A histomorphological analysis of human and nonhuman femora

[*]Desiré Brits[a,b], Maryna Steyn[a], Ericka N L'Abbé[a]

[a]Forensic Anthropology Research Centre, Department of Anatomy, University of Pretoria, PO Box 2034, Pretoria, Republic of South Africa

[b]School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2000, Republic of South Africa

[*]Corresponding author:

Desiré M Brits

University of the Witwatersrand,

Faculty of Health Sciences, School of Anatomical Sciences

7 York Road, Park Town, 2193

Johannesburg, Republic of South Africa

Tel: +27 11 717 2304 (Office)

Fax: 0867653383

E-mail: desire.brits@wits.ac.za

Abstract

Histology is used to describe post-mortem bone alterations, trauma, pathology, age estimation and to separate human and nonhuman bones. Many scholars are however not familiar with the intricate and variable microstructure of bone and due to the complex nature of some classification systems, bone histomorphology is often incorrectly described or identified. Little information is available on the histomorphology of nonhuman bones found in southern Africa and therefore the aim of this study was to describe the histomorphology of nonhuman species commonly found in southern Africa, namely, impala and monkeys, along with cat, dog, cow, sheep, equid and pig. Human femora were included for comparative purposes. The periosteal surface of femora was described and focused *only* on the arrangements of vascular canals, primary osteons and secondary osteons. The results compared favourably to other studies and also added a histomorphological description of impala femora which consisted of primary vascular longitudinal bone tissue. A large degree of overlap and combinations of bone tissue types was observed as well as evidence that allow animals from similar taxonomic orders to be grouped together. Primary vascular bone was primarily observed in artiodactyls (cow, pig, sheep and impala), while Haversian bone was recognised in carnivores (cat and dog), perissodactyla (horses and donkeys) and primates. These differences can be used to exclude human from unknown bone fragments and also serve to caution investigators when using animal models to infer human bone tissue responses to thermal damage, ballistic trauma, etc., as bone tissue types different to that of human bone may respond differently.

Keywords:
bone, histomorphology, human, nonhuman, femora, species differentiation

Introduction

The histological structure of bone has successfully been utilised in anthropology and archaeology to describe post-mortem alterations to bone [1-4], to analyse trauma and healing [5-8], to estimate age at death [9-11] and to diagnose pathology [12-17]. Additionally, bone histology has also been used to differentiate between human and nonhuman bone fragments [18-24]. This is possible due to a number of species-specific differences related to the organisation of the microstructure of bone [25, 26].

Enlow and Brown [25, 27, 28] have described the histomorphology of bone of a number of extinct and extant species. They concluded that most vertebrates do not exhibit Haversian bone [25, 26]. Haversian bone is, however, often the only bone tissue type described in many textbooks and is therefore habitually the only bone tissue type taught [29]. This creates the illusion that *all bone* conforms to this bone tissue type [26]. Osteons or Haversian systems are described as the basic structural unit of compact bone, however it may be completely absent in some animal species [25, 27, 28]. Primary vascular canals, on the other hand, are found in almost all vertebrate species during some stage of ontogeny or throughout life and yet these canals are scarcely mentioned and/or described in regular bone histology texts [26].

Small mammals and primates (including humans) have Haversian bone, while most medium to large size mammals have plexiform bone [18, 19, 22-24, 30]. Plexiform bone is described as primary bone with a dense network of vascular canals arranged longitudinally, circumferentially and radially and results in a characteristic "brick-wall" appearance [26, 31]. As plexiform bone is rarely observed in humans, the presence has been described as a nonhuman characteristic [18, 21, 23, 32].

Regardless of the voluminous literature available on bone histology, many scholars and investigators are not familiar with the complex and variable microstructure of bone in nonhumans [33]. Therefore the aim of this study was to describe the histomorphology of a number of species commonly found in southern Africa, namely impala, primates, cat, dog, cow, sheep, pig and equids, according to the second category of bone classification defined by Francillon-Vieillot *et al*. [31], i.e. bone vascularization patterns which is solely based on the arrangements of primary vascular canals, primary osteons and/or secondary osteons (Haversian systems) in an attempt to create a simplified methodical system for the qualitative description of bone histomorphology. Human specimens were included for comparative purposes. To our knowledge, this is the first study to specifically assess species in southern Africa, and the results may be of use if situations arise where it is necessary to exclude human bone fragments from nonhuman bone or to determine the possible origin of unidentified bone fragments (e.g. in smuggling of animal products). This study purely concentrated on the differences and similarities of the qualitative appearance of the bone microstructure of various species and no quantitative measures such as Haversian system or canal diameters were included.

Materials and methods

Bone samples were taken from the midshaft of the anterior diaphysis of 19 human adult and three juvenile (*Homo sapiens sapiens*) femora as well as four cat *(Felix catus)*, six dog *(Canis familiaris)*, five cow (*Bos taurus*), five sheep (*Ovis aries*), three impala (*Aepyceros melampus*), five pig (*Sus scrofa domestica*), six equid and three nonhuman primate femora. The equid mammals consisted of horses

(*Equus ferus caballus*) and donkeys (*Equus africanus asinus*) with vervet monkeys (*Chlorocebus pygerythrus*) and baboons (*Papio ursinus*) forming the nonhuman primates. Impalas are African antelope that is commonly found in the woodland parts of southern and eastern Africa. They are characterized by a distinct reddish coat and are described as "intermediate mixed feeders" that can either browse or graze, depending on the availability of food, the season and their location [34].

