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Comparative study of the histomorphometry of the femur in humans and *canis lupus famuliraris* (Dog)*:* Implication for forensic investigations

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Abstract
Skeletal remains are often subjected to adverse and unexpected conditions, and more so if comingled remains, which could eventually distort evidence. The destruction of such potential diagnostic features may, thus, obstruct a quick decision as to whether a bone fragment is human or non-human. The aim of our study therefore is to determine if the histomorphometric features of the femur bone can be used to distinguish a human from dog specie. A convenience sampling technique was adopted to select and harvest femur bones from a healthy canis lupus familiaris (dog) and cadaver. 30 fragmented bone samples which has 15 human (Homo sapiens) bone fragments and 15 non human (Canis lupus familiaris) were used. The morphometric analysis of the micrographs was obtained using the Image J software The data obtained from the parameters were then analyzed using an inferential and descriptive approach with SPSS version 23. The mean values of Haversian canal area and diameter were higher in humans when compared to those of Canis lupus familiaris (dog) for the distal, midshaft and proximal parts. The osteon count was higher in the dogs, and the variation was statistically significant (P<0.05). The results show that the histomorphometric features of the femur bone can distinguish between humans and dogs. This is a useful data for forensic scene investigation with comingled and altered skeletal remains.

Key words: *Femur, Humans, Dogs, Histomorphometry, Osteons, Haversian canal*

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I. Introduction

In forensic science, skeletal remains can reside at a site for an unknown amount of time, with the more important question seeking to know whether it is of human origin or not? As such, forensic anthropologists are frequently required to verify the human origin of complete and partial skeletal remains. This can be difficult for bone fragments with few or no morphological hallmarks, or made even more difficult following post-mortem distortion (Ubelaker, 1989).[1] The more fragile cancellous portions of the articular regions of long bones, especially of the humerus, femur and tibia, can often be targeted by carnivores (Haglund et al., 1988) [2] and rodents (Klippel et al., 2007).[3] The destruction of such potential diagnostic features may, thus, obstruct a quick decision as to whether a bone fragment is human or non-human. Currently, there are various methods available for species discrimination on the basis of skeletal structure, such as morphological, histological, molecular biology and protein radioimmunoassay (Chilvarquer *et al*., 1987; Hillier et al., 2007; Imaizumi et al., 2005; Nganvongpanit et al., 2015).[4,5,6 &7] However, even with the advent of molecular approaches that potentially offer positive identification accuracy from fragments, they are not without their own specific limitations, especially in terms of cost, time, and in the field real-time application. To determine the biological profiles from human skeletons, classical osteometric techniques have been successfully used to determine sexual dimorphism from long bones such as humerus, femur, tibia or fibula (De Mendonça, 2000; Duyar et al., 2003; Kranioti et al, 2009; Purkait, 2005; Rios Frutos, 2005; Wright et al., 2003).[8,9,10,11,12&13] In addition, osteometric analysis of long bones has been successfully used to determine the difference between dog breeds (Alpak *et al*., 2004),[14] red fox and arctic fox (Monchot et al., 2010),[15] sheep and goat (Salami *et al*., 2010).[16] However the identification of human and non-human mammalian bones by osteometric study is quite limited with population specific information.

Discrimination analysis of long bone morphometrics has been documented for humans and quadrupedal (sheep, dog, and pig) and bipedal (kangaroo and emu) animals common to Australia (Saulsman et al., 2010).[17] The results from this study was used to indicate enough variation between species to correctly assign an unknown bone to a human or non-human, with cross-validated classification accuracy of 95% or better.

Enlow et al., 1958 [18] provided a thorough description on the types of bone found in different species but their text lacks quantitative data and direct comparisons. Harsa´nyi, 1998 [19] however, provided quantitative data for one histological structure (Haversian canal diameter) but with limited qualitative description. Some have been concerned with distinguishing human from non human bone using a small and select group of mammalian species, most often dog and farm animals (Benedix, 2004; Lackey, 2001, Walter, 2004).[20,21&22] For example, Mulhern et al., 2001 [23] compared femoral midshaft sections of human sub adults and adults with that of subadult sheep and miniature swine in an attempt to distinguish between human and nonhuman bone. Their study revealed that osteon banding was useful in distinguishing between human and non human bone.

