Post-mortem feasibility of dual-energy computed tomography in the detection of bone edema-like lesions in the equine foot: a proof of concept

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Abstract

Introduction In this proof-of-concept study, the post-mortem feasibility of dual-energy computed tomography (DECT) in the detection of bone edema-like lesions in the equine foot is described in agreement with the golden standard imaging technique, magnetic resonance imaging (MRI).

Methods Five equine cadaver feet were studied, of which two were pathological and three were within normal limits and served as reference. A low-field MRI of each foot was performed, followed by a DECT acquisition. Multiplanar reformations of DECT Virtual Non-Calcium images were compared to MRI for the detection of bone edema-like lesions. Gross post-mortem was performed and histopathologic samples were obtained of the navicular and/or distal phalanx of the two feet selected based on pathology and one reference foot.

Results On DECT virtual-non-calcium imaging, both feet selected based on pathology showed bone abnormalities as diffuse increased attenuation corresponding with bone edema-like lesions, whereas the three reference feet were considered normal. These findings were in agreement with the findings on MRI. Histopathology of the two pathologic feet showed abnormalities in line with bone edema-like lesions. Histopathology of the reference foot was normal.

Conclusion DECT virtual-non-calcium can be a valuable diagnostic tool in the diagnosis of bone edema-like lesions in the equine foot. Further examination of DECT in equine diagnostic imaging is warranted in a larger cohort, different locations, and alive animals.

1 Introduction

Lameness is common in horses and is mostly diagnosed in the distal forelimbs [1]. When the diagnosis remains inconclusive based on radiography and ultrasonography, cross-sectional imaging methods can be performed, such as computed tomography (CT) and magnetic resonance imaging (MRI) [2-8].

MRI is a validated technique and the most used cross-sectional imaging modality for a wide range of pathologies in the equine foot [7, 9,10]. Hence, MRI provides a detailed image of both osseous and soft tissue structures of the equine lower limb [9, 11-13]. Moreover, MRI can be performed both standing (low-field magnet) as under general anesthesia (both low- and high-field magnets) [14].

The use of computed tomography (CT) imaging is emerging in equine diagnostic imaging, especially since the upcoming ‘standing CT’ technique, since it can be performed under sedation and with a short acquisition time of only several seconds [14-18]. Conventional CT imaging generates greyscale images that are a result of the differing attenuation values within the scanned tissue. However, attenuation coefficients are not unique for any given material; it is a function of the material composition, the incoming photon energy, and the mass density of the material [19, 20]. This limitation can make tissue and lesion characterization challenging, particularly in the evaluation of soft tissues.

The upcoming dual-energy CT (DECT) imaging technique offers an improved tissue characterization by obtaining a second attenuation measurement at a different photon energy. As a result, two attenuation coefficients for each voxel are obtained at two photon energy measurements. The ratio of these two attenuation coefficients allows the unique differentiation of tissues with differing atomic numbers, based on the energy- and element-dependent nature of x-ray attenuation [19, 21]. Hence, DECT allows the decomposition of tissues into its constituent elements, including soft tissues [22, 23]. Soft tissue characterization via DECT imaging is innovative, since the evaluation of soft tissues usually requires MRI. Therefore, DECT has allowed a new approach in human diagnostic imaging, including in the detection of bone marrow edema [24-28].

Bone marrow edema was first described by Wilson et al. (1988) to define the pathological appearance of bone marrow on MRI in human painful joints for which no specific radiographic abnormalities were detected [29]. Bone marrow edema is an umbrella term for various histopathologic findings that cause accumulation of fluid within the bone marrow, including hemorrhage, necrosis, fibrosis, and infrequently “true” edema [30-32]. Therefore, the term ‘bone marrow edema-like lesion’ is often preferred in human diagnostic imaging [31, 33]. In the horse, the navicular bone consists out of compacta and spongiosa without a medulla; therefore, the term ‘bone edema-like lesion’ is chosen in this article. In equine diagnostic imaging, the presence of bone edema-like lesions on MRI was observed in cases for which osseous injury of the distal limb was reported while the radiographs were unremarkable [34, 35]. Moreover, Mizobe et al. (2008) have found that the presence of bone edema-like lesions on MRI have been found clinically significant, since the application of appropriate care based on its presence would contribute to the prevention of further injury [34].

The mechanism behind the detection of bone edema-like lesions differs between MRI and DECT. On MRI, bone edema-like lesions demonstrate typical altered signal as a result of the increased water content, characteristically generating low-T1 and high-T2 signal intensity with a hyperintense signal in fat-suppressed sequences, such as the short-tau inversion recovery (STIR) sequence [28, 36-39]. The STIR sequence provides a homogenous fat suppression, which improves the contrast ratio between high-fluid bone edema-like lesion and the physiological, fat-rich yellow bone marrow. Additionally, water/fat separation can be achieved by the XBONE sequence; this is a gradient echo type sequence that generates two sets of images containing respectively only fat signal and only water signal. Contrarily, DECT allows detection of bone edema-like lesions via a three-material decomposition algorithm [22, 40]. This algorithm subtracts the calcium from cancellous bone, generating virtual non-calcium (VNCa) images to evaluate the fat and water components within the bone marrow.

The added value of DECT in the detection of bone edema-like lesions has already been thoroughly evaluated at different human body sites, including knee [40, 41], wrist and hand [26], ankle [42], hip [43, 44], and spine [45-47]. DECT has been found to be an accurate technique in humans for the detection of bone edema-like lesions, with a sensitivity, specificity, and accuracy of respectively 81-94 %, 91-98 %, and 90-91 % in comparison to the gold standard MRI [25, 28, 46-49]. However, this diagnostic accuracy is considered to be reduced by the presence of bone sclerosis, since it has been reported indistinguishable from bone edema-like lesions in some cases [50].

Despite the great benefits of DECT imaging in human medicine and the increasing availability of compatible refurbished CT scanners in veterinary medicine, the implementation of DECT in equine diagnostic medicine in the detection of bone edema-like lesions and other soft tissue lesions has not been investigated. The objective of this study is twofold; firstly, the hypothesis that DECT VNCa imaging is a feasible diagnostic technique to detect bone edema-like lesions in the equine foot in agreement with MRI. Secondly, this study will contribute to the further optimization of the DECT protocol for the equine distal limb.

2 Materials and methods

2.1 Material collection

In total, five unfrozen feet were collected from five different horse cadavers that were euthanized for reasons unrelated to this study. Two feet were selected based on pathology of the foot; these feet were collected from horses that were euthanized due to injury of the equine foot with poor prognosis. Three other feet were collected randomly as reference feet. Each foot was removed in the fetlock joint. The post-mortem interval between euthanasia and diagnostic imaging was noted for each foot. During this interval, the material was kept refrigerated up to maximum six hours prior to diagnostic imaging. For each foot, description of the cadaver (age, sex, and cause of death/euthanasia) were noted.

