the value of histological analysis in forensic contexts where skeletal remains are fragmented or altered. Future research should include larger, more diverse samples and multiple species to improve reliability. There is also a need for regional histological reference collections to support faster, more accurate forensic assessments, especially in resource-limited settings.

Conclusion

Histological features such as the presence of plexiform bone, osteon type, and their arrangement provide reliable diagnostic markers for differentiating human from pig bone. This study supports the integration of histological analysis into routine forensic protocols, especially in cases involving degraded, charred, or incomplete skeletal remains. Findings also highlight the need for localized histological reference collections.

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