Similarly, Whitman in 2004 [24] studied the presence of Haversian systems in the ribs of humans, beef cattle, and dogs and concluded that human Haversian systems and canals were larger in diameter than those of both beef, cattle and dog, but an overlap was present for all three mammals. Rajtova´et al., 1995 [25] compared cortical bone histology of sheep and goats while Hidaka et al., 1998 [26] compared cortical bone histology of raccoon dogs and badgers. More recently, Benedix, 2004 [20] looked specifically to mammalian species in Southeast Asia as bones from such are commonly recovered alongside human remains during Joint Accounting Command missions to locate and identify unrecovered and missing U.S. soldiers.

Research done by Enlow, 1966 [27] studied the differentiation of the human and non human cortical bone at a microscopic level, particularly for forensic situations in which small, non diagnostic bone fragments are recovered. Unfortunately, the major limitation in the examination of these fragments is that the anatomical and individual origin of the bone fragment is unknown and utilizing ‘‘normal’’ descriptions of human and non human bone microstructure to differentiate is problematic and may result in an erroneous differentiation. Factors such as specific bone, bone portion sampled, age, sex, and pathological conditions all affect the ‘‘normal’’ appearance of bone tissue, resulting in significant variation in bone tissue appearance throughout the skeleton, within a specific bone and even a portion of a single bone. Attention has been paid to the difficult task of assessing highly fragmented bone for identification (Singh *et al.*, 1974).[28]

Georgia et al., 1998 [29] carried out a study on the cortical bone of the ribs and long bones of mature dogs and observed that it is predominantly composed of dense Haversian bone. Periosteal and endosteal circumferential lamellae bone is well developed but often interrupted by scattered Haversian systems. Haversian systems are present in various shapes with Haversian canals classified as small. In immature dogs, remnants of osteonal banding and plexiform are present, particularly at the periosteal surface. Significant differences ($p<0.01$) were reported for the amount of Haversian bone in arboreal quadrupeds, terrestrial quadrupeds, suspensory animals (including chimpanzees and spider monkeys), and bipeds (humans). Suspensory animals and bipeds utilize the femur as the dominant limb in locomotion, causing this bone to experience an increased amount of loading as compared with the femur of arboreal and terrestrial quadrupeds, who utilize all four limbs equally. Accordingly, the femora of the suspensory animals and bipeds would contain more Haversian bone, and consequently, Haversian systems, as a response to an increase in remodeling (Schaffler et al., 1984).[30]

According to Enlow, 1958 [18] the long bone and rib cortical bone of skeletally mature hare consists primarily of dense Haversian bone tissue with small Haversian canals area. A wide ring of periosteal circumferential lamellae and a thinner, irregular ring of endosteal lamellae surround the middle component of dense Haversian bone. Remnants of primary longitudinal tissue with scattered primary osteons may be present in younger individuals.

Several other researchers documented dense haversian bone and small haversian area for the long bones of dogs, pigs, goats etc (Whitman, 2004; Lackey, 2001, Benedix, 2004; Rajtova et al., 1995).[24,21,20,&25] Mulhern et al. 2003 [23] study on the histology of juvenile chimpanzee cortical long bone of the lower limb reported increased number of Haversian systems in the femur as compared with the tibia and fibula. Our study therefore aims to compare the histomorphometric differences of femur bone of humans and *Canis lupus famuliraris.*

II. Materials and Methods

A convenience sampling technique was adopted to select and harvest femur bones from a healthy *canis lupus familiaris* (dog) and cadaver. 30 fragmented bone samples which has 15 human (Homo sapiens) bone fragments and 15 non human (*Canis lupus familiaris*). The protocol of this study was approved by Human and Animal Ethics Committee, faculty of Basic Medical Sciences, University of Port Harcourt to use human and animal bones. Human bone samples were obtained from Anatomy department, Faculty of Basic Medical Sciences, University of Port Harcourt. Dried bone samples were harvested from the animal after ascertaining the

health status of the animal by a Veterinary Bio-scientist in University of Port Harcourt. The bones used in this study were strictly femur bones from human and canine specie (dog).

Data collection for this study is the cumulative data collection protocol on records for measurable parameters of the femur bones.

Bone Preparation

The femur bone of an adult human cadaver and *canis lupus familiaris* (dog) was harvested from the dissecting laboratory. The cadaver at the time of harvesting was fixed in formalin and had been dissected to an extent with its limbs still intact. Cadavers with good femur bone architecture and a healthy *canis lupus familiaris* were carefully selected to avoid poor result presentation.