2.2 Diagnostic imaging

2.2.1 Computed tomography

The DECT acquisition was performed using a 320-slice single-source CT scanner (Canon Aquilion ONE Vision Edition, Canon Medical Systems, Tochigi, Japan). Each foot was placed parallel to the z-axis in the isocenter of the gantry in lateral recumbency. Firstly, the tube current for the DECT acquisition was determined via automated tube current modulation through the acquisition of an initial helical conventional CT scanogram. The DECT protocol was performed by acquiring two sequential volume scans: a low (80 kV) and a high (135 kV) dataset at both a rotation time of 1.5 seconds. The tube current-rotation time product was noted for each foot. The volume DECT scan acquisition time was a standard set time of 3.6 seconds with a scan length of 16 cm for all feet. In between the datasets, the positioning of the limbs remained unchanged and volume scans were centered on the navicular bone. The CT dose index and dose-length product and were noted for each foot.

2.2.1.1 Image reconstruction

DECT images were created on the workstation via post-processing software that was made available by the vendor. Virtual non-calcium (VNCa) images (two-dimensional multiplanar reformations, slice thickness 0.5 mm) were obtained using a three-material decomposition software to differentiate calcium, fat, and water. The dual-energy gradient for calcium was set at 0.70 and material formulas for fat and water were respectively -136/-106 and 0/0 (80 kV/135 kV). The low- and high-kilovoltage datasets were automatically reconstructed to conventional CT images (bone and soft tissue kernel) with a slice thickness of 0.5 mm and sent to PACS.

2.2.2 Magnetic resonance imaging

The MR acquisition was performed using a low-field MRI (Vet-MR Grande, 0.25-T, Esaote, Italy). Each foot was individually covered with plastic coating and placed in lateral recumbency onto the table in a (human) knee coil, centered on the navicular bone. Five sequences were acquired (table 1): a 3D SST1-weighted sequence (slice thickness: 0.35 mm; TE: 9 ms; TR: 22 ms; flip angle: 30°), a 3D SST2-weighted sequence (slice thickness: 0.43 mm; TE: 10 ms; TR: 20 ms; flip angle: 50°), a transverse fast proton density-T2 sequence (slice thickness: 4 mm; TE: 25 ms; echo train length: 8; TR: 4260 ms; flip angle: 90°; slice thickness: 4 mm), a sagittal short-tau inversion recovery (STIR) sequence (slice thickness: 4 mm; TE: 30 ms; TR: 4340 ms; IT: 70 seconds; flip angle: 90°; slice thickness: 3.5 mm), and a transverse XBONE sequence (TE: 21.2 ms; TR: 1440 ms; flip angle: 60°; slice thickness: 4 mm). For foot III and V, the 3D SST2-sequence was not included in the protocol.

2.2.3 Image reading

The MRI, conventional CT (bone and soft tissue kernels) and DECT VNCa datasets were transferred to PACS and retrieved in OsiriX (v.12.5.2, Geneva, Switzerland) for analysis. All datasets were randomized by a doctoral candidate and descriptively analyzed in consensus by two readers (European diplomates in veterinary diagnostic imaging) who were not present during material collection and data acquisition. Hence, the readers were unaware which feet were collected as reference and pathologic and were blinded from patient description, gross pathology, and histology findings. Firstly, the DECT VNCa images for each foot were evaluated for the presence of bone edema-like lesion. Next, the MR images for the same foot were evaluated. The presence of a bone edema-like lesions on DECT VNCa was based on an increased attenuation; for MRI, its presence was based on a hyperintense signal on short-tau inversion recovery (STIR) sequence with concomitant hypointense signal on T1-weighted sequence [28, 36, 39]. MRI was used as the golden standard for detection of bone edema-like lesions. The bone and soft tissue kernel CT images were evaluated in addition to MRI, to confirm the presence of pathology and sclerosis that might imitate bone edema-like lesions on DECT VNCa imaging.

2.3 Gross post-mortem dissection and histopathology

After image acquisition, the material was kept refrigerated at 2-5 °C up to 24 hours prior to gross post-mortem dissection and histopathological sample collection. For the feet selected based on pathology, bone samples were collected of the area where bone edema-like lesions were observed on MRI. For the third reference foot, bone samples were collected of the distal phalanx and the navicular bone. Bone samples were obtained by slicing the foot into sagittal slices using an automatic slicer. For the distal phalanx, cuboid samples were carefully collected using a manual saw in the dorsal plane of the mid-sagittal slice. For the navicular bone, the entire mid-sagittal slice was collected as a sample. Immediately following collection, the samples were fixated in formaldehyde solution (4 %) at room temperature for one to two weeks. Subsequently, the samples were transferred to an acidic decalcification solution. After a waiting period of 72-96 hours (depending on sample volume), the samples were sliced using tweezers and standard scalpel into block slices for histopathology. Successively, the slices were placed into individual cassettes per sample and immersed in buffer solution (Na_2SO_4 5 %) prior to microtomy. The slices were stained with standard hematoxylin and eosin stain. The histological samples were evaluated by a European diplomate in veterinary pathology, blinded from the MRI and DECT images.

3 Results

3.1 Subject description

Three reference feet (foot I-III) were collected from three different cadavers for which no orthopedic abnormalities were reported. Two other cadavers (foot IV-V) were diagnosed with lameness in the foot. For all cadavers, the left front foot was collected, except for foot V, which was collected from the right hind limb. For foot I, II, IV, and V, the average post-mortem interval to imaging was 33 hours (range 2-72). For foot III, the day of death was unknown.

Foot I was gathered from a 17-year-old mare that was euthanized following the complications of colic and foot II was a 3-year-old gelding that was euthanized following inguinal hernia after recent castration. Foot III was gathered from a mare with no anamnesis or description.

Foot IV was gathered from a 23-year-old gelding that was euthanized because of lameness of one month, because of a penetrating nail injury with an infectious navicular bursitis. This was confirmed on ultrasound, bacteriology synovial fluid of the navicular bursa and radiographic examinations (lateromedial and a dorsal 55° proximal to palmarodistal oblique projection (fig.1). On the radiographs, a chronic penetrating injury of the sole was observed. A metal probe was placed in the fistula in the lateral sulcus of the frog, which advanced in a mildly oblique fashion in dorsodistal to palmaroproximal direction, extending towards the navicular region.

