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Comparative Study of the Femoral Cortex of Human and *Bos Taurus* (Cow): Forensic Implication

Loveday Ese Oghenemavwe[1], Clinton David Orupabo[2], Esther Onome Esiobise[1]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria
[2]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nigeria

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ABSTRACT
Introduction: A frequent challenge at forensic scene is the need for the identification of bone remains, suspected to be of human origin. Often times, these remains may have been exposed to adverse conditions which may have caused severe degradation of the bone fragments. It has been postulated that histological features of animals may have been used in error during forensic case investigation especially in case of commingled remains. Therefore, the aim of this study is to evaluate the histomorphometry of femoral cortex of *Bos taurus* (cow) and human.
Methods: A convenience sampling technique was adopted to select and harvest femur bones from healthy *Bos taurus* (cow) and human cadaver. 20 femoral midshaft fragments, (10 from human and 10 from cow), were used. The fragments were processed using modified Frost's Manual Method of bone preparation.
Results: The morphometric analysis of the micrographs was obtained using Image J software and the data analyzed using SPSS version 23. Qualitative results showed the presence of plexiform bone pattern in cow which was completely absent in human. The mean HCA and HCD between human and cows showed no statistically significant difference. Further analysis showed the presence of fewer osteons in the cow bone. The osteon count was higher in human and the variation was statistically significant, ($p<0.05$).
Conclusion: The findings show that histomorphometry of femur bone can be used to distinguish human and cow remains.
Keywords: Cow; Femur; Forensic anthropology;, Haversian canal; Osteon.

Introduction

Forensic anthropologists are often tasked with the verification of human origin of complete or partial skeletal remains. The degradation of these bone fragments due to post mortem distortion makes identification more difficult and requiring more robust techniques[1]. Specie determination therefore is essential to the study of small bone fragments at crime scenes[2]. Human remains distortion has been recorded by some researchers to be commonly caused by carnivores[3] or rodents,[4] as these target the fragile cancellous portion of long bones. Examination of specie for discrimination has been widely done through qualitative and quantitative histological approaches. It was discovered that plexiform bone structure is common in large and medium sized animals but rare in smaller sized animals and never in humans[5,6]. Also according to Mulhern *et al.*, 2001[7], differences recorded in osteon morphology have been reported as important in specie determination. Thorough description of bone types found in various species in an attempt to distinguish between human and non-human bone fragments had been conducted by various researchers[8-10,7]. These studies showed that osteon banding was useful in identification of human and non-human bone. Upon this basis, Zedda *et al.*, 2008[11] determined that morphometrical and morphological differences exist in the compact bone structure of femur between horses and cows. Also in furtherance of the existing work, Zedda and Babosova, 2021[12], wrote that body mass does not affect the size of bone microstructure as well as the histomorphometric features since this factor could be considered in assuming existing variations in specie, but that the lifestyle and locomotor abilities of these species were highly implicated. This could hinge on the fact that anatomic changes seen in the microstructure of bones are more influenced by mechanical events occurring during the lifetime of the individual[13-16]. Brits *et al.*, 2014[17], described histomorphology of non-human species commonly found in South Africa and determined that similar taxonomic orders can be grouped together as a large degree of overlap and that combinations of bone tissue types was observed. According to Brits *et al.*, 2014[17], the differences in the taxonomic orders can be used to exclude human from unknown bone fragments. The aim of this study

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therefore is to evaluate the histomorphometry of femoral cortex of *Bos taurus* (cow) and human.

Materials and Methods

A convenience sampling technique was adopted to select and harvest healthy femur bones of *Bos taurus* and human. The study used 20 bone fragments from the midshaft of femur bones of Bos taurus and human. Ethical clearance for animal and human study was sought and approved by the Research Ethics Committee of the University of Port Harcourt. The human samples were obtained from the cadaver laboratory of the Department of Anatomy of University of Port Harcourt. Fresh Bos taurus samples were harvested from Okru slaughter after the designated Veterinary doctor ascertained the health status of the animal.