The human remains were obtained from the Student Bone Collection, Department of Anatomy, University of Pretoria. Adult humans consisted of 10 males and 9 females with ages ranging between 22 and 80 years (42.74 ± 18.06 years). Since no demographic information is available for the juvenile material in the collection, age at death had to be estimated and was done by measuring the diaphyseal lengths of the femur and then plotting them on a growth chart for femora, published by Steyn and Henneberg [35]. This method provides a broad indication of age at death categories and produced juvenile ages ranging between three (3) years and 13 years with a mean age of 6.3 years (SD ±5.8 years). All human remains were randomly selected, however specimens were excluded if either pathology was visible or if the person had died of a known nutritional deficiency, such as malnutrition, pellagra, or kwashiorkor.

Cat, dog and equid bones were collected from the Faculty of Veterinary Sciences, University of Pretoria. Cow, sheep and pig remains were obtained from butchers, while impala remains were acquired from local hunters. The nonhuman primates utilised in this study form part of a small animal bone collection housed in the Department of Anatomy, University of Pretoria. All animal remains were of adult ages, except for the cows (20-22 months), sheep (±8 months) and pigs (10-12 months). Subadult cows, sheep and pigs of comparable ages were utilized by Martiniaková *et al*. [18, 19, 21] and as such allowed for direct comparisons. The subadult ages of these animals have been ascribed to modern butchery practices in which subadult animals are commonly slaughtered [23]. Similar to the human sample, animal remains did not have any macroscopic indication of bone altering diseases.

A total of 59 ground bone sections were prepared according to the stipulations of Maat *et al*. [36]. Bone samples were removed by making two parallel cuts into the medullary cavity of the bone and were manually grounded on waterproof abrasive paper. Three random areas on the periosteal surface of each bone sample were observed and photographed with a Nikon transmission light microscope fitted with a 10x Plan DL Ph1 objective lens and a CFW 10x ocular lens. The periosteal surface of bone was specifically selected because of the presence of primary bone tissue, particularly plexiform bone that has successfully been utilized to differentiate between species [18, 20, 23]. Ground sections instead of embedded and sectioned slices were made as the method described above is a fast and affordable method that produces images of high quality suitable for descriptive and metric purposes.

The periosteal bone tissue microstructure of each species was described according to the definitions and bone tissue classification systems of Enlow and Brown [27] and Francillon-Vieillot *et al*. [31] and focussed *only* on vascular canal, primary osteon and secondary osteon arrangements. Secondary osteon arrangements were described as irregular or dense characterised by a few isolated and scattered or tightly packed osteons, respectively. Secondary osteons were characterised by a vascular canal (Haversian canals) surrounded by a number of lamellae demarcated by a cement line.

Vascular canals were identified as canals lacking concentric lamellae while primary osteons were recognised by vascular canals surrounded by a few concentric lamellae [23, 31]. Vascular canals and primary osteons were described as unidirectional (circumferential, radial, oblique, longitudinal) or multidirectional (laminar, plexiform, radiating, reticular) arrangements. These tissue types and descriptions are outlined in Table 1. In an attempt to simplify the complex and often misinterpreted bone tissue classification systems, observations were described with regard to bone tissue types formed based on the organization of the vascular canals found primarily at the periosteal surface.

Table 1 Bone tissue classification system amended from Enlow and Brown [27] and Francillon-Vieillot *et al.* [31]

Avascular bone tissue

    Bone tissue devoid of any vascular canals

Primary vascular bone tissue

    Characterized by the presence of primary vascular canals/osteons arranged:

        Unidirectional vascular canals arranged:

            Circumferential

                Around the medullary cavity

            Radial

                Radiate from the periosteum or endosteum

            Oblique

                Irregular arrangement

            Longitudinal

                Parallel to medullary cavity and further arranged:

                    Circular rows

                    Radial rows

                    Bundles

        Multidirectional orientated vascular canals arranged:

            Laminar

                Circumferentially and longitudinally orientated canals

            Plexiform

                Circumferentially, longitudinally and radially orientated canals

            Radiating

                Longitudinally and radially orientated canals

            Reticular

                Unorganised display of branching vascular canals

Haversian bone tissue

    Characterized by the presence of Haversian systems arranged as:

   *Irregular* - A few dispersed and isolated osteons within bone tissue

        Dense - Tightly packed osteons with little or no interstitial lamellae


Results

The qualitative characteristics of the periosteal surface of the anterior femoral midshaft of the abovementioned species were described and are summarized in Table 2. For a few of the small animals, such as cats and dogs, qualitative traits pertaining to the mesosteal surface was also included.

As illustrated in Fig. 1 and 2, cow and pig femora consisted of primary vascular plexiform bone which was characterised by sheets of bone separated from one another by circumferentially orientated vascular canals. Additionally a number of short radiating canals as well as longitudinally orientated vascular canals were observed. A small area of avascular bone was noted in a cow femur while a few primary osteons were documented in pig femora.
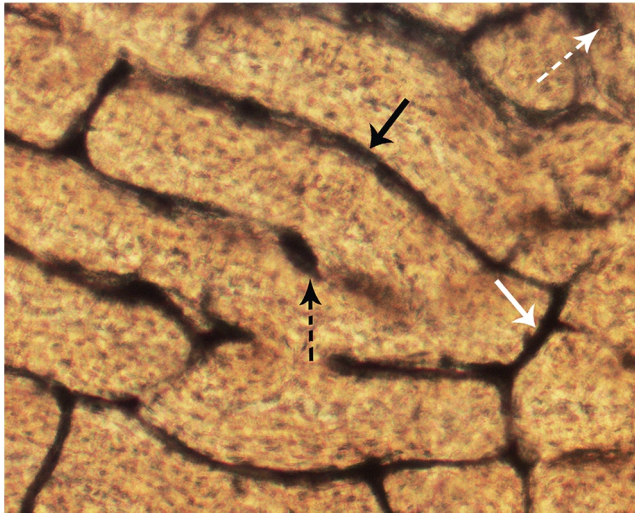
Fig. 1 Primary vascular plexiform bone of a cow (*Bos taurus*) femur characterised by vascular canals orientated circumferentially (black arrow), longitudinally (black dotted arrow) and radially (white arrow) (white dotted arrow points towards the periosteum, 100x magnification)
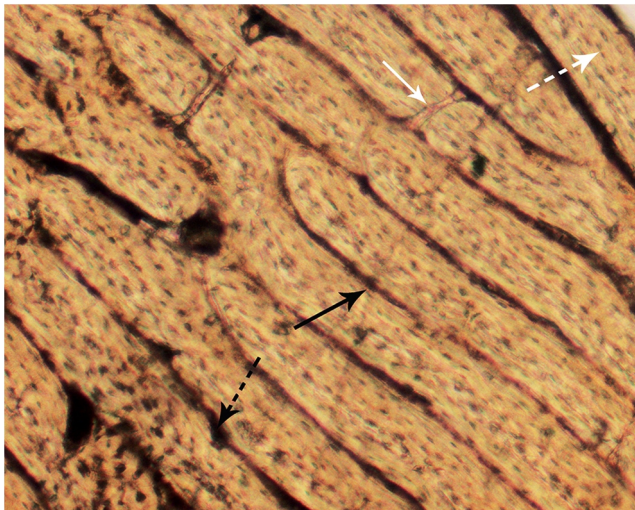