Foot V was gathered from an 8-year-old, Selle-Français gelding. The horse was presented with progressive, chronic lameness. On clinical examination, a diffuse swelling of the dorsal aspect of the coronary band of the right hind foot with a shortened stride in walk and lameness in straight line trot in the same foot. The lower limb flexion test of the right hind foot was strongly positive. Synovial fluid aspiration of right hind distal interphalangeal joint revealed a small amount of amber-colored, viscous fluid. Synovial fluid analysis and bacteriology confirmed a bacterial infection. The horse was euthanized.

3.2 Imaging

A summary of imaging findings on DECT VNCa, MR and CT and histopathology of the three reference feet and two pathologic feet is presented in table 1. An overview of the DECT VNCa, MR (T1 and STIR sequence) and CT images in the mid-sagittal plane of all five feet is shown in figure 2. With a rotation time of 1.5 seconds, the mean tube current-rotation time product was 270 (± 63) mAs (80 kV) and 111 (± 23) mAs (135 kV). With a DECT scan length of 16 cm for all feet, the mean CT dose index was 24.42 (± 5.21) mGy and the mean dose-length product was 390.58 (± 83.37) mGy.cm.

3.2.1 Reference feet (foot I-III)

On DECT VNCa of the reference feet, the normal areas of high attenuation in the distal phalanx, navicular, and middle phalanx are presented in table 1 and showed in figure 2. The reference feet were unremarkable on MR and CT imaging. Areas of high attenuation on DECT VNCa imaging corresponded to zones of high bone density on both MR-T1 sequence and CT (fig. 2).

3.2.2 Foot IV

On DECT VNCa imaging of foot IV (fig. 2), besides the areas of high attenuation as in the reference feet, an uniformly, increased attenuation in the spongiosa of the navicular bone and a mottled area of increased attenuation in the proximal half of the distal phalanx, which decreased gradually towards the distal tip, was present.

The MR examination showed a marked hypointense T1 signal in the distal phalanx involving the palmar surface, extending into both lateral and medial palmar processes, with corresponding hyperintense STIR and XBONE signal. In the navicular bone, a diffuse, hypointense T1 signal with corresponding diffuse hyperintense STIR and XBONE signal was observed; this diffuse signal intensity was more pronounced slightly lateral to the mid-sagittal plane with diffuse disruption to erosion of the palmar compacta, which was observed on both MRI and conventional CT. Focal osseous resorption of the lateral aspect of the flexor surface of the distal phalanx was present, visualized by an irregular hypointense T1 signal, hyperintense STIR and XBONE signal, and diffuse hypoattenuation with irregular outline on CT. Moderate to severe distention of the navicular bursa and the distal interphalangeal joint was present.

The following soft tissue injury/abnormalities were present: dorsal margin lesion with focal thickening of the lateral lobe of the deep digital flexor tendon at the level of the insertion on the distal phalanx; moderate tendinopathy of the lateral lobe of the deep digital flexor tendon proximal to the suprasesamoidean region with marked enlargement and dorsal bulging with a moderate T1 hyperintensity/hypodense lesion on CT ; thickening of the distal sesamoidean impar ligament. Additionally, a linear, small (0.3 cm in length), well-defined mineral body was present on CT that was located just proximal to the proximal ligamentous border of the navicular bone.

3.2.3 Foot V

On DECT VNCa imaging of foot V (fig. 2 and 3), besides the areas of high attenuation as in the reference feet, a spherically-shaped low attenuation was observed in the mid-plantar part of the spongiosa of the navicular bone, surrounded by diffusely increased attenuation in the spongiosa.

The MR and conventional CT examination revealed a rounded cyst-like lesion in the mid-plantar compacta of the navicular bone, extending dorsally into the navicular spongiosa and plantarly towards the navicular bursa. The content of the lesion demonstrated a mixed hypo- and isointense signal on T1 sequence and hyperintense signal on STIR and XBONE sequences surrounded by T1 hypointense and STIR hyperintense rim (fig. 2, 3). On CT, the cyst-like lesion was 5.5 cm in diameter with a diffuse, low-attenuating content surrounded by a smooth, sclerotic rim. The flexor surface of the distal phalanx showed a diffuse irregular outline with a demineralized aspect. Additionally, a small enthesophyte was observed at the distal margin of the navicular bone.

Evaluation of the soft tissues revealed dorsal margin irregularity of the lateral lobe of the deep digital flexor tendon, starting from just proximal to the navicular bone until the level of the proximal interphalangeal joint. The soft tissue lesions were not conclusive on soft tissue kernel CT.

3.3 Gross post-mortem findings and histopathology

For foot II and IV, two histological samples were taken of both the navicular bone and distal phalanx. For foot V, two samples were collected of the navicular bone. No samples were collected of foot I and III.

In foot II, no gross changes were observed. At histological examination, samples of the navicular bone were unremarkable. In the distal tip of the distal phalanx, a well delineated, irregular focal zone of central fibrosis with loss of adipocytes was found, rimmed by sclerotic bone trabeculae.

In foot IV, a diffuse indentation of the articular cartilage and palmar compacta of the navicular bone was present at gross examination with hemorrhagic appearance of the underlying spongiosa (fig. 4 A). For the distal phalanx, a resorption lesion of the flexor surface was observed (fig. 4 B). At histology of the navicular bone, multifocal moderately large areas of extravasated erythrocytes were present in the spongiosa, surrounded with a mild proliferation of fibroblasts and fibrous tissue (fig. 5 A). In other areas, the fibrosis was more pronounced demonstrating densely packed fibrous tissue with osteoclastic resorption of trabecular bone as observed by the presence of multiple Howship's lacunae (fig. 5 B). In the distal phalanx, infrequent lumina of intramedullary capillaries were dilated containing lightly stained eosinophilic material (protein-rich fluid). In addition, multiple foci of expansion of the interstitium of the adipose tissue with similar material (edema) were present (fig. 5 C).

In foot V, an indentation of the plantar compacta of the navicular bone was present at gross examination. Histologically, a cyst-like lesion lined by sclerotic trabeculae was present adjacent to this lesion. The center of the cyst consisted of a dense, uniform fibrous connective tissue, surrounded by adipocytes and sporadic capillaries (fig. 6). In the spongiosa surrounding the cyst-like lesion, multifocal small areas of extravasated erythrocytes with proliferation of fibrous tissue and trabecular osteolysis were observed.

4 Discussion

This initial experience with DECT imaging of the equine foot describes a feasible DECT protocol in the horse, including its appearance in both physiological and pathological cadaver feet. This study shows that DECT VNCa imaging appears to be a feasible technique in the detection of bone edema-like lesions in the equine foot.

Therefore, further examination with blinded approach in a larger cohort study is warranted to determine the accuracy of DECT VNCa in equine foot in agreement to the gold standard, MRI.