The femur bones were harvested using a scalpel with the cadaver positioned on the dissecting table.

Soft tissue removal: The skin of the cadaver and canis lupus familiaris was removed completely and separated from the body using a scalpel with blade. The muscles attached to the bones and cartilages were cut and separated. Proper care was taken not to damage the cartilages and the joints. Extra precaution was applied in removing the muscles. The bony frame with the remains of muscles was submerged in water and covered in a container for a period of time to allow the remaining flesh to soften for easy removal. After allowing the tissue remains on the bone to soften, the bone sample was transferred to a large tray containing water. The muscles, tendons and ligaments were carefully removed with a pair of forceps and scalpel. A fine brush was used to remove traces of muscles.

Fat curing: Fat and other materials still present in the bones were removed with lime water to preserve the bones. The bones are kept immersed in the fluid for a few hours, thoroughly washed with water and dried in an open Space.

Bone tissue preparation: The modified Frost's method was adopted for tissue preparation.

Histomorphometric Parameters of the Femur

Osteon Count (OC): The counting of osteon was done by taking note of the structure in the haversian system.

Osteon Diameter (OD): The OD was measured by taking the mean of the maximum and minimum OD across the osteon, drawn perpendicular to each other.

Osteon Area (OA) and Perimeter (OP): The OA and OP were measured by marking the boundary within an osteon.

Haversian Canal Diameter (HCD): The diameter of Haversian canal was measured by taking the mean of the maximum and minimum HCD across the Haversian canal, drawn perpendicular to each other.

Haversian Canal Area (HCA) and Perimeter (HCP): HCA and HCP were measured by marking the boundary of the Haversian canal.

Data Analysis

Light microscopy was used to examine the specimens without a staining method. Photomicrographs were taken using the LEICA ICC50 E microscope and at a magnification level of ×100 for clearer visualization. The morphometric analysis of the micrographs was obtained using the Image J software (US National Institute of Health, Bethesda, MD, USA). The data obtained from the parameters were then analyzed using an inferential and descriptive approach with SPSS version 23.

III. Results

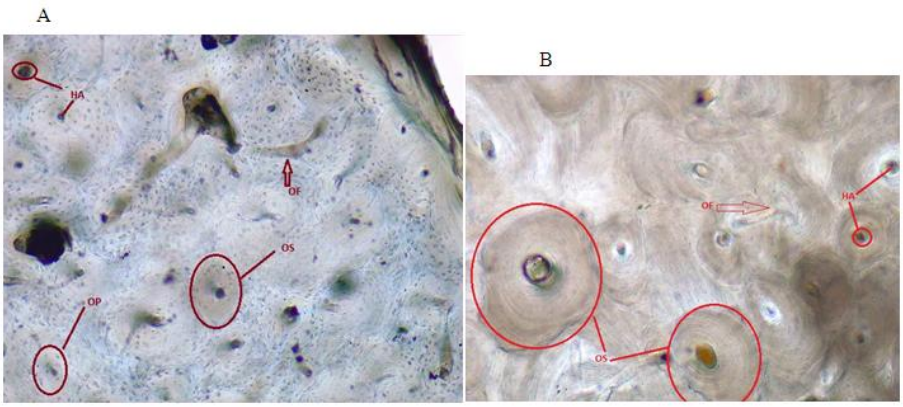

Figure 1: Distal part of the bone tissue (A-Dog, B-Human)