Fig. 2 Primary vascular plexiform bone of a pig (*Sus scrofa domestica*) femur characterised by vascular canals orientated circumferentially (black arrow), longitudinally (black dotted arrow) and radially (white arrow) (white dotted arrow points towards the periosteum, 100x magnification)

Primary vascular longitudinal bone tissue was mainly observed in sheep and impala femora (Fig. 3 and 4) with longitudinally arranged primary osteons. These primary osteons were arranged in

Table 2 Summary of the qualitative characteristics of human and nonhuman bone tissue types as observed at the periosteal surface of femora

Order	Animal	Bone tissue types	Histological characteristics
Artiodactyla	Cows (*Bos taurus*)	Primary vascular plexiform bone	Short radiating vascular canals
		Avascular bone	Longitudinal vascular canals
	Pigs (*Sus scrofa domestica*)	Primary vascular plexiform bone	Numerous short radially orientated vascular canals
			Few primary osteons
			Longitudinal vascular canals
	Sheep (*Ovis aries*)	Primary vascular longitudinal bone	Elongated primary osteons
			Osteon bands consisting of primary osteons
			Numerous short radially orientated vascular canals
	Impala (*Aepyceros melampus*)	Primary vascular longitudinal bone	Rounded primary osteons
			Osteon bands consisting of primary osteons
			Numerous reticularly orientated vascular canals
Carnivora	Cats *(Felix catus)*	Primary vascular longitudinal bone	Small osteons with equally small Haversian canals
		Combination of avascular and irregular Haversian bone	Circular rows or bundles of vascular canals
	Dogs *(Canis familiaris)*	Combination of primary vascular longitudinal and irregular Haversian bone	Circular rows of small vascular canals
			Few reticularly orientated vascular canals
		Avascular bone	Small, oval Haversian system with centrally
		Primary vascular plexiform bone	located Haversian canals
Perissodactyla	Equids	Combination of primary vascular longitudinal and irregular Haversian bone	Osteon bands consisting of primary osteons and secondary osteons
			Long radially orientated vascular canals
			Oval osteons with small Haversian canals

Table 2. (*continue*) Summary of qualitative characteristics of human and nonhuman bone tissue observed at the periosteal surface of femora

Order	Animal	Bone tissue types	Histological characteristics
Primates	Nonhuman primates	Avascular bone or in combination with irregular Haversian bone	Few longitudinally orientated vascular canals
			Osteon bands consisting of secondary osteons

		Haversian systems with large Haversian canals
Humans – adult	Irregular and/or dense Haversian bone	Few resorption spaces
(Homo sapiens sapiens)	Primary vascular longitudinal bone	Few radially orientated vascular canals
	Avascular bone	Scattered longitudinally orientated vascular canals
Humans –juvenile	Irregular Haversian bone	Large resorption spaces
(Homo sapiens sapiens)	Primary vascular longitudinal bone	Few radially orientated vascular canals
		Numerous longitudinally orientated primary osteons
		Osteons of various sizes with large Haversian canals
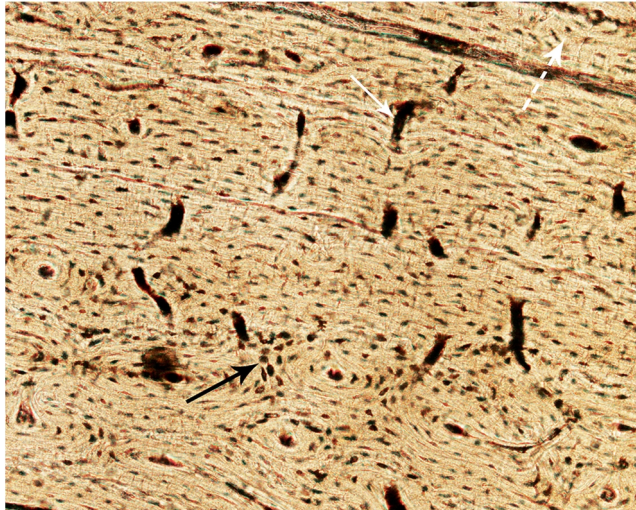
Fig. 3 Primary vascular longitudinal bone of a sheep (*Ovis aries*) femur. Black arrow indicates a few longitudinally orientated primary osteons arranged around the medullary cavity of the bone. White arrow indicates a short radially orientated vascular canal (white dotted arrow points towards the periosteum, 100x magnification)
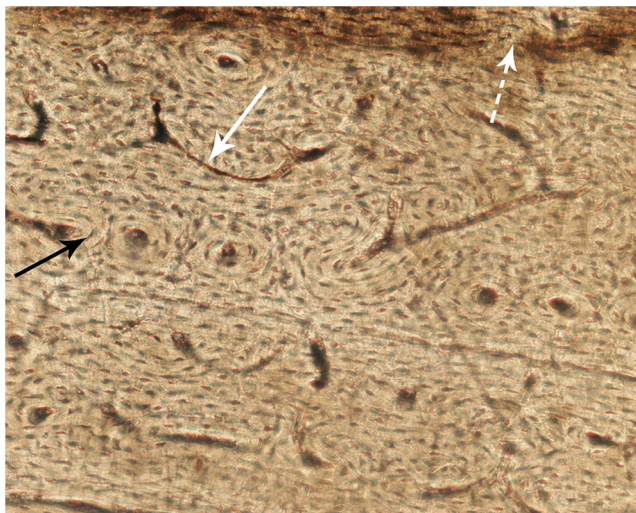