Bone Tissue Processing: After harvesting the femur bones, cross sections from the midshaft were carefully cut out with the help of a hacksaw. These fragments were obtained from the midshaft of the femur bones. The bone fragments were collected and ground sections were prepared using Modified Frost's Manual method[18-20].

Histological Features

Primary Osteons, (Os-P): Characterized by few or 3 rings of concentric lamellae surrounding them, possessing a small central canal with a diameter of about 100microns.

Secondary Osteons, (Os-S): Mature remodeled bone, possessing a Haversian canal with blood vessels, surrounded by lots of concentric lamellae.

Osteon Fragments, (Os-F): Remnants of old secondary osteons with partially visible haversian canals obscured by neighboring osteons. Sometimes described as secondary osteons which have lost their haversian canals.

Haversian Canal Diameter, (HCD): It is the distance measured from one end of the haversian canal to the other, covered within its largest possible circumference.

Haversian Canal Area, (HCA): Measured by marking the boundary of the haversian canal.

Micrographic and Data Analysis: Slides were mounted and viewed with a Leica ICC 50E photomicroscope to demonstrate histological features. Images were captured at x4, x10 and x100 magnifications. The data obtained were analyzed using SPSS version 23.

Results

Table 1. Histological differences between the human and cow femoral cortex

Table 2: Independent t test for differences in the mean histomorphometric parameters in Humans and Cow femur.

Table 1. Histological differences between the human and cow femoral cortex.

Features	Human	*Bos taurus* (COW)
Histoarchitecture	Presence of dense haversian system as shown in fig. 2	Presence of plexiform bone pattern as shown in fig. 1 at the periosteal end
Haversian system	Presence of dense prominent haversian system throughout the femoral cortex as shown in fig.4.	Presence of sparse and irregular haversian systems but denser towards the endosteal end of femoral cortex as shown in fig. 3
Haversian canal	Haversian canals are visibly larger in size as shown in fig. 8	Haversian canals are smaller in femoral cortex of cows as shown in fig 7.
Concentric lamellae	Concentric lamellae are packed together as shown in fig. 8	Sparse concentric lamellae as shown in fig. 7
Volkmann's canal	Less number of Volkmann's canals as shown in fig. 6	More Volkmann's canals when compared to human cortex as shown in fig. 5
Primary Osteons	Primary osteons (PO) are found as shown in fig. 8	Primary osteons appears more as shown in fig. 7
Secondary Osteons	More secondary osteons (SO) in femoral cortex as shown in fig. 4	Few secondary osteons in cow femoral cortex shown in fig. 3
Osteon fragments	More osteon fragments are found in human femoral cortex	Less osteon fragments are found in cow femoral cortex.
Endosteal characteristics	Presence of haversian system as shown in fig. 9	Poor presentation of haversian system as shown in fig. 10
Periosteal characteristics	Presence of haversian system as seen in fig. 9 Secondary osteons (SO) present.	Presence of plexiform bone pattern as seen in fig. 10 No secondary osteons (SO) observed

Table 2. Independent t test for differences in the mean histomorphometric parameters in Humans and Cow femur.

Parameters (μm²)	t score	t critical	P value
HCA	0.30	2.11	0.77
HCD	-0.27	2.11	0.79
PO	2.78	2.11	0.01*
SO	4.40	2.11	0.00*
OF	4.38	2.11	0.00*

*Significant (P≤0.05) HCA-haversian canal area, HCD-haversian canal diameter, PO-primary osteons, SO-secondary osteons, OF-osteon fragments

Table 2 showed that the difference in the mean haversian canal area and diameter of human and cow bones were not significant. The table also showed that the differences in their primary and secondary osteons as well as the osteon fragments were significant.

