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Computed Tomography in Veterinary Medicine: Currently Published and Tomorrow's Vision

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Abstract

The utilisation of computed tomography (CT) in veterinary practise has been increasing rapidly in line with reduced cost, improved availability and the increase in expertise and technology. This review briefly examines the recent technological advancements in imaging in the veterinary sector, and explores how CT and micro-computed tomography (μ CT) have furthered basic understanding and knowledge, and influenced clinical practise and medicine. The uses of CT technology in veterinary research, especially in relation to bone, vasculature and soft tissues are explored and compared in relation to the different species. CT is essential not only for the diagnosis and treatment of many disorders, but it is now being used to understand areas ranging from drug delivery and surgical advancements through to anatomical and educational uses throughout the world.

Keywords: veterinary, computed tomography, clinical, research, bone, vasculature

1. Overview of computed tomography in veterinary medicine

The introduction of computed tomography (CT) has provided one of the most important advancements in diagnostic imaging in the veterinary sector. In contrast to standard diagnostic radiography, CT produces an axial slice of the area under investigation and a resultant three-dimensional image. CT also allows greater differentiation between individual soft tissue structures than diagnostic radiography. This is due to the ability of CT to accurately measure the tissue absorption of X-ray beams as they pass through the patient [1].

Since the inception of CT, the technology has been developed to yield further improvements. The original first generation of CT scanners consisted of a single detector and an X-ray tube

which produced a single narrow beam. The assembly of X-ray and detector linearly scanned the whole patient in the axial plane. Together, the X-ray beam and detector are rotated by 1° after each single line image. At the end of the process, each individual scan was then compiled to produce an image in a process known as reconstruction [1].

A pitfall of the first generation of CT scanners was the time taken to acquire an image, with a single slice taking up to 6 min [2]. The development of second generation CT scanners aimed to address this with the introduction of an X-ray tube which produced several narrow beams and generated a fan-shaped projection. The fan-shaped beam was directed at multiple detectors and together this system would rotate as a unit around 360° to generate an image. As fewer incremental steps were required whilst scanning the whole patient, this resulted in shorter scan times of up to 20 s for each slice [3, 4]. But even with this marked improvement in time taken to generate each slice, image quality was still affected by artefacts associated with the technology and movement blur [2, 5].

Further advancements of the technology resulted in third and fourth generation scanners which could acquire individual image slices considerable faster at a rate of one image per second from a patient. Third generation scanners consisted of an X-ray beam which spanned the entire width of the patient which was directed at an assembly of detectors. Both the X-ray tube and the assembly of detectors rotate 360° around the patient on a fixed frame (gantry) to produce a movement known as rotation-rotation. Fourth generation CT scanners are composed of an X-ray tube which rotates around the patient and directs its beam at a ring of fixed stationary detectors built into the machine housing [6].

2. Clinical uses of computed tomography in veterinary medicine

The use of CT in veterinary medicine in a clinical setting was first documented in the 1980s for the investigation of disease of the central nervous system and neoplasia in canines [7–10]. CT has become more common in veterinary medicine due to the technological advancements of CT and its increased availability in general practise. Another imaging modality which is becoming increasingly available in the veterinary sector is magnetic resonance imaging (MRI). The use of MRI is most commonly indicated in conditions that require differentiation between soft tissues, such as in the field of neurology, whereas CT is useful for imaging both bones and soft tissues [11].

In small animals, the use of CT is most commonly indicated in patients with thoracic and abdominal disease, intracranial and extracranial lesions, and disorders of the musculoskeletal system including the appendicular skeleton and spine [12–17]. As the generation of images in CT is so rapid, this diagnostic modality is important in cases where anaesthesia and sedation are not an option. CT is therefore useful in emergency critical cases or disorders which may be compromised by anaesthesia or sedation [18, 19].

In equine veterinary medicine, the use of CT is most appropriate in the assessment of structures with mixed tissue thickness and thus differing levels of tissue absorption of X-rays. Therefore,

the structures most commonly assessed are the appendicular skeleton for diagnostic lameness work-ups, the dental arcade, paranasal sinuses and the skull [20–28]. In the clinic, patient positioning provides complications due to the size of the horse, although a hovercraft-design table alongside horse sedation (a technique revolutionised by the late Alastair Nelson) has enabled considerable development of scans involving the head [29].