Fig. 4 Primary vascular longitudinal bone of an impala (*Aepyceros melampus*) femur. White arrow indicates a reticular vascular canal. Black arrow illustrates a row of primary osteons also known as an osteon band (white dotted arrow points towards the periosteum, 100x magnification)

circular rows around the medullary cavity of the bone, often forming strings of connected osteons also described by Mulhern and Ubelaker [37] as osteon bands. A number of short radial and/or reticular vascular canals were also recorded.

Cat femora comprised of primary vascular longitudinal bone with vascular canals arranged in either circular rows or bundles. Femora with avascular bone in combination with dense Haversian bone located towards the mesosteal zone were also observed (Fig. 5). These secondary osteons varied in shape and size and contained small Haversian canals.

A combination of primary vascular longitudinal and irregular Haversian bone, with scattered osteons, was observed in dog femora (Fig. 6). The primary vascular bone contained small primary vascular canals arranged in circular rows. A small number of reticular canals were also noted. Areas of avascular and plexiform bone tissue were observed towards the periosteum with dense Haversian bone
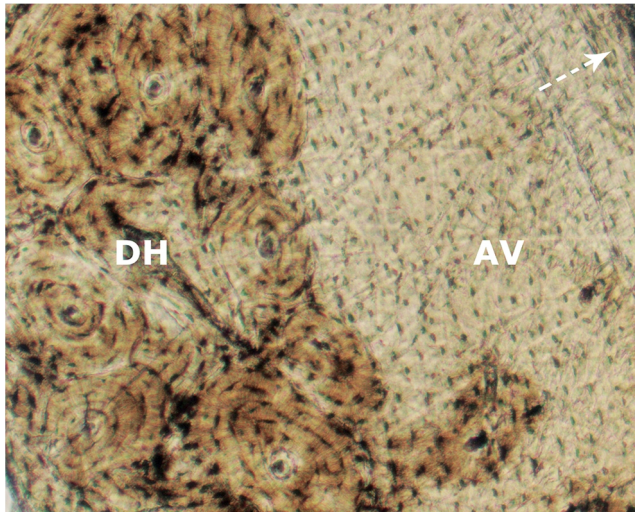
Fig. 5 Avascular bone (AV) in combination with dense Haversian bone (DH) of a cat (*Felix catus*) femur (white dotted arrow points towards the periosteum, 100x magnification)
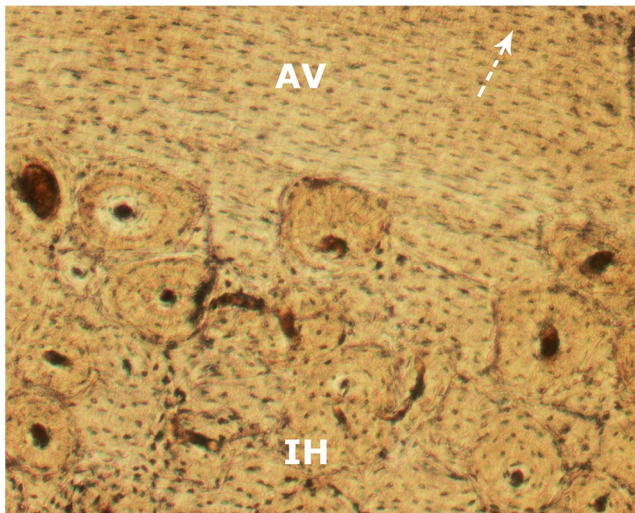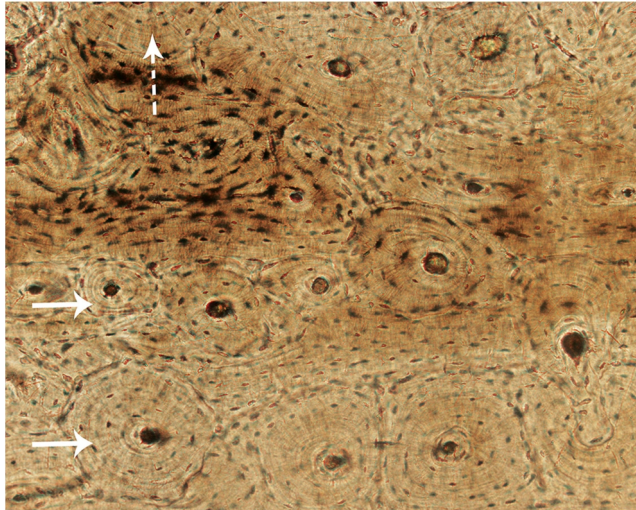

Fig. 6 Avascular bone (AV) in combination with irregular Haversian bone (IH) of a dog (*Canis familiaris*) femur (white dotted arrow points towards the periosteum, 100x magnification)

observed mainly towards the mesosteal zone. Osteons were mostly small and oval with a centrally located Haversian canal.