Discussion

This study compares the histological features of the femoral cortex of human and cow using the histomorphometry parameters. Qualitative analysis show that human femurs have dense and numerous haversian systems as compared to cows that show a plexiform bone pattern (Table 1) (Fig.1 & 2). Although cows have sparse haversian system, they appear to be denser towards the endosteal end of the femoral cortex as against human femurs that show even distribution of numerous haversian systems across the cortical regions (Table 1) (Fig.3 & 4). The histomorphometry of cows shows the plexiform pattern markedly observed towards the periosteal surface whereas the haversian systems were better observed towards the endosteal surface (Fig.2 & 4). Some authors who had conducted similar studies documented very close findings to that obtained in the present study[8, 21–23, 17].

The present study also showed very numerous Volkmann's canals in the cow femur as compared

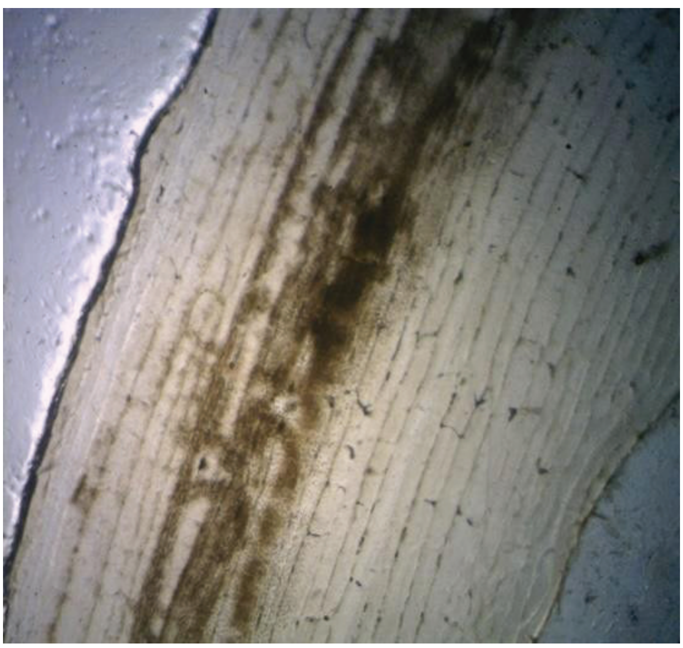

Figure 1. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

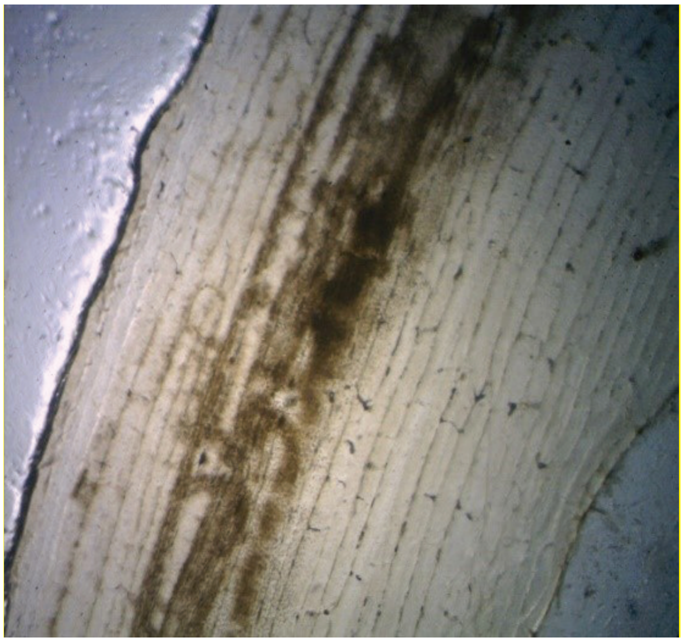

Figure 2. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

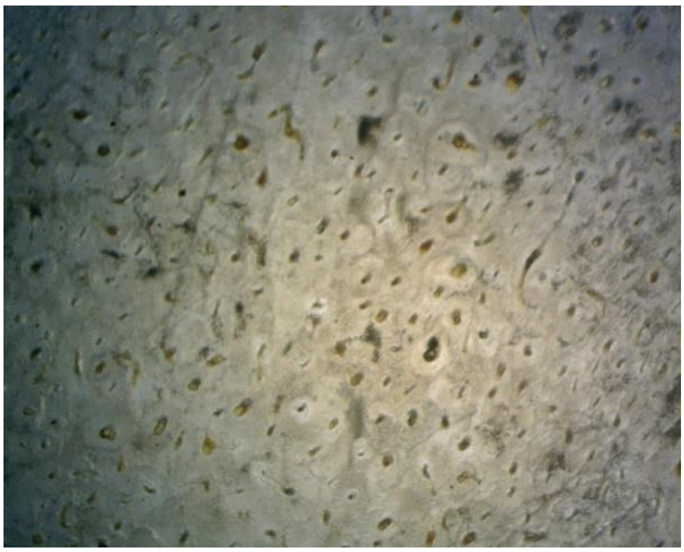

Figure 3. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

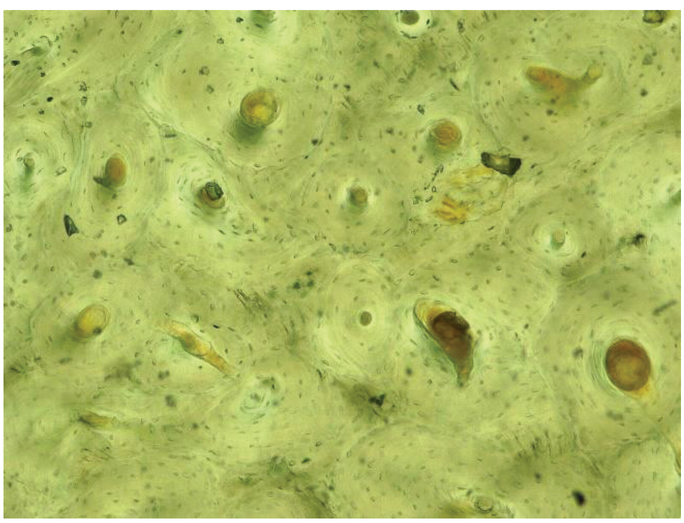

Figure 4. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

to human femur (Fig.5 & 6). Brits 2014[17] in his study described results on cow bone fragments as basically primary vascular plexiform bone. This is thus in agreement with the present study which obtained a predominantly plexiform bone pattern with cows and as well more numerous Volkmann's canals, hence affirming the term 'a vascular plexiform bone'. This is also in consent with Hillier and Bell, 2007[24] whose study suggested that appearance of plexiform bone may mean that the bone fragment identified most likely is animal bone as this characteristic is also found in other animals. According to Enlow 1963[25], this characteristic reflects in the cortical bone of large and fast-growing animals such as cow, pig, goat, sheep and horse. This consistency of plexiform bone type in cows allow it to be differentiated from human bone[26,21]. Whitman 2004[27] in his study noted that the appearance of plexiform bone was linked to sub-adult cows and therefore, it is possible that plexiform bone arrangement may be absent in adult cows. It is also important to note that in another study by Hillier and Bell, 2007[24], they observed that the plexiform pattern of bone disappears in the post mortem leaving only the haversian pattern to be recognized.