The application of CT in a clinical setting to produce diagnostic images in cattle is not common. CT is often reserved for valuable cattle, primarily due to its expense but also due to the use of general anaesthetics and off-label drugs [30]. Unlike small animal and equine imaging, it is not often used for the appendicular skeleton or spine. The most common indications for its use are disease of the central nervous system, otitis media and dental disease.

3. The technical use of micro-computed tomography (μ CT) in veterinary medicine and research

Recent technological advances are rendering the use of CT imaging as a diagnostic technique and preoperative tool increasingly common in veterinary medicine. These technological advances have, similarly, opened new possibilities in the field of research, which include the investigation of both hard and soft tissues, at and below the micrometre scale, providing physiological information non-destructively on the sample [31].

X-ray imaging is based around the principle of attenuation—the reduction of signal as the photons interact with electrons in the matter, known as the absorber, through which they are being passed [31]. The linear attenuation coefficient (μ), defined as the proportion of incident photon intensity reduction per unit length of absorber and expressed in cm^{-1} , is dictated by photon energy (E) and atomic number (Z). Photon intensity (I) decreases exponentially as a function of absorber thickness (t) in a homogenous absorber, as shown in the equation below [32, 33].

$$I = I_0 \exp(-\mu t) \quad (1)$$

I_0 is the incident radiation intensity, μ = linear attenuation coefficient, I = photon intensity, t = absorber thickness.

Attenuation principally arises from two processes: Compton scattering and photoelectric absorption [32]. Compton scattering involves the transfer of a proportion of the energy of the incident photon to an electron, resulting in the emission of a lower energy photon [34], and photoelectric absorption is the complete transfer of the incident photon energy [35]. Compton scattering is determined principally by Z , with the effect of E being only minimal, while photoelectric absorption is strongly dependent on both [32]. The attenuation of biological soft tissues, which display relative uniformity in their low- Z constituents [31], and bones is predominantly in the form of photoelectric absorption at low energy ranges ($E = 30$ – 50 keV) and Compton scattering and at higher energy levels ($E = 200$ – 1000 keV). With E ranging from 30 to 130 keV in X-ray radiographic imaging, attenuation in CT imaging usually results from a

combination of photoelectric absorption and Compton scattering [32]. As CT detectors are not energy-discriminative and solely detect I , greater contrast is achieved at lower E levels where photoelectric absorption prevails [31, 32].

The X-rays used in μ CT imaging may come from a laboratory X-ray generator or a synchrotron source. Synchrotron-source X-rays tend to be used monochromatically, with an E selected from a range, while laboratory-generated X-ray beams usually consist of peaks of characteristic X-rays with white (polychromatic) beams from bremsstrahlung radiation (bremsstrahlung radiation). Thus voxel (volumetric pixel) values directly represent μ in synchrotron but not in laboratory CT [31].

4. Clinical and research investigations into bone tissue

CT has been used to investigate a number of bone and growth disorders. The key to CT scanning is that it can be used to visualise not only gross anatomy, such as fractures and general morphology, but can also show micro fractures, bone thickness, trabecular bone distortion and architecture, and bone curvature and angles in situ. When the variety of functions that can be applied to the normal body is considered, the uses for CT and μ CT in diseases, disorders and in other studies are wide ranging. Cortical bone thickness and trabecular bone distortion can be used as indicators of localised mechanical strain [36] and it is likely that it is linked to many bone disorders in addition to fractures and trauma incidents.