Equid femora consisted of a combination of primary vascular longitudinal and irregular Haversian bone. The primary vascular longitudinal canals were circularly arranged, often forming osteon bands, similar to that seen in sheep and impala femora. A few long radially orientated vascular canals were also noted and often resulted in primary bone tissue similar to that of plexiform bone. The irregular Haversian bone was characterised by a few secondary osteons that mainly formed part of parallel osteon bands, as illustrated in Fig. 7. These osteons were of varying sizes with small centrally located Haversian canals.

Nonhuman primates exhibited large areas of avascular bone with a few scattered longitudinally arranged vascular canals (Fig. 8). Towards the mesosteal surface irregular Haversian bone was observed with scattered osteons often arranged in bands. The osteons were medium in size with large centrally located Haversian canals. Interestingly nonhuman primate bone tissue was not very
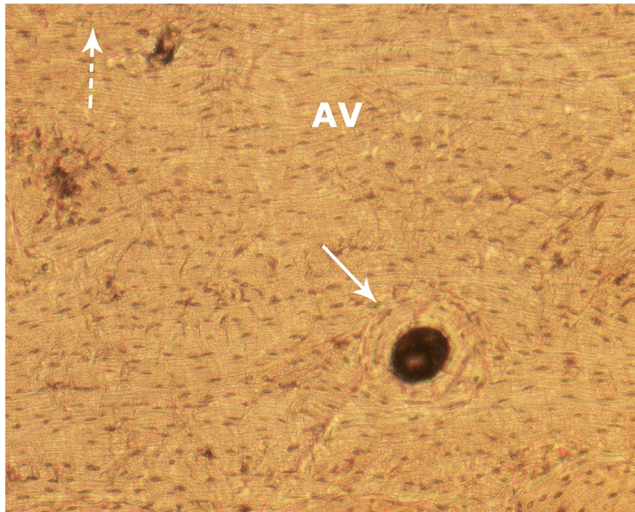
Fig. 7 Irregular Haversian bone of an equid femur illustrating two parallel osteon bands consisting of secondary osteons (white arrows) (white dotted arrow points towards the periosteum, 100x magnification)
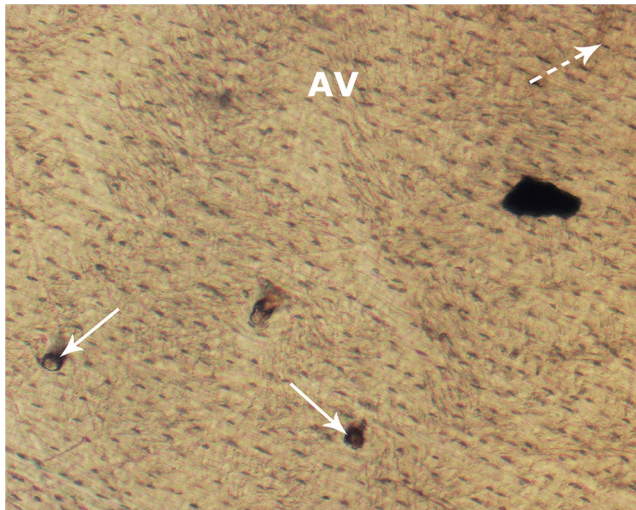

Fig. 8 Avascular bone (AV) with a few isolated, scattered longitudinal vascular canals, indicated by white arrows, of a nonhuman primate femur (white dotted arrow points towards the periosteum, 100x magnification)

similar to that of humans as the general microscopic appearance of adult human femora varied between irregular and dense Haversian bone. Combinations of Haversian bone and primary vascular longitudinal bone was also observed. Osteon arrangements varied from scattered to tightly packed osteons. Small scattered longitudinal vascular canals as well as short radially orientated vascular canals (also known as Volkmann's canals), resorption spaces and areas of avascular bone were also recorded. Fig. 9 illustrates areas of avascular bone with a single isolated osteon as observed in an adult human femur.

Only a few juvenile femora were available for histological analysis, limiting the qualitative data available for description. Irregular Haversian bone tissue was frequently encountered, characterised by a few scattered secondary osteons. The osteons varied in size with large centrally located Haversian canals (Fig. 10). A few radially orientated vascular canals or Volkmann's canals were observed together with large irregularly shaped resorption lacunae. A number of longitudinally arranged primary osteons were also recorded.

Fig. 9 Avascular bone (AV) with an isolated Haversian system (white arrow) of an adult human (*Homo sapiens sapiens*) femur (white dotted arrow points towards the periosteum, 100x magnification)
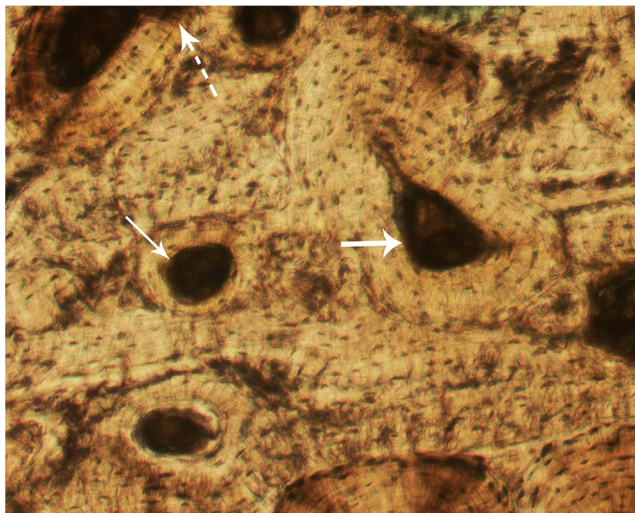
Fig. 10 Irregular Haversian bone of a juvenile (*Homo sapiens sapiens*) femur with a few scattered osteons characterised by large Haversian canals (white dotted arrow points towards the periosteum, 100x magnification)

Discussion

Due to the complex nature of some classification systems some tissue types are often incorrectly described and/or identified [29, 31, 33], while other scholars identify bone tissue types, such as laminar bone as plexiform bone [38]. This potentially hampers the direct comparison of bone histomorphology of diverse nonhuman species and emphasises the need for a simplified classification system. The aim of this study was to describe the bone microstructure of femora of human and various animal species, according to bone vascularization patterns as defined by Francillon-Vieillot *et al.* [31] which relied exclusively on the arrangement of primary vascular canals, primary osteons and/or secondary osteons, creating a simplified classification system. The results of this study parallel those of others and add to the current body of knowledge in describing the histology of impala femora.