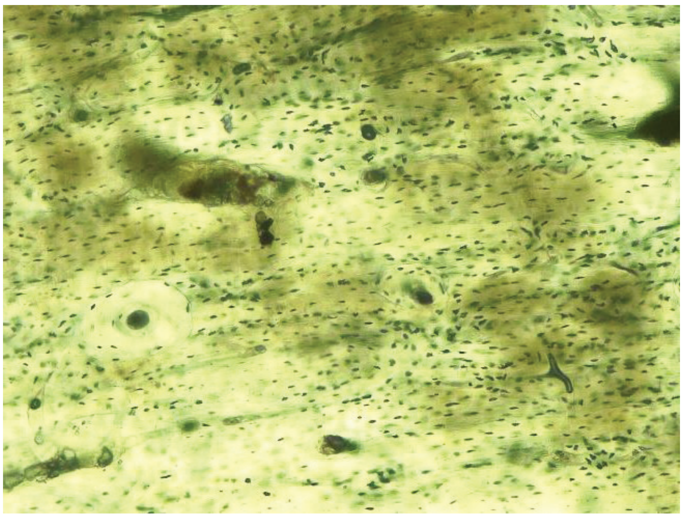

Figure 5. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

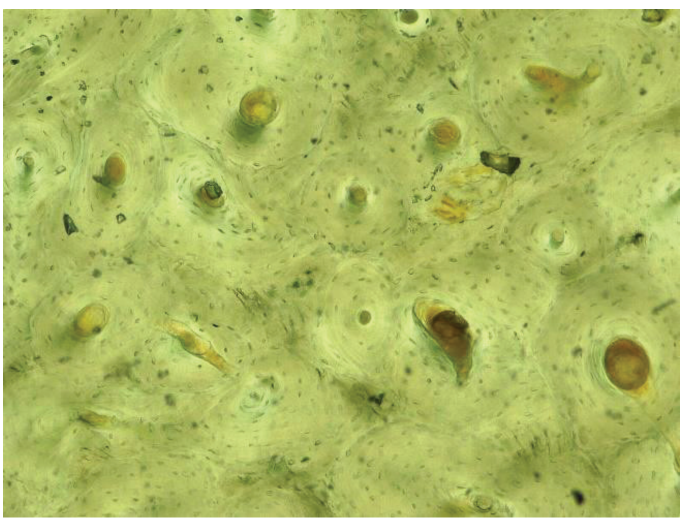

Figure 6. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

The present study noted that plexiform bone pattern was more numerous in the periosteal region (Fig.2), however Lyman 1994[28] and Ericksen 1997[29] stated that exfoliation of bone due to weathering and extreme fragmentation from perimortem trauma or fire may result in the removal of the plexiform bone tissue. This is important and crucial for forensic purposes especially when handling mutilated bodies and distorted skeletal remains. Hence histomorphology could pose some difficulty if to be solely utilized to differentiate between the femoral cortex of cow and human.

It was noted that human femurs have a more regularly-shaped haversian system showing continuous circles of lamellae called concentric lamellae while those of cow were sparse and more irregular (Fig. 7 & 8).