Normal growth and development can be observed using CT scanning. As part of a study into human trabecular bone ontogeny, the femur trabecular number, thickness, and bone volume fraction were investigated from the foetus and youths up to 9 years of age [37]. These studies showed an increase in trabecular bone thickness and bone volume fraction, but a decrease in trabecular number at around a year old, coinciding with the onset of unaided walking and, as a consequence, load bearing was concluded to be causal of the changes observed. Similar observations have also been noted in nonhuman animals. An example is shown in the cat, where bone material density was used in the diagnosis of osteopenia and in order to quantify the benefits of the applied treatments [38]. μ CT has shown the effectiveness of titanium lattice implants in relation to bone ingrowth and bone contact in rat, which has implications for not only veterinary but also human medicine [39]. Results from guinea pig have shown how bone research can help identify differences in bone development and structure. Despite adult weight being achieved at around 9–12 months of age, the study showed that bone development continued beyond 12 months [40]. The authors were also able to give detailed anatomical descriptions of the bones, show where weaker areas might occur, (which is useful in understanding fractures) and show that differing bones had different growth rates. Examples of the high quality of images and cortical bone thickness are shown in **Figure 1**. Using CT measurements has been shown to be more accurate than callipers in humans [41] and although the guinea pig study showed no significant differences between the two methods, the largest variation was observed within the smallest bones [40] indicating that for smaller measurements CT may be more applicable but more research needs to be undertaken in this area in differing measurement sizes to understand the limitations of each technique.

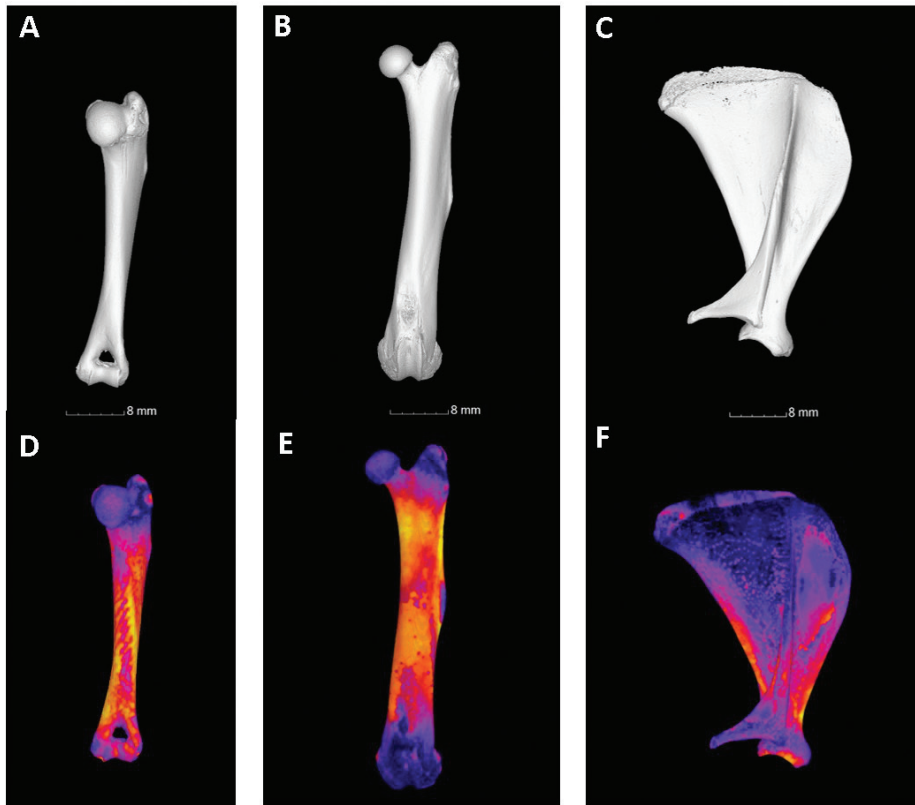


Figure 1. 3D rendered 4-year old guinea pig bones showing surface morphology (A, C, E) and cortical bone thickness (B, D, F) of the humerus (A, D), femur (B, E) and scapula (C, F). Bone thickness was mapped, where increasing brightness indicates thicker bone. Bone] plugin for ImageJ [42] was utilised to calculate bone thickness.