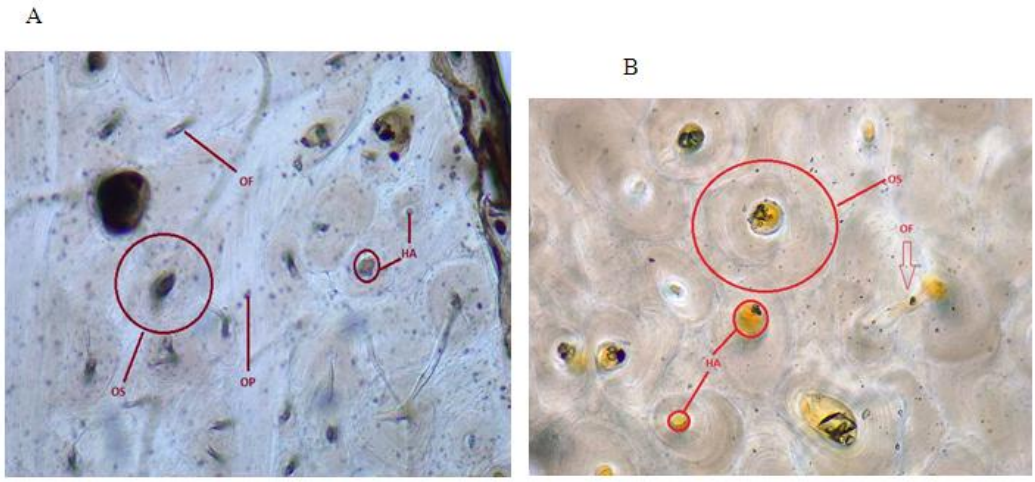

Figure 2: Midshaft of the bone tissue (A-Dog, B-Human)

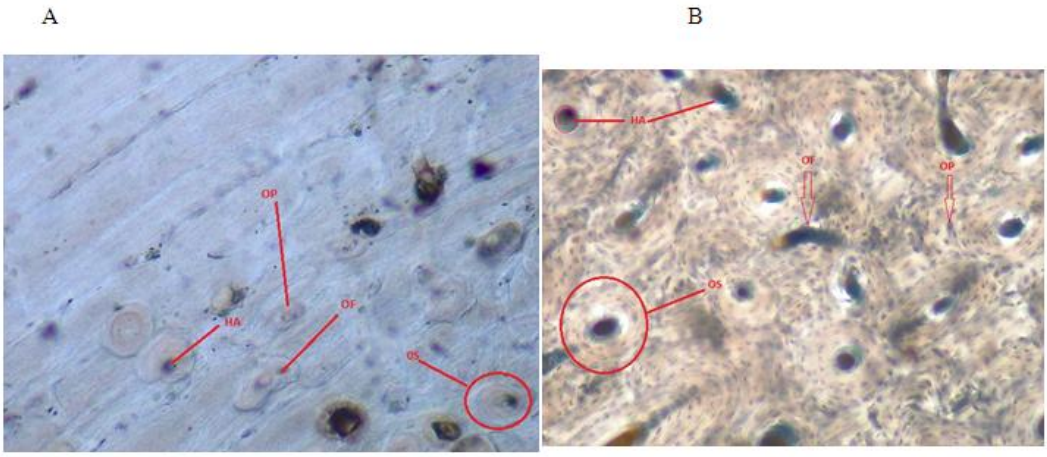

Figure 3: Proximal part of the bone tissue (A-Dog, B-Human)

Table 1. Descriptive statistics for Haversian canal area and Haversian canal diameter

Parameters	Sample size	Mean	SEM	SD	Var	MinV	MaxV
Haversian canal area							
Human (distal)	5	90.76	4.06	63.77	4067.05	7.50	469.33
Dog (distal)	5	52.52	3.57	64.03	4100.38	9.42	405.25
Human (mid shaft)	5	146.30	7.63	115.22	13274.52	24.25	918.83
Dog (mid shaft)	5	52.93	2.70	55.82	3116.29	9.92	664.75
Human (proximal)	5	125.47	5.72	87.32	7624.18	17.00	673.58
Dog (proximal)	5	53.60	2.12	41.86	1752.49	13.00	372.00
Haversian canal diameter							
Human (distal)	5	10.17	0.22	3.48	12.08	3.09	24.44
Dog (distal)	5	7.40	0.19	3.48	12.13	3.46	22.71
Human (mid shaft)	5	12.93	0.29	4.38	19.16	5.56	34.20
Dog (mid shaft)	5	7.61	0.15	3.08	9.49	3.55	29.09
Human (proximal)	5	12.07	0.25	3.77	14.21	4.65	29.28
Dog (proximal)	5	7.83	0.13	2.64	6.99	4.07	21.76

Table 2: Descriptive statistics for number of osteon count, primary osteon, secondary osteon and osteon fragments