In the three reference feet (foot I-III), no bone edema-like lesions were detected on MRI, which was confirmed on histology for foot II. Yet, on DECT VNCa imaging, high attenuation was observed in certain anatomic zones within the feet of all reference feet for which no abnormalities were noted on MRI/CT. This includes the spongiosa and bone marrow directly adjacent to the cortical edge in respectively the navicular bone and the distal, middle, and proximal phalanx, the proximal part of the distal phalanx, and adjacent to the proximal articulate surfaces in the distal phalanx and the middle phalanx (fig. 2). Similar recurring zones of high attenuation have already been described by Pache et al. (2010) and Guggenberger et al. (2012) in humans [40, 42]; an increased ratio of bone cortex to bone marrow leads to higher variance in the calcium subtraction on DECT VNCa reconstructions, caused by beam hardening and filtering effects. In this study, the high attenuation observed in the proximal part of the distal phalanx and articular surfaces are likely to be caused by physiological high bone density, which can be observed by a hypointense signal on MR-T1 sequence [51] and increased attenuation on bone kernel CT in all feet (fig. 2). These DECT VNCa artefacts appear on similar locations in the equine foot with a dual-source CT scanner (Siemens) (unpublished data same research team; see supplementary data figure 1). Moreover, the role of increased cortical bone thickness was apparent in DECT VNCa images of the foot of a draft horse, in which these artefacts were more pronounced (unpublished data; supplementary data figure 2). This breed shows a physiological marked thickening of cortical bone of the distal phalanx. Here, the beam hardening artefact was present in an increased extent. Future studies need to be aware of this artefact and determine the effect of pathologically increased bone density (i.e. sclerosis) on DECT VNCa in the equine foot. The accuracy and inter-reader variability of DECT VNCa imaging should be determined for each anatomical location separately and the diagnostic value of a DECT scan could possibly be breed-dependent. Lastly, future studies cannot directly extrapolate the accuracy of DECT imaging from adult to adolescent animals, since the bone marrow in these patients is still immature and undergoing red-to-yellow bone marrow conversion. Similar to MRI, the immature, red bone marrow can be wrongfully interpreted as bone edema-like lesions, since both have a high-water composition [52]. Furthermore, horses with systemic diseases were not included in this study to avoid the presence of yellow-to-red bone marrow reconversion, which has been described in human cases secondary to an increased physiological demand or an ongoing systemic stress reaction [53, 54].

In two feet (IV-V), bone edema-like lesions were detected on MRI, defined by a typical altered MR signal, including a hyperintense STIR signal and a hypointense T1 signal [37]. In this study, in case of bone edema-like lesions on MRI, VNCa imaging showed a diffuse high attenuation in the affected bone (i.e. navicular bone and/or distal phalanx). The presence of bone edema-like lesions on MRI was confirmed via histopathology and corresponded to the findings described in literature (fig. 5, 6) [30-32, 55, 56]; the bone edema-like lesion area observed on diagnostic imaging consisted of normal bone marrow/spongiosa, in which there were focal zones of hemorrhage, fibrosis, foci of necrosis, osteolytic trabeculae, increase vascularization, and/or 'true' edema (defined in this study as the accumulation of extracellular protein-rich fluid, swollen fat cells and by the incipient disintegration of fat cells). Of all these abnormalities that are categorized under the umbrella term 'bone edema-like lesion', there was only a selected range of the abnormalities detected in every case. Furthermore, for each case a different abnormality dominated the histological sample, being hemorrhage in foot IV and fibrosis and "true" edema in foot V. Consequently, future studies should determine the influence of the lesion characterization on the accuracy of DECT VNCa imaging, since DECT VNCa imaging solely evaluates the fat and water components within the bone marrow [22, 40]. For example, in foot IV, the presence of iron in the hemorrhage zone in the navicular bone could be a determining factor for the increased attenuation on DECT [57]. Histology also confirmed the presence of bone edema-like lesions surrounding the sclerotic rim of the cyst-like lesion in the navicular bone. In comparison to MRI, sclerosis and bone edema-like lesions cannot solely be differentiated on DECT VNCa imaging; therefore, the evaluation of the DECT VNCa images should be performed along with the conventional CT images. Lastly, the etiology of bone edema-like lesions in the horse differs from humans. In the horse, bone edema-like lesions are often associated with osteoarthritis, soft tissue injury, acute trauma, or biomechanical stress [35], whereas in humans it is also often seen in non-traumatic diseases, including rheumatoid arthritis and gout [26, 28, 41].

Regarding the DECT scan protocol, the tube current-rotation time product was calculated via automatic tube current modulation for each foot via an initial helical conventional CT scanogram. In this study, the mean CTDIvol was 24.42 mGy (range 19.5-33.3) and the mean DLP was 390.58 mGy.cm (range 311.20-532.40) for a 16 cm DECT volume scan with rotation time of 1.5 seconds; although radiation dose restrictions are less strict in animals compared to humans, future studied could determine the minimal requirement in rotation time per anatomical location to obtain an acceptable signal-to-noise ratio and minimize radiation dose. Currently, only limited information is in human studies regarding radiation doses associated with multi-energy imaging compared to platforms that use the dual-source technology [58]. Apart from the scan parameter settings, it is crucial to position the patient or material in the isocenter of the gantry to generate the most optimal results, especially when using automated tube current modulation [59]. Therefore, future studies should determine the accuracy of DECT VNCa imaging with the upcoming standing CT technique, since a scanogram and automatic tube current modulation could not be feasible.

Another important consideration when evaluating DECT images, is that the DECT material decomposition only works for the defined substances. Therefore, bone tissue in the DECT image is not classified correctly. Hence, the conventional CT dataset, which is reconstructed from the DECT datasets, should still be used to evaluate the bone tissue. Additionally, future studies are advised to focus the application of DECT on the equine lower limb (i.e. distal to metacarpal and metatarsal region), as the accuracy of material characterization in more proximal image acquisition can be hampered by a photon starvation artifact [46]. Moreover, as previously mentioned while discussing radiation dose, DECT can be performed by different techniques depending on the vendor; readers must be aware of the specific limitations of the technique they are using [60]. Comparable to traditional CT imaging, scan protocol will need small adjustment in between different anatomical locations.