CT for the assessment of equine disorders such as complex foot lameness cases is expanding [20]. Recent studies have shown visible thinning and fractures within bones of chronically laminitic horses, using μ CT and histopathology in parallel [43]. μ CT studies have also given enormous insights into bovine lameness. By combining clinical data with μ CT images and measurements, direct correlations between bone damage, remodelling and growth were made, thus giving new insights into the mechanisms behind bovine lameness [44]. In addition to visualising bone measurements such as thickness, trabeculation and anatomical size, CT is an excellent platform for understanding bone angle and rotation, useful in understand deformities, dysplasia, neoplasia, osteopathies and degenerative diseases in addition to normal anatomy or in trauma situations. A good example of monitoring bone angles is some of the early imaging of the canine and feline temporomandibular joint, as this joint is particularly difficult to visualise using traditional radiographic techniques [45], and its

use during/postsurgery to assess bone angle and healing, particularly in companion animals such as cats and dogs [46, 47].

CT is not restricted to small animal analysis and diagnosis. Although an elephant may be difficult to scan whilst alive, post-mortem tissue gives valuable insights into pathologies. An example was the work carried out into elephant foot pathology and anatomy. In this species, foot problems cause a substantial number of morbidity and mortality issues, and work undertaken to understand these showed a range of complications, from bone remodelling through to osteoarthritis and fractures [48]. Similar work has also been carried out in the rhinoceros [49]. Comparisons between elephants of differing ages, sexes and species (African vs. Asian) were made and, although captive (zoo) animals were used [48], there is potential for assessing and comparing wild animals in the future. Studies such as these can have beneficial outcomes on the way that animals are managed in captivity. Understanding what may influence disease and cause damage can help provide management mechanisms, thus enhancing animal health and welfare.

As a physiologically active tissue, bone's high adaptability to its environment can provide insight into the pathophysiological status of its surroundings [50]. While the osseous remodelling processes may be induced through a number of mechanisms such as trauma, ageing and disease, CT imaging can provide valuable insights into the bone's adaptive capabilities in terms of gross shape, cortical thickness, trabecular anisotropy and position within the body and in relation to other structures.

5. Clinical and research investigations into soft tissues

Visualising soft tissue and achieving contrast between the differing tissues can be a challenge [31]. Due to these difficulties there are numerous uses and techniques being developed in order to investigate soft tissue and liquids using CT. A separate section on vasculature CT is given below (Section 6).

The current method of staging canine appendicular osteosarcoma relies on radiography alongside scintigraphy, however work is being undertaken to try to use CT as an alternative. One such study showed that CT could effectively show malignancies in the thorax and abdomen, and lung lesions but it had a lower detection of appendicular osteosarcoma than the present methods [51]. It was suggested that diagnosis may be reduced due to reader fatigue, as shown in human radiology, but that slice thickness and lesion size may also play important roles. More development is needed in this area before CT can be used as a standalone tool for diagnosis. In other tumour types, CT is more successful. In the case of canine thyroid tumours, CT is recommended for both preoperative diagnosis and for staging [52, 53]. It has also been recommended that any middle aged dog that has a body CT should be checked for incidental thyroid nodules as, although rare, they are identifiable [54]. CT is already regularly used for staging cancers, and each tumour type must be individually assessed as to which method is most appropriate for this vital process.

Significant improvements in dogs with nasal neoplasia are observed when CT is utilised to stage tumours [55] and whereas the World Health Organisation staging guidance was originally based around radiography, this has since been updated to include CT [56, 57].

CT can reduce the number of surgical procedures undertaken or enable keyhole surgeries. Thoracic duct lymphography has been undertaken under research conditions and in canine patients with chylothorax, using CT and iodine as a contrasting agent. Furthermore, the technique was demonstrated to be beneficial when used post-surgery to check for recurrence [58, 59] as it was described as minimally invasive and easy to perform. A similar technique was used to look at feline lymphography. CT was able to show the small mammary lymphatic vessels and lymph nodes with minimal side effects [60].