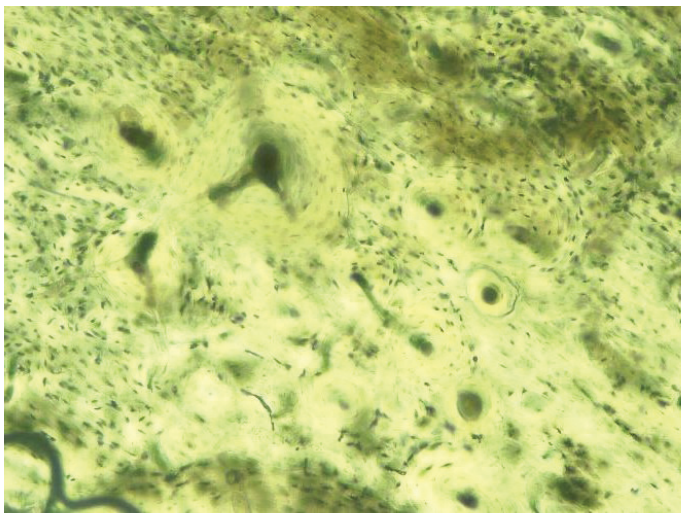

Figure 7. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

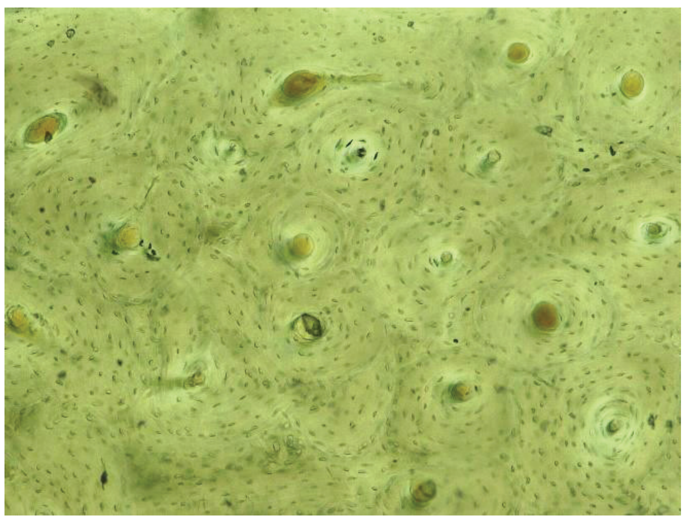

Figure 8. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

In attempt to look at the quantitative differences the study considered some histomorphometric features

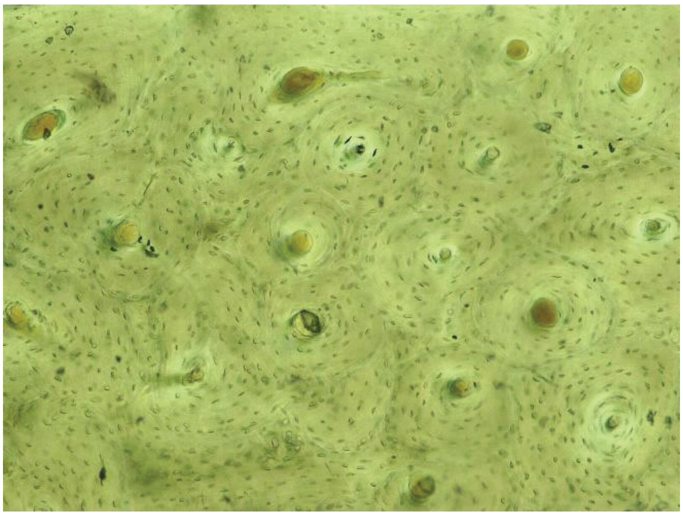

Figure 9. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

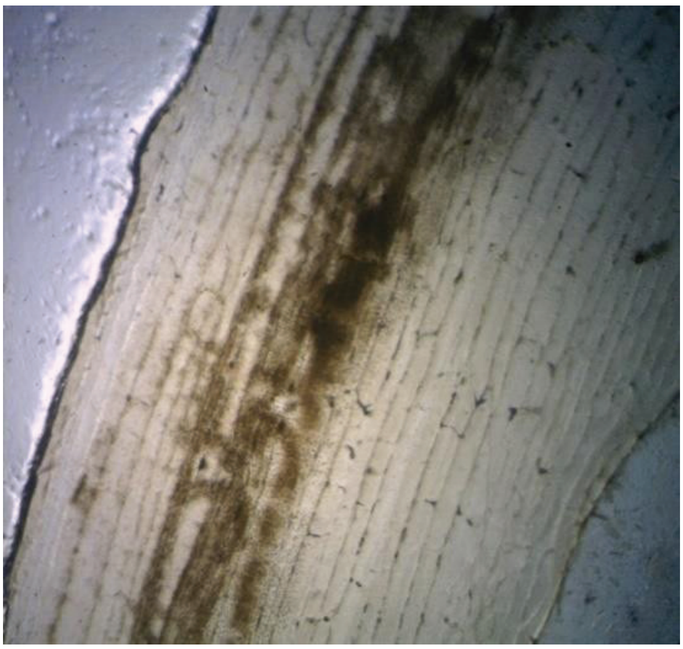

Figure 10. Micrographs of the femoral cortex of humans and cow at the periosteal and endosteal ends. X100