Certain limitations of this proof-of-concept study should be considered. Since this study was performed on post-mortem material, the appearance of bone edema-like lesions on MRI and DECT VNCa could differ in comparison to the ante-mortem equine foot. As a result of tissue autolysis, the intra-osseous STIR hyperintensity may be affected [61, 62]. Therefore, histopathology of areas where bone edema-like lesions was observed on MRI was included to confirm the presence of histopathological changes in line with bone edema-like lesions described by Zanetti et al. (2000), Thiryayi et al. (2008), and Plenk et al. (1997) [30-32]. Future examinations should also include histopathology of all reference subjects, since no consensus has been reached yet on the definition of normal bone marrow in DECT [63]. It is hypothesized that differing anatomical regions or DECT technology affect the optimal cut-off value to differentiate bone edema-like lesions from normal bone marrow on DECT VNCa; therefore, the proposed values range between -80 and 6 HU [64]. Furthermore, the sample size was limited. The two feet that showed bone edema-like lesions on MRI and DECT VNCa are both of an infectious-inflammatory nature. Nevertheless, as mentioned above, bone edema-like lesions in the horse are often of traumatic nature [35]. Hence, further examinations should be made to determine if differing etiopathogeneses affects the accuracy of DECT VNCa in the detection of bone edema-like lesions. Moreover, DECT VNCa could have been preferably compared to high-field MRI as a golden standard for detection of bone edema-like lesions, since for low-field MRI a lower spectral separation between fat and water has been observed, which imposes limits on the ability to perform frequency-selective fat suppression [65].

Overall, DECT VNCa imaging encompasses great advantages, since it combines the advantages of high spatial resolution conventional CT with great visualization of bone structures, complemented with the ability to detect bone edema-like lesions, which was previously limited to MRI. Moreover, a range of additional images can be reconstructed via the post-processing software apart from DECT VNCa imaging, including DECT collagen maps or virtual-non-contrast images. This initial study can contribute to protocol optimization and future clinical use of DECT in veterinary diagnostic imaging.

5 Conclusion

In this proof-of-concept study, DECT VNCa imaging allows the evaluation of bone edema-like lesions in the equine foot. The appearance of the normal equine foot and feet with bone edema-like lesions on DECT VNCa imaging are discussed, including the most important caveats of this initial experience with DECT in the equine foot. Further examination is warranted in a larger cohort, different locations, different diseases, different gradations of lesions, and alive animals, which will simultaneously increase the experience in DECT in the veterinary diagnostic imaging.

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Conflict of interests

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References

1. Schramme MC, Schumacher J, Schumacher J. Clinical Examination, Differential Analgesia and Imaging Modalities for Investigation of Distal Limb Lameness. Proceedings Am Assoc Equine Pract (2007) 53:51-69.
2. Dyson S, Murray R, Schramme M, Branch M. Magnetic resonance imaging of the equine foot: 15 horses. Equine Vet J (2003) 35(1):18-26. doi: 10.2746/042516403775467531
3. Dyson S, Blunden T, Murray R. The collateral ligaments of the distal interphalangeal joint: magnetic resonance imaging and post mortem observations in 25 lame and 12 control horses. Equine Vet (2008) 40:538-544. doi: 10.2746/042516408X313661
4. Vanderperren K, Ghaye B, Snaps F, Saunders J. Evaluation of computed tomographic anatomy of the equine metacarpophalangeal joint. Am J Vet Res (2008) 69(5):631–638. doi:10.2460/ajvr.69.5.631
5. Werpy NM. Recheck magnetic resonance imaging examinations for evaluation of musculoskeletal injury. Vet Clin N Am Equine Pract (2012) 28:659-680. doi: 10.1016/j.cveq.2012.09.003
6. Claerhoudt S, Bergman EH, Saunders JH. Computed tomographic anatomy of the equine foot. Ana Histol Embryol (2014) 43(5):395-402. doi: 10.1111/ahe.12091
7. Barrett MF, Frisbie DD, King MR, Werpy NM, C. E. Kawcak CE. A review of how magnetic resonance imaging can aid in case management of common pathological conditions of the equine foot. Equine vet. Educ.(2017)29(12) 683-693doi: 10.1111/eve.12542
8. Vandekerckhove L, Raes E, Dumoulin M, Martens A, Vanderperren K. The benefits of computed tomography imaging in the diagnosis, prognosis and treatment planning of equine orthopedic patients illustrated by six cases. Vlaams Diergeneeskd Tijdschr, (2021) 90(6), 299–312. doi:10.21825/vdt.v90i6.21088
9. Dyson S, Murray R. Magnetic resonance imaging evaluation of 264 horses with foot pain: the podotrochlear apparatus, deep digital flexor tendon and collateral ligaments of the distal interphalangeal joint. Equine Vet J (2007) 39(4):340-3. doi: 10.2746/042516407x185566
10. Bolen G, Audigié F, Spriet M, Vandenberghe F, Busoni V. Qualitative Comparison of 0.27T, 1.5T, and 3T Magnetic Resonance Images of the Normal Equine Foot. J Equine Vet Sci (2010) 30(1):9-20. doi: doi:10.1016/j.jevs.2009.11.002.
11. Dyson S, Murray R, Schramme M, Branch M. Lameness in 46 horses associated with deep digital flexor tendonitis in the digit: diagnosis confirmed with magnetic resonance imaging. Equine Vet J (2003) 35(7):681-90. doi: 10.2746/042516403775696294

12. Mair TS, Kinns J, Jones RD, Bolas NM. Magnetic resonance imaging of the distal limb of the standing horse. Equine Vet Educ (2010) 17:74-8. doi:10.1111/j.2042-3292.2005.tb00340.x

13. van Veggel E, Selberg K, van der Velde-Hoogelander B, Bolas N, Vanderperren K, Bergman HJ. Magnetic Resonance Imaging Findings of the Proximal Metacarpal Region in Warmblood Horses: 36 Lame and 26 Control Limbs (2015-2021). Front Vet Sci (2021) 8:714423. doi: 10.3389/fvets.2021.714423

14. Desbrosse F, Vandeweerd JM, Perrin R, Clegg P, Launois T, Brogniez L, Gehin, S. A technique for computed tomography (CT) of the foot in the standing horse. Equine Vet Educ (2008) 20. 93-8. doi: 10.2746/095777308X272085

15. Porter EG, Werpy NM. New Concepts in Standing Advanced Diagnostic Equine Imaging. Vet Clin North Am (2014) 30(1):239-68. doi: 10.1016/j.cveq.2013.11.001

16. Dixon J, Müksch G, Witte T, Perkins J, Weller R. Standing Equine Computed Tomography: Technique and Clinical Use. In book: EAVDI Yearbook 2016. Cambridge, UK: European Association of Veterinary Diagnostic Imaging (2016). p.31-50.