CT has shown considerable promise for study of lesions such as cysts, abscesses, hydrocephalus and coenurosis lesions in ruminants, including sheep, cattle and the alpaca [61–64]. Ruminant brain disorders and malformations have been observed [61, 65]. These are increasingly used as the lower cost and reduced anaesthesia required in comparison to MRI is seen as favourable, especially in small ruminants and calves [66]. CT is presently used for assessing muscle mass, and is considered as the 'gold standard' alongside MRI. The technique is able to successfully differentiate between differing soft tissues such as skin and muscle. Muscle mass is critical in a number of situations including injury, chronic wasting, malnourishment, and during hospital and rest phases. In an interesting study, urine was examined rather than soft tissues. CT was used in a non-invasive manner to gauge whether urine concentration could be assessed in canine patients undergoing abdominal imaging [67]. The work even showed that the X-ray attenuation of urine could be measured. This has significant implications not only for measuring urine in differing species, but holds the potential for measuring other types of biological fluids.

Echocardiograms are frequently used for cardiovascular disorders, but CT is increasingly being utilised in research and in the clinical setting. One of the attractions of using CT is that second or third generation dual source scanner can scan animals at high speed and therefore within a heartbeat—if the heart is not beating too quickly [68]. Frequently CT is used to locate physical deformities such as atrial and ventricular septal defects, following device placement and surgery. It can also be used to look at general heart morphology and development in models of disease, and in animals with abnormalities such as endocarditis and regurgitation, to look at narrowing of the blood vessels such as the aorta, and to look for occlusions, seromas and abscesses [69].

6. Clinical and research investigations into vasculature

Vascular disturbances have long been associated to the pathogenesis of differing disorders [70], and digital venography is a commonly employed technique providing vital information for treatment options and for monitoring their progress [71]. CT images provide higher levels of quantitative information than venograms, enabling the visualisation of discrete areas

rather than an overall impression of perfusion rate based on X-ray attenuation of contrast agents in numerous vessels simultaneously. Naturally, in the case of μ CT, the post-mortem nature of the samples renders speculation on vasoactivity impossible, but this technique can still provide insights into vascularisation.

A number of different functions can now be investigated in relation to vasculature using CT. Complex 3D models of whole or partial organism vasculature can show areas of angiogenesis and neovascularisation. This technique can also show network interactions, show where vascular junctions and branching occurs, and indicate lumen diameter within a given area. There is an added complexity with blood vessels in that once blood flow ceases, the vascular morphology is altered. In order to preserve the tissues and permit a good visibility of the vasculature once scanned, tissues can be perfused and fixed while fresh. The aim of fixation is to maintain tissues in a life-like state, and perfusion fixation provides the optimal route for the fixative to reach the tissues upon which it can quickly act [72], while fixing the blood vessels in such a way to prevent their collapse and allow them to fill with air, providing the contrast between vessel lumen and the surrounding tissues. Achieving a balance in contrast between soft tissues, vasculature and hard tissue such as bone is complex. Previous studies have indicated that the perfusion of a high-Z contrast agent resulted in images where vasculature and bone were indistinguishable [73]. In one of our studies, the vasculature of the equine foot was flushed and fixed with paraformaldehyde (PFA). As the PFA was absorbed into the surrounding tissues, the empty vascular lumen filled with air. This air acted as the perfect contrast medium, allowing the vasculature not only to be distinguished from the surrounding soft tissues, but also from the bone (**Figures 2–4**).

One criticism of any vascular fixation method is that it could be argued that manually pressurising the vessels is subjective and could lead to a degree of variability in visible vessel diameter. This method may therefore be useful when comparing similarly fixed tissues, but its variable nature should be kept in mind when direct measurements are being taken. The system is not being visualised *in vivo*, but this does reflect observations made under histopathology conditions for example.

In vivo CT scanning is advancing rapidly and the use of single photon emission computed tomography (SPECT) that utilises a radiolabelled tracer has shown that the technique works well in animals. Following its use in humans, SPECT was used to assess cerebral blood flow in canine hepatic encephalopathy patients [74]. Comparing both healthy and hepatic encephalopathy canine patients, hypoperfusion was observed for the first time in the temporal cortex subcortical region. Not only was this condition comparable to humans, but also showed that the scanning method was well tolerated by the animals and that the technique itself was comparable to human studies [74]. In addition to the use of tracers and air to differentiate vasculature, a number of researchers have used corrosions casting in order to understand vascularisation in a number of disorders and systems, ranging from kidney development to ocular disorders in species from mice through to sheep [75–77].