Plexiform bone was typical of cow and pig femora as described in Enlow and Brown [25], Martiniaková *et al.* [18, 19, 22], Cuijpers [20] and Crescimanno and Stout [38]. No secondary osteons were observed in the periosteal surface of cow or pig femora similar to reports by Martiniaková *et al.*

9

[18, 19, 22]. Likewise no secondary osteons were observed in sheep femora contrary to reports of scattered secondary osteons in the periosteal surface by Martiniaková *et al.* [22]. In accordance with Mulhern and Ubelaker [37] the absence of secondary osteons could in part be related to the subadult ages of the specimens utilized. Additionally variations to osteon patterns have been linked to different breeds of animals [39], however discussions on differences found amongst diverse animal breeds are very scarce in the literature. Also, unlike reports by Martiniaková *et al.* [18, 21, 22] sheep femora did not comprise of plexiform bone but rather of primary vascular longitudinal bone with a number of primary osteons. This dissimilarity can also be ascribed to the differences associated with various breeds of sheep utilised in the several studies [26, 39]. To the knowledge of the authors this is the first study to describe the histological arrangement of impala femora and a number of histomorphological similarities between impala and sheep femora were reported. Only a few impala specimens were, however, available for examination and future studies would greatly benefit from increased sample sizes, describing various skeletal elements represented by several age groups.

As described by Mulhern and Ubelaker [40], this study also observed primary bone with circularly arranged vascular canals in the periosteal surface and Haversian bone towards the mesosteal zone, in carnivore femora. Additionally areas of plexiform bone were noted in dog femora, consistent with reports by Enlow and Brown [25] and Greenle and Dunnell [32].

As equids form part of the Order Perissodactyla, which represents odd-toed hoofed animals, their microstructure was compared to that of horse bone microstructure and a close resemblance was noted [41]. The general bone tissue type associated with horse long bones, as described in the literature, is primary vascular reticular bone and plexiform bone [20, 23, 25]. Some areas of primary vascular plexiform bone were noted in the current study characterised by radially orientated vascular canals comparable to descriptions by Cuijpers and Lauwerier [42]. Bone tissue varied from primary bone to Haversian bone, with a number of primary vascular canals, and primary and secondary osteons [20, 25, 39]. Some of the secondary osteons were often linearly arranged in bands similar to observations by Cuijpers [20].

Primate femora followed the general descriptions of Haversian bone, with irregular Haversian bone most frequently encountered. The large areas of avascular bone, the number of resorption spaces and the presence of irregular Haversian bone observed in the nonhuman primates could be indicative of young adults [26]. These results are congruent with reports by Mulhern & Ubelaker (2003, 2009) who also noted fewer osteons in the periosteal zone as well as fewer osteons in juvenile chimpanzees compared to an adult chimpanzees (35 years) [30, 43]. Reports of primary vascular canals and primary osteons in nonhumans primates are also consistent with observations made by Burr (1992) and Mulhern & Ubelaker (2003) [30, 44]. Burr (1992) noted primary vascular spaces in macaques femora, while Mulhern & Ubelaker (2003) reported primary vascular canals as well as primary osteons in juvenile chimpanzees [30, 44]. Burr (1992) also reported fewer osteons in macaques femora compared to human femora which is comparable to the few, scattered osteons observed in the irregular Haversian bone of nonhuman primates and the increased number of closely packed osteons observed in the dense Haversian bone of humans [44]. In agreement with the literature, human bone tissue consisted of

primary and secondary osteons [20, 37] with little variation noted between juvenile and adult humans corresponding to reports by Cuijpers [20].

Areas of avascular bone were documented in cow, cat, dog, nonhuman primates and humans and according to Enlow [26] this is seen in most vertebrates and forms as a result of a slow growth process. Future research examining the organization of areas of the bone matrix thereof could potentially assist with specie separation as reports by Cuijpers and Lauwerier [42] showed that a fibrous component was dominant in cattle while a lamellar component dominated in horses.

Osteon bands, which are characterized by a number of primary or secondary osteons arranged in rows or bands [37], were observed in sheep, impala, equids and nonhuman primates. Osteon bands were also documented in sheep femora by Mulhern and Ubelaker [37] and according to these authors the presence of osteon bands could also be considered when excluding human bone fragments from other nonhuman species. According to Enlow (1963), as cited by Mulhern and Ubelaker [37], these bands are frequently observed in subadults, characterised by rapid growth and often disappear during life.

Enlow [26] described typical histomorphology associated with various taxonomic groups. These group characteristics were also evident in the current study (Table 2) with primary vascular bone tissue mainly identified in artiodactyls (cow, pig, sheep and impala) and Haversian bone recognised in carnivores (cat and dog), perissodactyla (horses and donkeys) and primates. Combinations of primary and Haversian bone, such as observed in cat, dog, equids and primates, were however more commonly observed. Similar combinations are also regularly described in the literature and are believed to be related to the gradual replacement of primary bone by Haversian bone, during growth [19, 22, 26]. Bone histomorphology differs among species mainly due to differences related to growth and development rates [19, 22], forms of locomotion [39], adaptive responses, mechanical influences, total body size [26] and differences related to metabolism [32]. Apart from factors associated with these specie differences, a number of features have also been identified that affects the general histological appearance of bone and include factors such as pathology, nutritional deficiencies, postural and weight changes and even variations in feeding habits [26]. The sex, age, skeletal element and area of bone studied can also influence the histological appearance of bone [18, 22, 24, 26, 27, 38]. The effects of these factors on bone histology were not within the scope of the current study and future in depth explorations of such effects will appraise our current knowledge. It is therefore important to emphasize that the current study examined only the anterior midshaft of femora and as significant differences within a single bone and between various bones has been documented [26], researchers describing and comparing unknown fragmentary remains are cautioned.