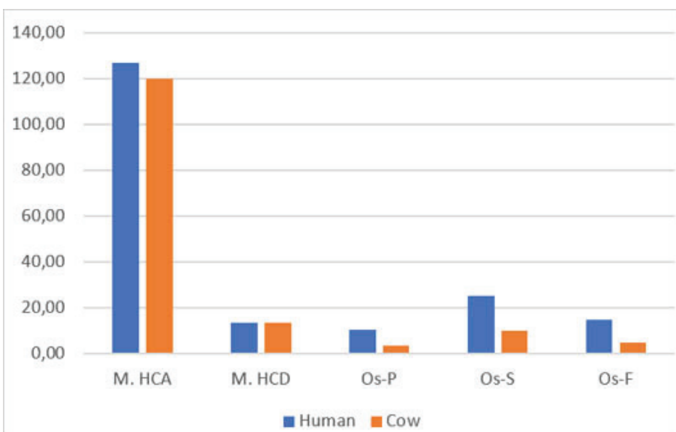

Figure 11. Schematic presentation of the mean histomorphometric parameters of humans and cows.

like the haversian canal area, haversian canal diameter, number of primary and secondary osteons and as well the osteon fragments. There were no statistically significant differences (p> 0.05) in haversian canal area and diameter of the cow and human femoral cortex (Table 2). These parameters therefore may not be accurate if employed in specie variation between humans and cows. These findings are in accordance with those documented by Jowsey 1966[30], Hillier *et al.*, 2007[24] and Labuschagne 2020[31]. The primary and secondary osteons as well as the osteon fragments showed statistically significant difference between humans and cows (p< 0.05) (Table 2). Humans in our study also possessed more number of total osteons when compared to the cows, and this variation was noted to be statistically significant (p≤0.05) (Fig.2). Nor *et al.*, 2014[32] in their study documented that the mean osteon count in humans were higher than animals at 14.57 and 7.45 respectively. Porto *et al.*, 2022[33] also confirmed higher number of secondary osteons and osteon fragments in humans when compared to animals.

On the contrary however, Owsley *et al.*, 1985[34] reported greater number of osteons in animals and as well larger haversian canals. Jowsey 1966[30] reported that the smaller the animal the smaller the osteon size, hence body size affects the presentation of the osteon features. It is evident as seen in the present study that human femoral cortex should have larger osteons compared to cows.

The difference in the osteon count between humans and cows are significant and therefore can be used in distinguishing between these species. The secondary osteons and osteon fragments were seen in our study to have more statistically significant variation between human and cow. This according to some authors may be linked to bone remodelling events[22,35,31]. The difference in the number of secondary osteons can also be attributed to the sparse secondary osteons at the periosteal end of the cow femoral cortex, predominantly filled with plexiform bone (Fig.2).

Conclusion

The findings of the study showed that the presence of plexiform bone pattern and fewer osteons in the cow bone is of forensic value in distinguishing between the femur of humans and cows.

Ethical approval

The authors state that every effort was made to follow all local and international ethical guidelines and laws that pertain to the use of human cadaveric donors in anatomical research[36].

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Mini Curriculum and Author's Contribution

1. Loveday Ese Oghenemavwe 1. MSc, 2. PhD. He constructed the study design, statistical analysis, review and editing of whole manuscript. ORCID: 0000-0002-3575-9987

2. Clinton David Orupabo 1. MB.BCh, 2. MSc, 3. PhD. He did the Interpretation of images, data analysis, microscopic imaging and arrangement, laboratory work, as well as the manuscript construction. ORCID: 0000 0002 4784 4273

3. Onome Esther 1. B.Sc. She did the Literature review, field work, laboratory work, as well as sourcing for materials.

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Corresponding author
Clinton David Orupabo
E-mail: Clinton.orupabo@ust.edu.ng