17. Mageed M. Standing computed tomography of the equine limb using a multi-slice helical scanner: Technique and feasibility study. Equine Vet Educ (2020) 34. doi:10.1111/eve.13388

18. Moliner L, Bolas N, Pallas E, Rodríguez- Álvarez MJ, Benlloch JM. Development of a Standing Equine Leg CT (slCT). JINST (2022) 17(3):C03003. Doi: 10.1088/1748-0221/17/03/C03003

19. Alvarez RE, Macovski A. Energy-selective reconstructions in X-ray computerized tomography. Phys Med Biol (1976) 21:733–7442. doi: 10.1088/0031-9155/21/5/002

20. Avrin DE, Macovski A, Zatz LE. Clinical application of Compton and photo-electric reconstruction in computed tomography: preliminary results. Invest Radiol (1978) 13:217–22. doi: 10.1097/00004424-197805000-00007

21. McCollough CH, Leng S, Yu L, Fletcher JG. Dual- and Multi-Energy CT: Principles, Technical Approaches, and Clinical Applications. Radiol (2015) 276(3):637-653. doi:10.1148/radiol.2015142631

22. Johnson TR, Krauss B, Sedlmair M, Grasruck M, Bruder H, Morhard D, Fink C, Weckbach S, Lenhard M, Schmidt B, Flohr T, Reiser MF, Becker CR. Material differentiation by dual energy CT: initial experience. Eur Radiol (2007) 17:1510–7. doi: 10.1007/s00330-006-0517-6.

23. Fletcher JG, Takahashi N, Hartman R, Guimaraes L, Huprich JE, Hough DM et al. Dual-energy and dual-source CT: is there a role in the abdomen and pelvis? Radiol Clin North Am (2009) 47:41–57. doi: 10.1016/j.rcl.2008.10.003.

24. Mallinson PI, Coupal TM, McLaughlin PD, Nicolaou S, Munk PL, Ouellette HA. Dual-Energy CT for the Musculoskeletal System. Radiology (2016) 281(3):690-707. doi: 10.1148/radiol.2016151109. PMID: 27870622.

25. Diekhoff T, Hermann KG, Pumberger M, Hamm B, Putzier M, Fuchs M. Dual-energy CT virtual non-calcium technique for detection of bone marrow edema in patients with vertebral fractures: A prospective feasibility study on a single- source volume CT scanner. Eur J Radiol (2017) 87:59-65. doi: 10.1016/j.ejrad.2016.12.008.

26. Jans L, De Kock I, Herregods N, Verstraete K, Van den Bosch F, Carron P, Oei EH, Elewaut D, Jacques P. Dual-energy CT: a new imaging modality for bone marrow oedema in rheumatoid arthritis. Ann Rheum Dis (2018) 77(6):958-60. doi: 10.1136/annrheumdis-2018-213152

27. Diekhoff T, Engelhard N, Fuchs M, Pumberger M, Putzier M, Mews J, Makowski M, Hamm B, Hermann KA. Single-source dual-energy computed tomography for the assessment of bone marrow oedema in vertebral compression fractures: a prospective diagnostic accuracy study. Eur Radiol (2019) 29(1):31-39. doi: 10.1007/s00330-018-5568-y.

28. Chen M, Herregods N, Jaremko JL, Carron P, Elewaut D, Van den Bosch F, Jans L. Bone marrow edema in sacroiliitis: detection with dual-energy CT. Eur Radiol (2020) 30(6):3393-3400. doi: 10.1007/s00330-020-06670-7.

29. Wilson AJ, Murphy WA, Hardy DC, Totty WG. Transient osteoporosis: transient bone marrow edema? Radiology (1988) 167(3):757-60. doi: 10.1148/radiology.167.3.3363136.

30. Plenk H, Hofmann S, Eschberger J, Gstettner M, Kramer J, Schneider W, Engel A. Histomorphology and bone morphometry of the bone marrow edema syndrome of the hip. Clin Orthop Relat Res. (1997) 334:73-84.

31. Zanetti M, Bruder E, Romero J et al. Bone marrow edema pattern in osteoarthritic knees: correlation between MR imaging and histologic findings. Radiology (2000) 215:835–840. doi: 10.1148/radiology.215.3.r00jn05835

32. Thiryayi WA, Thiryayi S, Freemont A. Histopathological perspective on bone marrow oedema, reactive bone change and haemorrhage. Eur J Radiol (2008) 67:62-7. doi:10.1016/j.ejrad.2008.01.056.

33. Martig S, Boisclair J, Konar M, Spreng D, Lang J. MRI characteristics and histology of bone marrow lesions in dogs with experimentally induced osteoarthritis. Vet Radiol Ultrasound (2007) 48(2):105-12. doi: 10.1111/j.1740-8261.2007.00213.x.

34. Mizobe F, Nomura M, Ueno T, Yamada K. Bone marrow oedema-type signal in the proximal phalanx of Thoroughbred racehorses. J Vet Med Sci (2019) 81. doi:10.1292/jvms.18-0530.

35. Olive J, Mair T, Charles B. Use of standing low-field magnetic resonance Imaging to diagnose middle phalanx bone marrow lesions in horses. Equine Vet Educ (2009) 21. 116 - 123. 10.2746/095777309X383612.

36. Hayes CW, Conway WF, Daniel WW. MR imaging of bone marrow edema pattern: transient osteoporosis, transient bone marrow edema syndrome, or osteonecrosis. Radiographics (1993) 13(5):1001-11. doi: 10.1148/radiographics.13.5.8210586.

37. Dyson SJ, Murray R, Schramme MC. Lameness associated with foot pain: results of magnetic resonance imaging in 199 horses (January 2001-December 2003) and response to treatment. Equine Vet J (2005) 37(2):113-21. doi: 10.2746/0425164054223804.

38. Starr AM, Wessely MA, Albastaki U, Pierre-Jerome C, Kettner NW. Bone marrow edema: pathophysiology, differential diagnosis, and imaging. Acta Radiol (2008) 49(7):771-86. doi: 10.1080/02841850802161023.

39. Schett G. Bone Marrow Edema. Ann N Y Acad Sci (2009) 1154:35-40. doi: 10.1111/j.1749-6632.2009.04383.x

40. Pache G, Krauss B, Strohm P, Saueressig U, Blanke P, Bulla S, Schäfer O, Helwig P, Kotter E, Langer M, Baumann T. Dual-energy CT virtual noncalcium technique: detecting posttraumatic bone marrow lesions—feasibility study. Radiology (2010) 256:617–24. doi: 10.1148/radiol.10091230.

41. Booz C, Nöske J, Lenga L, Martin SS, Yel I, Eichler K, Gruber-Rouh T, Huizinga N, Albrecht MH, Vogl TJ, Wichmann JL. Color-coded virtual non-calcium dual-energy CT for the depiction of bone marrow edema in patients with acute knee trauma: a multireader diagnostic accuracy study. Eur Radiol. (2020) 30(1):141-50. doi: 10.1007/s00330-019-06304-7

42. Guggenberger R, Gnannt R, Hodler J, Krauss B, Wanner GA, Csuka E, Payne B, Frauenfelder T, Andreisek G, Alkadhi H. Diagnostic performance of dual-energy CT for the detection of traumatic bone marrow lesions in the ankle: comparison with MR imaging. Radiology (2012) 264(1):164-73. doi: 10.1148/radiol.12112217.