Apart from combinations of bone tissue types observed, a great deal of overlap with regard to these bone tissue types among species was also reported. This is important to note as it can potentially obstruct species separations and consequently future investigators are encouraged to not only examine the qualitative, but also the quantitative characteristics of bone microstructure. Examining the organisation of the bone matrix has also proved successful in the separation of animal species [42] and future research describing the arrangement of the collagenous fibres of bone is encouraged. Additionally primary bone tissue is predominantly located near the periosteal surface of many

vertebrates and as a number of peri- and post-mortem factors can impede the integrity of this layer [23] descriptions of the mesosteal and endosteal layers of bone are also essential.

Conclusion

The current study provides a baseline description of bone histomorphology of animals specific to southern Africa according to the bone vascularization patterns. Only the anterior midshaft of femora was examined and as such this information cannot be extrapolated to other bones of the skeleton or other parts of the femur. The proposed simplified classification system by no means aspires to replace the more extensive classifications systems, but rather aims to provide scholars with a methodical system to easily identify and describe the microscopic organization of unknown bone fragments.

This study clearly illustrated differences in the histological arrangement of bone that can potentially be used to separate or exclude human bone from nonhuman species such as cow, pig, sheep and impala, however in agreement with Enlow and Brown [25] it is not always possible to confidently identify and/or distinguish between human and nonhuman bone. A large degree of overlap in bone microstructure exists between the various species, however some evidence is available to group some animals from similar orders together.

Future research will greatly benefit from histological analysis of larger animals such as elephant and rhinoceros as it will help scholars dealing with skeletal remains, to better assess and interpret bone microstructure. Additionally, since so many species-specific histomorphological differences are present, future investigators are cautioned when using animal models in research to examine fracture patterns, burning, healing, etc., before inferring any human responses, as bone tissue types different to that of human bone may respond differently.

References

1. Hackett CJ (1981) Microscopical focal destruction (tunnels) in exhumed human bones. Med Sci Law 21:243-267
2. Jans MME, Nielsen-Marsh CM, Smith CI, Collins MJ, Kars H (2004) Characterisation of microbial attack on archaeological bone. J Archaeol Science 31:87-95

3. Hollund HI, Jans MME, Collins MJ, Kars H, Joosten I, Kars SM (2011) What happened here? Bone histology as a tool in decoding the postmortem histories of archaeological bone from castricum, The Netherlands. Int J Osteoarchaeol. doi: 10.1002/oa.1273

4. Bell LS (2012) Histotaphonomy. In: Crowder C, Stout S (eds) Bone histology. An anthropological perspective. CRC Press, Taylor & Francis Group, pp 241-251

5. Boyde A, Hendei P, Hende R, Maconnachie PE, Jones SJ (1990) Human cranial bone structure and the healing of cranial bone grafts: a study using backscattered electron imaging and confocal microscopy. Anat Embryology 181:235-251. doi: 10.1007/BF00174618

6. De Boer HH, Aarents M.J, Maat GJR (2010) Staining ground sections of natural dry bone tissue for microscopy. Int J Osteoarchaeol. doi: 10.1002/oa.1208

7. Bartelink EJ, Wiersema JM, Demaree RS (2001) Quantitative analysis of sharp-force trauma: an application of scanning electron microscopy in forensic anthropology. J Forensic Sci 46:1288-1293

8. Agnew AM, Bolte JH IV (2012) Bone fracture: biomechanics and risk. In: Crowder C, Stout S (eds) Bone histology. An anthropological perspective. CRC Press, Taylor & Francis Group, pp 221-240

9. Kerley ER (1965) The microscopic determination of age in human bone. Am J Phys Anthropol 23:149-163. doi: 10.1002/ajpa.1330230215

10. Keough N, L'Abbé EN, Steyn M (2009) The evaluation of age-related histomorphometric variables in a cadaver sample of lower socioeconomic status: implications for estimating age at death. Forensic Sci Int 191:114.e1–114.e6. doi: 10.1016/j.forsciint.2009.07.012

11. Streeter M (2012) Histological age-ate-death estimation. In: Crowder C, Stout S (eds) Bone histology. An anthropological perspective. CRC Press, Taylor & Francis Group, pp 135-152

12. Ortner DJ (2003) Identification of pathological conditions in human skeletal remains, 2nd edn. Academic Press, Amsterdam

13. Maat GJR (2004) Scurvy in adults and youngsters: the Dutch experience. A review of the history and pathology of a disregarded disease. Int J Osteoarchaeol 14:77-81. doi: 10.1002/oa.708

14. Schultz M (2001) Paleohistopathology of bone: A new approach to the study of ancient diseases. Yrbk Phys Anthropol 44:106-108. doi: 10.1002/ajpa.10024

15. Schultz M (2003) Light microscopic analysis in skeletal paleopathology. In: Ortner D (ed) Identification of pathological conditions in human skeletal remains, 2nd edn. Academic Press, Amsterdam

16. Schultz M, Parzinger H, Posdnjakov DV, Chikisheva TA, Schmidt-Schultz TH (2007) Oldest known case of metastasizing prostate carcinoma diagnosed in the skeleton of a 2,700-year-old Scythian King from Arzhan (Siberia, Russia). Int J Cancer 121:2591–2595. doi: 10.1002/ijc.23073