Parameter	Sample size	Mean	SEM	SD	Var	MaxV	MinV
Total Osteon Count							
Human (distal)	5	40.60	1.94	4.34	18.80	35.00	45.00
Dog (distal)	5	57.60	2.71	6.07	36.80	50.00	66.00
Human (mid shaft)	5	42.00	2.35	5.24	27.50	36.00	48.00
Dog (mid shaft)	5	75.00	6.22	13.91	193.50	56.00	93.00
Human (proximal)	5	42.80	3.12	6.98	48.70	33.00	52.00
Dog (proximal)	5	67.00	4.04	9.03	81.50	56.00	79.00
Secondary Osteon							
Human (distal)	5	20.40	2.58	5.77	33.30	15.00	30.00
Dog (distal)	5	30.80	2.15	4.82	23.20	23.00	
Human (mid shaft)	5	20.80	1.39	3.11	9.70	17.00	24.00
Dog (mid shaft)	5	33.40	2.50	5.59	31.30	26.00	39.00
Human (proximal)	5	20.00	1.67	3.74	14.00	15.00	23.00
Dog (proximal)	5	27.80	1.98	4.44	19.70	24.00	35.00
Primary Osteon							
Human (distal)	5	4.40	2.79	6.23	38.80	0.00	15.00
Dog (distal)	5	1.00	0.55	1.22	1.50	0.00	3.00
Human (mid shaft)	5	0.80	0.37	0.84	0.70	0.00	2.00
Dog (mid shaft)	5	1.40	0.51	1.14	1.30	0.00	3.00
Human (proximal)	5	0.60	0.24	0.55	0.30	0.00	1.00
Dog (proximal)	5	1.80	0.73	1.64	2.70	0.00	4.00
Osteon Fragment							
Human (distal)	5	15.60	1.75	3.91	15.30	12.00	22.00
Dog (distal)	5	25.20	2.01	4.49	20.20	19.00	29.00
Human (mid shaft)	5	20.40	1.57	3.51	12.30	17.00	24.00
Dog (mid shaft)	5	40.20	3.92	8.76	76.70	29.00	52.00
Human (proximal)	5	22.20	1.85	4.15	17.20	18.00	29.00
Dog (proximal)	5	37.40	3.06	6.84	46.80	31.00	47.00

Table 3: Test for differences in Haversian canal area and Haversian diameter between human and *canis lupus familiaris*

Parameter	**t value**	**P value**	**Inference**
*HCA (human distal) & HCA (dog-**distal)***	7.08	0.00	Significant
HCA** (human mid-shaft) & HCA (dog mid-**shaft)	11.53	0.00	Significant
HCA (human proximal) & HCA (dog proximal)	11.78	0.00	Significant
HCD (human distal) & HCD (dog distal)	9.42	0.00	Significant
HCD (human mid-shaft) & HCD (dog mid-shaft)	16.31	0.00	Significant
HCD (human proximal) & HCD (dog proximal)	15.00	0.00	Significant

T test was conducted to check for differences of the HCA for the distal part, mid shaft and proximal part of the femur and a P value of 0.00 indicates that there is a significant difference in the HCA of man and dog. A significant difference was also noted for the HCD for man and dog.

Table 4: Test for differences in number of osteon count, secondary osteon, primary osteon and osteon fragment between human and *canis lupus familiaris*

Parameter	**t value**	**p value**	**Reference**
Osteon C (human distal)& Osteon C (dog distal)	5.10	0.00	Significant
Osteon S (human distal)& Osteon S (dog distal)	3.09	0.01	Significant
Osteon P (human distal) & Osteon P (dog distal)	1.19	0.22	Not significant
Osteon F (human distal) & Osteon F (dog distal)	3.60	0.00	significant
Osteon C (human mid-shaft)& Osteon C (dog mid-shaft)	4.96	0.00	Significant
Osteon S (human mid-shaft)& Osteon S (dogmid-shaft)	4.40	0.00	Significant
Osteon P (human mid-shaft) & Osteon P (dog mid-shaft)	0.94	0.37	Not significant
Osteon F (human mid-shaft) & Osteon F (dog mid-shaft)	4.69	0.00	Significant
Osteon C (human proximal)& Osteon C (dog proximal)	4.74	0.00	Significant
Osteon S (human proximal)& Osteon S (dog proximal)	3.00	0.01	Significant

Osteon P (human proximal) & Osteon P (dog proximal)	1.55	0.18	Not significant
Osteon F (human proximal) & Osteon F (dog proximal)	4.24	0.00	Significant

Table 4 also shows a statistically significant variation (P<0.05) in the histomorphometric parameters for man and dogs except for the primary osteons at the proximal, midshaft and distal segments.