43. Jang SW, Chung BM, Kim WT, Gil JR. Nondisplaced fractures on hip CT: added value of dual-energy CT virtual non- calcium imaging for detection of bone marrow edema using visual and quantitative analyses. Acta Radiol. (2019) 60:1465-73. doi: 10.1177/0284185119831690

44. Son W, Park C, Jeong HS, Song YS, Lee IS. Bone marrow edema in non-traumatic hip: high accuracy of dual-energy CT with water-hydroxyapatite decomposition imaging. Eur Radiology (2020) 30(4):2191-8. doi: 10.1007/s00330-019-06519-8

45. Wang CK, Tsai JM, Chuang MT, Wang MT, Huang KY, Lin RM. Bone marrow edema in vertebral compression fractures: detection with dual- energy CT. Radiology (2013) 269:525-33. doi: 10.1148/radiology.13122577

46. Foti G, Beltramello A, Catania M, Rigotti S, Serra G, Carbognin G. Diagnostic accuracy of dual-energy CT and virtual non-calcium techniques to evaluate bone marrow edema in vertebral compression fractures. Radiol Med (2019) 124:487-94. DOI: 10.1007/s11547-019-00998-x

47. Yadav H, Khanduri S, Yadav P, Pandey S, Yadav VK, Khan S. Diagnostic accuracy of dual energy CT in the assessment of traumatic bone marrow edema of lower limb and its correlation with MRI. Indian J Radiol Imaging (2020) 30(1):59-63. doi:10.4103/ijri.IJRI_59_19

48. Booz C, Nöske J, Albrecht MH, Lenga L, Martin SS, Bucher AM, Huizinga NA, Wichmann JL, Vogl TJ, Yel I. Diagnostic accuracy of color-coded virtual noncalcium dual-energy CT for the assessment of bone marrow edema in sacral insufficiency fracture in comparison to MRI. Eur J Radiol (2020) 129:109046 https://doi.org/10.1016/j.ejrad.2020.109046

49. Yang P, Wu G, Chang X. Diagnostic accuracy of dual-energy computed tomography in bone marrow edema with vertebral compression fractures: A meta-analysis. Eur J Radiol (2018) 99:124–129 https://doi.org/10.1016/j.ejrad.2017.12.018

50. Diekhoff T, Hermann KG, Lambert RG. Future of Low-Dose Computed Tomography and Dual-Energy Computed Tomography in Axial Spondyloarthritis. Curr Rheumatol Rep (2022) 24(6):198-205. doi: 10.1007/s11926-022-01075-5.

51. Werpy N, Ho C, Kawcak C, Rantanen N, McIlwraith C. Review of principles and clinical applications of magnetic resonance imaging in the horse. Proc Am Ass equine Practnrs (2006) 52:427-440.

52. Gosangi B, Mandell JC, Weaver MJ, Uyeda JW, Smith SE, Sodickson AD, Khurana B. Bone Marrow Edema at Dual-Energy CT: A Game Changer in the Emergency Department. Radiographics (2020) 40(3):859-874. doi: 10.1148/rg.2020190173.

53. Cosentini T, Le Donne R, Mancini D et al: Magnetic Resonance imaging evaluation of bone marrow changes in obstructive sleep apnoea in adults. Radiol Med (2006) 111(4): 572–84. doi: 10.1007/s11547-006-0052-1

54. Hwang S, Panicek DM. Magnetic resonance of bone marrow in oncology, Part 1–2. Skeletal Radiol (2007) 36: 913–20. doi: 10.1007/s00256-007-0309-3

55. Busoni V, Heimann M, Trenteseaux J, Snaps F, Dondelinger R. Magnetic resonance imaging findings in the equine deep digital flexor tendon and distal sesamoid bone in advanced navicular disease – an ex vivo study. Vet Radiol Ultrasound (2005) 46:279-286.

56. Murray RC, Blunden TS, Schramme MC, Dyson SJ. How does magnetic resonance imaging represent histologic findings in the equine digit? Vet Radiol Ultrasound (2006) 47:17-31. doi: 10.1111/j.1740-8261.2005.00101.x

57. Pumberger M, Fuchs M, Engelhard N, Hermann KG, Putzier M, Makowski MR, Hamm B, Diekhoff T. Disk injury in patients with vertebral fractures-a prospective diagnostic accuracy study using dual-energy computed tomography. Eur Radiol (2019) 29(8):4495-4502. doi: 10.1007/s00330-018-5963-4.

58. Henzler T, Fink C, Schoenberg SO, Schoepf UJ. Dual-energy CT: radiation dose aspects. AJR Am J Roentgenol (2012) 199:S16-25. doi: 10.2214/AJR.12.9210

59. Matsubara K, Koshida K, Ichikawa K, Suzuki M, Takata T, Yamamoto T, Matsui O. Misoperation of CT automatic tube current modulation systems with inappropriate patient centering: phantom studies. AJR Am J Roentgenol (2009) 192(4):862-5. doi: 10.2214/AJR.08.1472.

60. Omoumi P, Becce F, Racine D, Ott JG, Andreisek G, Verdun FR. Dual-Energy CT: Basic Principles, Technical Approaches, and Applications in Musculoskeletal Imaging (Part 1). Semin Musculoskelet Radiol (2015) 19(5):431-7. doi: 10.1055/s-0035-1569253.

61. Bolen G, Haye D, Dondelinger R, Busoni V. Magnetic resonance signal changes during time in equine limbs refrigerated at 4 degrees C. Vet Radiol Ultrasound (2010) 51(1):19-24. doi: 10.1111/j.1740-8261.2009.01615.x.

62. Johnston GCA, Ahern BJ, Woldeyohannes SM, Young AC. Does the Low-Field MRI Appearance of Intraosseous STIR Hyperintensity in Equine Cadaver Limbs Change when Subjected to a Freeze-Thaw Process? Animals (Basel). (2021) 11(2):475. doi: 10.3390/ani11020475.

63. Mourad C, Cosentino A, Nicod Lalonde M, Omoumi P. Advances in Bone Marrow Imaging: Strengths and limitations from a clinical perspective. Semin Musculoskelet Radiol (2023) 27(1):3-21. doi:10.1055/s-0043-1761612).