17. Schultz M (2012) Light microscopic analysis of macerated pathologically changed bone. In: Crowder C, Stout S (eds) Bone histology. An anthropological perspective. CRC Press, Taylor & Francis Group, pp 253-296

18. Martiniaková M, Grosskopf B, Omelka R, Vondráková M, Bauerová M, (2006a) Differences among species in compact bone tissue microstructure of mammalian skeleton: use of a

discriminant function analyses for species identification. J Forensic Sci 51:1235-1239. doi: 10.1111/j.1556-4029.2006.00260.x

19. Martiniaková M, Grosskopf B, Vondráková M, Omelka R, Fabĭs M (2006b) Differences in femoral compact bone tissue microscopic structure between adult cows (*Bos taurus*) and pigs (*Sus scrofa domestics*). Anat Histol Embryol 35:167-170. doi: 10.1111/j.1439-0264.2005.00652.x

20. Cuijpers AGFM (2006) Histological identification of bone fragments in archaeology: telling humans apart from horses and cattle. Int J Osteoarchaeol 16:465-480. doi: 10.1002/oa.848

21. Martiniaková M, Grosskopf B, Omelka R, Vondráková M, Bauerová M (2007a) Histological analysis of ovine compact bone tissue. J Vet Med Sci 69:409-411. doi:10.1292/jvms.69.409

22. Martiniaková M, Grosskopf B, Omelka R, Dammers K, Vondráková M, Bauerová M (2007b) Histological study of compact bone tissue in some mammals: a method for species determination. Int J Osteoarchaeol 17:82-90. doi: 10.1002/oa.856

23. Hillier ML, Bell LS (2007) Differentiating human bone from animal bone: a review of histological methods. J Forensic Sci 52:249-263. doi:10.1111/j.1556-4029.2006.00368.x

24. Cattaneo C, Porta D, Gibelli D and Gamba C (2009) Histological determination of the human origin of bone fragments. J Forensic Sci 54:531-533. Doi: 10.1111/j.1556-4029.2009.01000.x

25. Enlow DH, Brown SO (1958) A comparative histological study of fossil and recent bone tissue, part III. Tex J Sci 10:187-230

26. Enlow DH (1966) An evaluation of the use of bone histology in forensic medicine and anthropology. In: Evans FG (ed) Studies on the anatomy and function of bone and joints. Springer Verlag, Heidelberg, pp 93-112

27. Enlow DH, Brown SO (1956) A comparative histological study of fossil and recent bone tissues, part I. Tex J Sci 7:405-443

28. Enlow DH, Brown SO (1957) A comparative histological study of fossil and recent bone tissues, part II. Tex J Sci 9:186-214

29. Locke M, Dean RL (2003) Vascular spaces in compact bone: A technique to correct a common misinterpretation of structure. Am Biol Teach 65:701-707

30. Mulhern DM, Ubelaker DH (2003) Histological examination of bone development in juvenile chimpanzees. Am J Phys Anthropol 122:127-133. doi: 10.1002/ajpa.10294

31. Francillon-Vieillot H, de Buffrénil V, Castanet J, Géraudie J, Meunier FJ, Sire J, Zylberberg L, de Ricqlès A (1990) Microstructure and mineralization of vertebrate skeletal tissues. In: Carter JG (ed) Skeletal biomineralization: patterns, processes and evolutionary trends. Van Nostrand Reinhold, New York, pp 471-530

32. Greenle DM, Dunnell RC (2010) Identification of fragmentary bone from the Pacific. J Archaeol Sci 37:957-970

33. Locke M (2004) Structure of long bones in mammals. J Morphol 262:546-565. doi: 10.1002/jmor.10282

34. Skinner JD, Chimimba CT (2005) The Mammals of the Southern African, 3rd edn. Cambridge University Press, Cape Town.

35. Steyn M, Henneberg M (1996) Skeletal growth of children from the Iron Age site at K2 (South Africa). Am J Phys Anthropol 100:389-396

36. Maat GJR, Van den Bos RPM, Aarents MJ (2001) Manual preparation of ground sections for the microscopy of natural bone tissue: update and modification of Frost's 'rapid manual method'. Int J Osteoarchaeol 11:366-374. doi: 10.1002/oa.578

37. Mulhern DM, Ubelaker DH (2001) Differences in osteon banding between human and nonhuman bone. J Forensic Sci 46:220-222

38. Crescimanno A, Stout SD (2011) Differentiating fragmented human and nonhuman long bone using osteon circularity. J Forensic Sci. doi: 10.1111/j.1556-4029.2011.01973.x

39. Zedda M, Lepore G, Manca P, Chisu V, Farina V (2008) Comparative bone histology of adult horses (*Equus caballus*) and cows (*Bos taurus*). Anat Histol Embryol 37:442-445. doi: 10.1111/j.1439-0264.2008.00878.x

40. Mulhern DM, Ubelaker DH (2012) Differentiating human from nonhuman bone microstructure. In: Crowder C, Stout S (eds) Bone histology. An anthropological perspective. CRC Press, Taylor & Francis Group, pp 109-134

41. Norman JE, Ashley MV (2000) Phylogenetics of Perissodactyla and Tests of the Molecular Clock. J Mol Evol 50:11–21. doi: 10.1007/s002399910002

42. Cuijpers S, Lauwerier RCGM (2008) Differentiating between bone fragments from horses and cattle: a histological identification method for archaeology. Environ Archaeol 13:165-179. doi: http://dx.doi.org/10.1179/174963108X343281

43. Mulhern DM, Ubelaker DH (2009) Bone microstructure in juvenile chimpanzees. Am J Phys Anthropol 140:368-375. doi: 10.1002/ajpa.20959

44. Burr DB (1992) Estimated intracortical bone turnover in the femur of growing macaques: Implications for their use as models in skeletal pathology. Anat Rec 232:180-189. doi: 10.1002/ar.1092320203