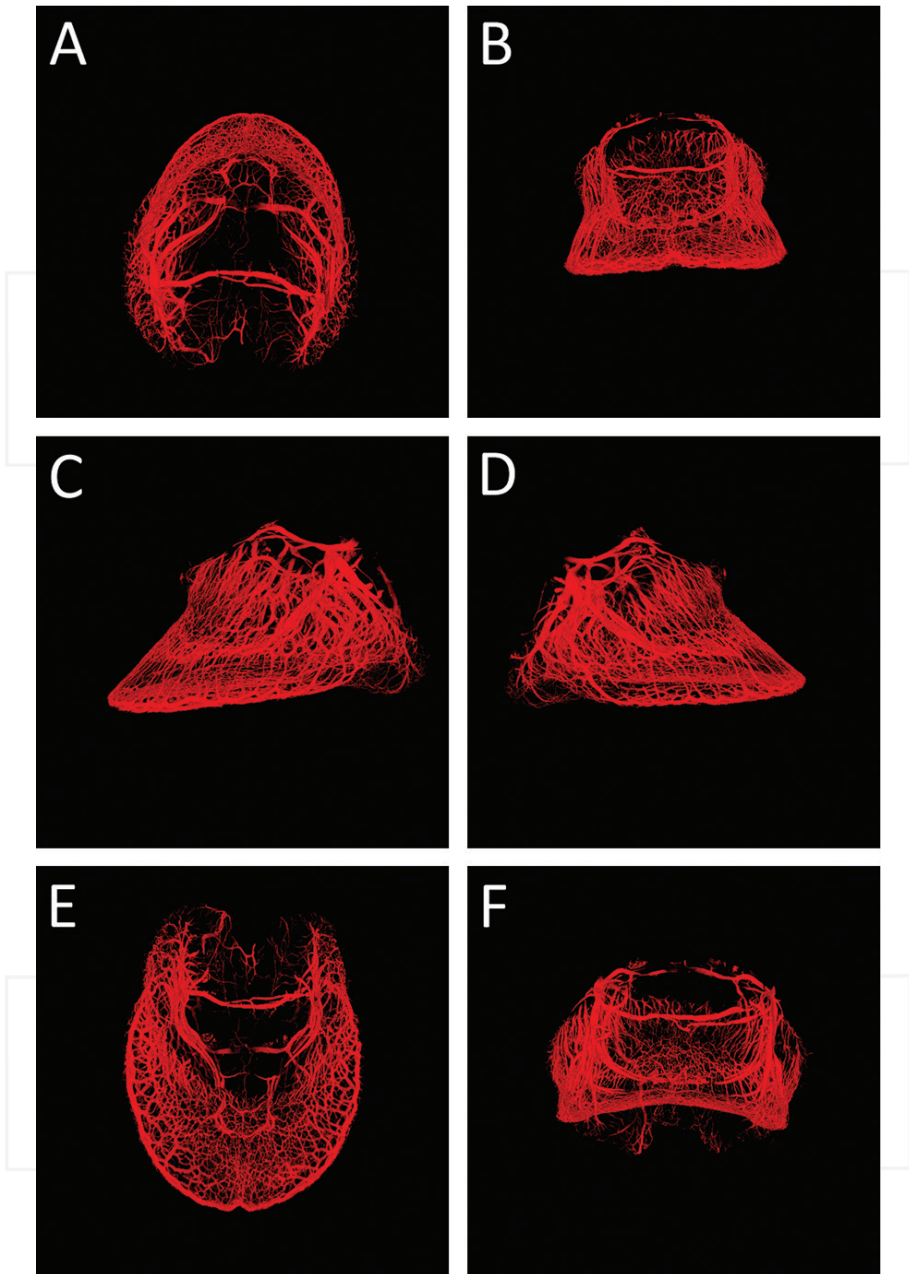


Figure 2. Vasculature of the equine foot. (A) Dorsally, (B) cranially, (C) laterally/medially, (D) laterally/medially, (E) ventrally, and (F) caudally. Scan spatial resolution = 120 μm .