64. D'Angelo T, Albrecht MH, Caudo D, et al. Virtual non-calcium dual-energy CT: clinical applications. Eur Radiol Exp (2021) 5(1):38. doi:10.1186/s41747-021-00228-y.

65. Ghazinoor S, Crues JV 3rd, Crowley C. Low-field musculoskeletal MRI. J Magn Reson Imaging. 2007 Feb;25(2):234-44. doi: 10.1002/jmri.20854. PMID: 17260396.

Figures

Figure 1: Radiographic examinations of foot IV (A-B): left front foot of a 23-year-old horse with a chronic penetrating nail injury revealed a chronic penetrating injury of the foot sole. Lateromedial (A) and a dorsal 55° proximal to palmarodistal oblique projection (B). A metal probe was placed in the fistula of the lateral fossa of the frog, which advanced in a mildly oblique fashion in dorsodistal to palmaroproximal direction, extending towards the navicular region.

Figure 2: overview of the DECT VNCa, MRI, and CT images in de mid-sagittal plane of all five feet. For each foot: (left to right) DECT VNCa image, MR T1, MR short-tau inversion recovery (STIR), and bone kernel reconstructions of DECT scans are shown. In all feet, areas of high attenuation was present on the DECT VNCa image in the bone marrow/spongiosa that correspond to zones of high bone density on both MR-T1 sequence and CT (dashed arrow), negative for bone edema-like lesions. Feet (I-III) were unremarkable in all modalities. (IV) In a 23-year-old horse with a chronic penetrating nail injury, DECT VNCa showed an uniformly, increased attenuation in the navicular spongiosa and a mottled area of increased attenuation in the proximal half of distal phalanx, decreasing gradually towards the distal tip (full arrow). On MRI, this corresponded to a diffuse hypointense T1 signal with corresponding diffuse hyperintense STIR signal (full arrow), indicating the presence of bone edema-like lesions. On CT, diffuse disruption to erosion of the palmar compacta was observed. (V) In an 8-year-old horse with a chronic lameness with confirmed bacterial infection of the distal interphalangeal joint, DECT VNCa showed a spherically shaped, decreased attenuation in the mid-plantar part of the compacta, surrounded by diffusely increased attenuation in the spongiosa (bold arrow). On MRI, a cyst-like lesion in the mid-plantar compacta of the navicular bone with sclerotic rim was observed, surrounded by a T1 hypointense and STIR hyperintense signal, indicating the presence of bone edema-like lesions. The content of the lesion generated a mixed hypo- and isointense T1 signal and hyperintense STIR signal, and was diffuse hypodense on CT (bold arrow).

Table 1: Overview of the findings of all feet (I-V) on DECT VNCa, MRI, CT, and histopathology. Legend: NB: navicular bone; P3: distal phalanx.

Foot	DECT	MRI and CT	Histopathology
I-III: reference	Areas of high attenuation: • directly adjacent to the cortical edge; • linearly oblique orientated within the proximal part of P3; • adjacent to the proximal articulate surfaces.	Areas of high attenuation on DECT VNCa corresponded to zones of high bone density on both MR-T1 sequence and CT.	Foot II NB and P3: within normal limits.
IV: Chronic penetrating nail injury	**As listed for foot I-III, including:** • NB: diffuse increased attenuation. • P3: mottled increased attenuation in the proximal half, decreasing gradually towards the distal tip.	**As listed for foot I-III, including:** • NB: diffuse, hypointense T1/ hyperintense STIR and XBONE signal. Diffuse disruption to erosion of the palmar compacta of the NB. • P3: marked hypointense T1/ hyperintense STIR and XBONE signal involving the palmar surface and palmar processes.	• NB: extravasated erythrocytes, fibroblasts and fibrous tissue in the spongiosa. Osteoclastic resorption of trabecular bone. • P3: eosinophilic material (protein-rich fluid) in dilated intra-medullary capillaries and in the interstitium of the adipose tissue.
V: bacterial infection of the distal inter-phalangeal joint	**As listed for foot I-III, including:** • NB: spherically shaped, decreased attenuation in the mid-plantar part of the compacta, surrounded by diffusely increased attenuation in the spongiosa.	**As listed for foot I-III, including**: • NB: cyst-like lesion in the mid-plantar compacta with sclerotic rim, surrounded by a T1 hypointense and STIR hyperintense signal. Content: mixed hypo- and isointense T1 signal, hyperintense STIR and XBONE signal, and diffuse hypodense on CT.	• NB: cyst-like lesion lined by sclerotic trabeculae adjacent to the macroscopic indentation of the plantar compacta. Content: dense, uniform fibrous tissue. Spongiosa surrounding the lesion: Extravasated erythrocytes with proliferation of fibrous tissue and trabecular osteolysis.

Figure 3: transverse view of the navicular bone of foot V, an 8-year-old horse with chronic lameness and confirmed bacterial infection of the distal interphalangeal joint (A-D). (A) CT: a cyst-like lesion was observed in the mid-plantar compacta with a diffuse, low-attenuating content surrounded by a smooth, sclerotic rim. (B) MR T1 sequence: the content of the lesion demonstrated a mixed hypo- and isointense signal, while the rim showed a hypointense signal. (C) MR short-tau inversion recovery sequence: the content of the cyst demonstrated a mild

hyperintense signal on STIR, while the rim showed a hyperintense signal. (D) DECT VNCa: a moderate, diffuse increased attenuation was present in the spongiosa surrounding a spherically-shaped decreased attenuation in the mid-plantar part.

Figure 4: overview of the macroscopic findings of the navicular bone and distal phalanx of foot IV, a 23-year-old horse with a chronic penetrating nail injury (hematoxylin and eosin stain) (A-B). (A) Mid-sagittal view of the navicular bone with diffuse indention articular cartilage and the palmar compacta of the navicular bone (arrow). (B) mid-sagittal view of the distal phalanx with resorption lesion of the flexor surface (arrow).

Figure 5: overview of the histopathological findings of the navicular bone and distal phalanx of foot IV, a 23-year-old horse with a chronic penetrating nail injury (hematoxylin and eosin stain) (A-C). (A) navicular bone: intertrabecular space within a pathogenic zone showing the free red blood cells with intervening formation of fibrous strands (arrows). (B) navicular bone: multiple Howship's lacunae (bold arrow) in the bone trabeculae of the medullar space with adjacent presence of osteoclasts (dashed arrow). (C) distal phalanx: increase of the interstitial space with pale eosinophilic staining (edema).

Figure 6: detail of the navicular bone of foot V, an 8-year-old horse with chronic lameness and confirmed bacterial infection of the distal interphalangeal joint (hematoxylin and eosin stain). Detail of the content of the cyst-like lesion with sclerotic rim, adipose cells in peripheral zone, and fibrous tissue in the center.