IV. Discussion

Several studies have evaluated the histomorphometry of bones from various species to observe and compare the differences in the morphology of human and nonhuman bone fragments during forensic investigations. This present study evaluated the qualitative and quantitative features of the femur bone and observed that the mean values of Haversian canal area and diameter were larger in humans when compared to those of Canis lupus familiaris (dog) for the distal, midshaft and proximal parts (Table 1). This is in consent with the study by Whitman, 2004 [24] who documented that haversian canals are larger in humans. The micrograph also showed a broad view of the haversian systems which are sparser in humans than that of Canis lupus (Figure 1, 2 and 3). These findings correlates with literatures and research work carried out by Jowsey, 1966.[31] Stout et al., 1982 [32] documented that as animals increase in size, their micro structural parameters will increase in size up to the size of a human and become constant after a certain size is reached, but have a rather smaller size in their growing stage. Also Mulhem et al., 2001 [23] reported increased number of haversian systems in the femur of animals compared to the tibia and fibula.

Also the mean values of the Osteon counts or numbers were observed to be higher in dogs when compared to human bone in the distal, midshaft and proximal parts of the femur bone (Table 2). This observation can be used as a marker to differentiate human and dog bone fragments. The appearances of the osteons are smaller in size and scattered in the Haversian system of the dog bone, as contrary to human bone which are larger in size and distinct causing a reduction in the number of its osteon count. This is in consent with the study by Jowsey, 1966 [31] who also documented that the mean osteon diameter was the smallest in rabbit, followed by monkey, human, dog and cow. Enlow, 1958 [18] also reported few primary osteons in non humans and hare. Although our study shows reduced number of primary osteons in all bone segments compared to other osteon types, it was the distal segment that had very low numbers of primary osteons in dogs compared to humans (Table 2).

Our findings however showed a statistically significant difference in the Haversian canal area and diameter between human and dog on the distal, midshaft and proximal parts of the fragmented femur bone at p<0.05 (Table 3). This variation could be due to the thickness of the cortex in human bones, which appears to be thicker and denser in contrast to that of nonhuman bones. The primary osteons on the other hand showed no statistically significant variation between human and dog for the proximal, midshaft and distal parts of the femur bone (P >0.05) (Table 4). Meanwhile all other histomorphometric features (osteon count, secondary osteons, osteon fragments) studied in our work had statistically significant variation (P<0.05) in all the bone segments examined between humans and dogs (Table 4). This shows that histological examination of histomorphometric features can be used to distinguish between humans and non humans, especially that of dogs if what is available at a forensic scene were just bone fragments with difficulty identifying whether they are humans or non humans. Our findings also show no peculiarity to choice of a particular segment, as statistically significant variation was noted across segments. Therefore the availability of what bone segment and to which section of the bone it belongs would not pose a challenge to species identification. More so the haversian canal diameter and haversian canal area have been demonstrated to be useful parameters if to ascertain which specie a bone would belong. This however suggests that bone histomorphometry could be the final step in forensic investigation of bone specie when all others have failed to justify the case under investigation.

Our findings are partly consistent with those documented by several other researchers. (Jowsey, 1966; Osterhoff et al., 2011).[31&33] This study has been able to contribute to the classification of higher microstrutctural parameters for humans and nonhumans. In some of these studies the values of osteon count and haversian canal diameter were found to be higher in humans than dog and deer, which is in agreement with those reported by Osterhoff et al., 2011.[33] Our findings however show that osteon count is higher in the dog bones as compared to human bone while the haversian canal diameter and haversian canal area are larger in humans. The higher number of osteon counts in dog bones could be as a result of frequent bone remodeling and increased remodeling rates following activity level. This fact which explains the reason we could have had higher numbers of osteons in dog femur was documented in the study by Schaffter et al., 1984. [30] They reported in their work that the femur of quadrupeds and arboreal animals have increased number of osteons following increased rates of bone remodeling. It can therefore be affirmed that histomorphometric parameters with the utilization of a good and accurate histological method can be reliably used in the assessment of skeletal remains to establish which bones are human and those of nonhuman.

V. Conclusion

This study has been able to show that there is significant variation in the histomorphometric features of humans and dogs, which is a useful aspect of forensic scene investigations. Our findings show significant variation in the haversian canal diameter, haversian canal area as well as the various distribution of the osteons between human and dog femur bone. The primary osteons although are not reliable indicators as seen in our study. Hence it is fact to affirm that bone histomorphometry can help assessment and identification of comingled bones to distinguish humans from non human bone species.

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