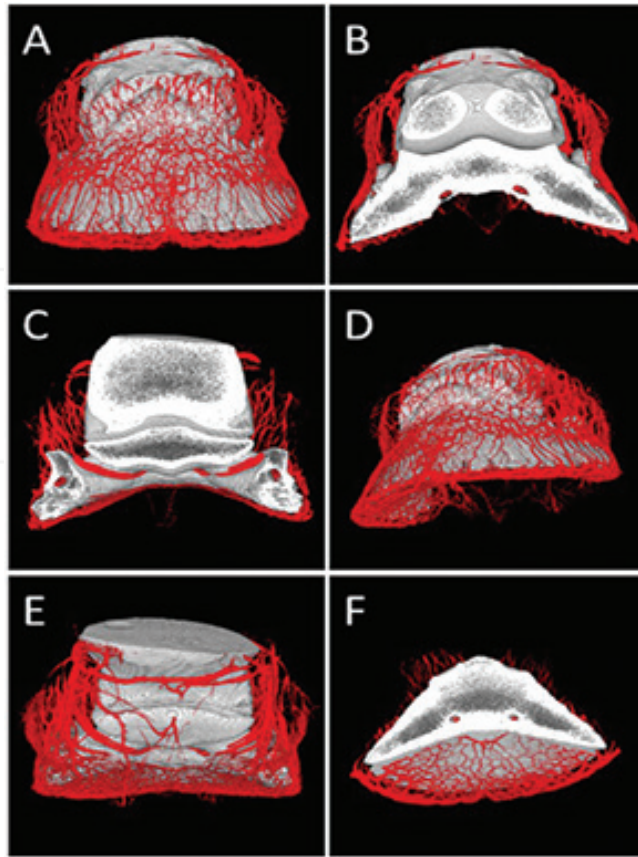


Figure 3. Cranial and caudal views of equine hoof bones and vasculature. (A) Cranially as a whole, (B) cranially as a mid-P3 coronal cut, (C) caudally as a mid-navicular coronal cut, (D) cranolaterally/cranomedially as a whole, (E) caudally as a whole, and (F) ventrocaudally as a coronal cut just cranial of P2 to include only P3. Scan spatial resolution = 120 μm .

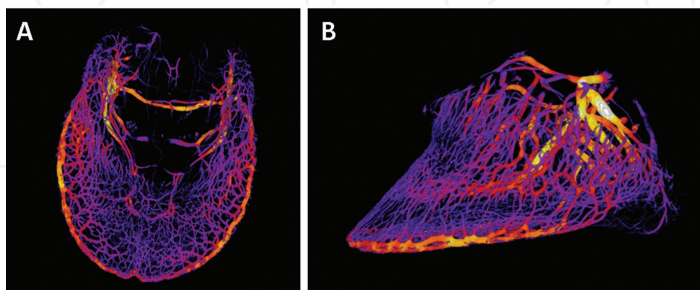


Figure 4. Blood vessel lumen rendered CT images. (A) Entire equine foot from a palmar/plantar perspective, and (B) from a lateral perspective. Lumen size was mapped, where increasing brightness indicates thicker vessels using BoneJ plugin for ImageJ [42]. Scan spatial resolution = 120 μm .

7. The future of CT in veterinary medicine and research

This chapter has explored the development of CT techniques and their uses, and has shown some of the present research in both the clinical and laboratory setting. Many of the examples shown throughout present ideas for uses in veterinary medicine and science, in addition to indications about where further research is required. Further advancements of CT in the clinic have frequently been directed at using the technology available alongside movement-restricting devices to produce images without general anaesthesia. This is important in patients who may be compromised by the use of anaesthetic drugs. The use of movement-restricting devices with or without sedation can be used to produce diagnostic CT images, and can thus be used to decrease the morbidity rates associated with the use of general anaesthetics [78].

Dynamic imaging, using contrast-enhanced CT and MRI, for the exploration of cerebral and tumour microvasculature is an ever-expanding area of interest [79]. As it stands, such dynamic imaging techniques have not been employed in all disorders but would be of benefit, especially in other highly vascularised structures which can undergo extreme pathogenic changes. The utilisation of such techniques could revolutionise our understanding of the complex pathologies of many areas of the body and differing pathological situations.

Exploitation of the unique characteristics of a synchrotron radiation based μ CT facilities could render dynamic experimentation possible, enabling the full elucidation the pathogenic mechanisms involved in differing diseases and disorders in addition to understanding basic anatomical structures. This might involve the visualisation of cellular changes, in addition to tissue alterations. One significant advancement would be to keep tissues metabolically alive and submit them to a variety of physical and chemical stressors, measuring cellular response with the aid of antibody-conjugated high-Z nanoparticles in conjunction with synchrotron-sourced X-ray CT. Synchrotron-based μ CT offers high spatial resolution which, when wishing to view microscopic components of large, intact specimens, could very rapidly become a limiting factor. It is this feature that would render dynamic imaging of constantly evolving structures possible. Studies in live blowfly showed the mechanisms behind their flight motors however it also caused damage to the organisms which died shortly after the experiments [80]. A study designed to show the effects of synchrotron-sourced X-ray CT in ants, grasshoppers, beetles and fruit flies indicated that differing protocols could attenuate cell and system damage, thus making it a more viable imaging source if the protocols were carefully developed [81]. μ CT has been utilised to study insects such as the Painted Lady chrysalis, many of the pupae hatched despite multiple scans, but the samples were also immobile thus making μ CT scans possible [82]. It should also be highlighted that insects generally tolerate radiation much better than mammalian cells [83]. Naturally the expense and space requirement needed for such high calibre machines and experimental set up restricts these possibilities in the normal clinical setting but is increasingly possible under research conditions.

An equally important and expanding use of CT in veterinary medicine and research is the use of images in order to create 3D reconstructions. This may assist the surgeon prior to surgery or during the recovery period. In addition, the images and 3D reconstructions can be an invaluable teaching tool. Whether teaching young children, undergraduates or surgeons,

they are a reusable and valuable addition to the mechanisms available. The uses range from teaching anatomy and physiology using 2D pictures, 3D videos, in virtual museums or even 3D printed examples and providing virtual dissection experiences through to their use as moulds for creating devices and as practise for surgery [84]. The use of CT in forensics and archaeology has also risen in line with the technologies available, although in many cases it would be suggested that this field is still 'emerging'. To date in forensics, this has included identifying tool marks on bones, age determination, assessing gunshot wounds, analysing teeth, understanding the pathology of bones and estimating post-mortem intervals [85, 86]. These and the use of CT in many other situations are essential in the development of the veterinary profession and research. A high profile human example was the use of CT and μ CT in establishing the cause of death, and injuries sustained by King Richard III who died in the Battle of Bosworth, England in 1485 [87]. His skeleton was excavated in 2012 and thereafter researchers sought to identify the body and to understand the skeleton and injuries. The information from the CT analysis, alongside DNA evidence [88] was used to help unravel the story behind the royal skeleton.

The laboratory CT, synchrotron imaging and software developed, is increasingly utilised to investigate areas previously imaged using histological and gross anatomical techniques, such as measuring vasculature and angiogenesis, bone morphology, assessing cell proliferation and identifying soft tissue structure and morphology. A number of studies use a variety of these techniques simultaneously to achieve insights into veterinary medicine and science. Many of the techniques discussed in this chapter have been used in the research and/or university setting. A challenge to the frequency of use and to use in the clinical setting of these techniques is the availability of equipment and expertise in these very challenging methodologies. The research does enable studies to be carried out to show proof of concept and to develop protocols, which can then be used within the clinical setting. With increasing levels of sophistication of both CT scanning units and associated software, this field presents an ever changing and dynamic field. The next generation of imaging techniques includes nano-CT, which can already achieve resolutions of 400 nm [89], and new software and algorithms that are frequently being designed and advance the present uses of available hardware. Nano-CT has been used in a number of animal based studies ranging from morphological features of osteocyte lacunae in murine bones [90] and comprehending cephalopod chamber formation, morphology and evolution [91], through to musculoskeletal and vascular research in the rat [92, 93]. As always, the key to advancing clinical techniques is the sharing of world class research alongside the financial ability to provide a service according to the needs of the patient.

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