Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Fakulteten för veterinärmedicin
och husdjursvetenskap**

Occurrence of a recently described and validated subchondral defect in the distal tarsus in adult Icelandic horses

Förekomst av en nyligen beskriven och validerad subkondral defekt i distala tarsus hos islandshästar

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Uppsala
2020

Degree Project 30 credits within the Veterinary Medicine Programme

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Degree Project in Veterinary Medicine

Credits: *30 HEC*
Level: *Second cycle, A2E*
Course code: *EX0869*
Course coordinating department: *Department of Clinical Sciences*

Place of publication: *Uppsala*
Year of publication: *2020*
Online publication: *https://stud.epsilon.slu.se*
Cover illustration: *Photo by Kerstin Hansson*

Keywords: *Osteoarthritis, radiography, subchondral defect, Icelandic horses, distal tarsal joints*

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SUMMARY

This study sought to retrospectively investigate the presence of a subchondral bone defect and its relation to lesions associated with osteoarthritis (OA) in the distal tarsal joints of Icelandic horses. The subchondral bone defect has earlier been described and validated in this breed, and is a potential biomarker for early signs of OA. The initial material used for this study was analogue radiographic projections from 788 joints in 394 adult skeletally mature horses. All the material was initially evaluated with respect to image quality, number of projections taken for each joint, if the structures of interest can be seen in the projections, if the radiographs showed open physes at the distal tibia or proximal metatarsus and if the joints had advanced stages of OA which might obscure the subchondral bone defect. Out of the initial 788 joints, 130 joints from 117 different horses were selected for further evaluation. Three projections were used for each joint in order to assess the presence of the subchondral bone defect and OA lesions in the centrodistal (CD) and tarsometatarsal (TMT) joint. The joints were assessed by the student (SW) and supervisor (KH) by grading each lesion 0 - 3, including the subchondral bone defect depending on the severity of the findings. The lesions associated with OA that were graded in the study were periarticular osteophytes, narrowing/lack of joint width space, subchondral sclerosis, entheseophytes and cyst-like lesions. All joints were also given a subjective OA grading between normal, suspected mild, mild, moderate and severe (0 - 4). Out of the 130 joints evaluated SW identified the subchondral bone defect in 38 (29.2%) of the joints, while KH saw the defect in 35 joints (26.9%). The defect was detected by both KH and SW in 18 joints with or without concurrent OA, and in 7 out of these 18 joints the defects were seen as the only finding. In a majority of the cases, the defect was observed in the CD joint, which is in agreement with earlier research. However, in a number of projections, the subchondral bone defect could also be identified in the TMT joint by both assessors. One may speculate that the different levels of experience with interpreting distal tarsal joints on analogue radiographs could explain why there was a notable difference in which joints the defect was identified. The result show that the subchondral bone defect is present and could be identified in (combination with concurrent OA and as the only finding) in a material consisting of analogue radiographs taken of adult Icelandic horses. As for the subchondral bone defects in relation to clinically manifest and significant OA, longitudinal studies are needed in order to further evaluate the defect as a possible biomarker for early OA.

Keywords: Osteoarthritis, radiography, subchondral defect, Icelandic horses, distal tarsal joints

SAMMANFATTNING

I denna studie görs en ansats att undersöka förekomsten av en subkondral bendefekt och dess relation till förändringar som är förknippas med osteoartrit (OA) i den distala tarsalledens glidleder hos islandshästar. Den subkondrala bendefekten har tidigare beskrivits och validerats för denna ras, och är en potentiell biomarkör för tidigt stadie av OA. Det material som användes till denna studie bestod av analoga röntgenprojektioner på 788 leder från 394 olika vuxna hästar. Inledningsvis undersöktes materialet med hänsyn till bildkvalitet, antal projektioner som var tagna för varje led, om projektionerna kunde visualisera de strukturer som skulle undersökas, om tillväxtzoner sågs vid distala tibia eller proximala metatarsus på röntbenbilderna och om lederna hade långt gångna OA-förändringar som försvårar bedömningen av den subkondrala bendefektens förekomst. Efter gallring kvarstod 130 leder från 117 olika hästar som användes för vidare utvärdering. Tre stycken projektioner användes från varje led för att undersöka förekomst av den subkondrala bendefekten samt förändringar kopplade till OA. Lederna som undersöktes var centrodistal- (CD) och tarsometatarsalleden (TMT). Lederna bedömdes av studenten (SW) och handledaren (KH) genom att gradera varje förändring samt den subkondrala bendefekten mellan 0 - 3 avseende hur grava fynden var. De förändringar som är kopplade till OA som graderades i studien var periartikulära osteofyter, förträngning/avsaknad av ledspringa, subkondral skleros, enteseofyter och cystliknande förändringar. Alla leder bedömdes också med hänsyn till den subjektiva grad av OA som upplevdes av den som graderade och gavs en grad mellan normal, misstänkt mild, mild, måttlig och grav (0 - 4). Av de 130 leder som bedömdes, identifierade SW den subkondrala bendefekten i 38 (29,2%) olika leder, medan KH såg defekten i 35 (26,9%) olika leder. Defekten sågs av både SW och KH i 18 leder med eller utan samtidig förekomst av andra fynd med koppling till OA, samt i 7 av dessa 18 leder som ensamt fynd. Majoriteten av de defekter som identifierades förekom i CD-leden, som också har bekräftats av tidigare studier. Defekten kunde även ses, om än i mindre usträckning, i TMT-leden av både studenten och handledaren. Olika grad av erfarenhet med att bedöma distala tarsalleder med hjälp av analoga röntgenprojektioner kan ha gett upphov till en påfallande skillnad gällande vilken led som defekten upptäcktes i. Resultaten visar att den subkondrala bendefekten kan ses (i kombination med förändringar kopplade till OA och som ensamt fynd) i det material som undersökts. Kopplingen mellan den subkondrala bendefekten och dess möjliga koppling till kliniskt manifesterad OA kräver ytterligare forskning av longitudinell karaktär för att utröna om defekten är en möjligt markör för tidig OA.

Nyckelord: Osteoartrit, radiografi, subkondral bendefekt, islandshästar, distala tarsalleder.

CONTENT

Abbreviations

CT	Computed Tomography
CD	Centrodistal
DLPlM-O	Dorsolateral-plantaromedial-oblique
DPl	Dorsoplantar
ECM	Extracellular matrix
KH	Kerstin Hansson
LM	Lateromedial
MRI	Magnetic Resonance Imaging
OA	Osteoarthritis
PlLDM-O	Plantarolateral-dorsomedial-oblique
RU	Radiopharmaceutical uptake
SW	Simon Wester
SZP	Superficial zone protein
TMT	Tarsometatarsal
γ	Gamma

INTRODUCTION

The Icelandic horse is thought to have been taken to Iceland along with the settlements over eleven centuries ago. It is known for its capability of handling five different gaits. These are the walk, trot, pace, gallop and toelt, with the latter being the one gait that distinguishes the Icelandic horse from most other horse breeds (Hugason, 1994).

Many Icelandic horses are exported. As of 2011, Sweden had 24 715 Icelandic horses registered through World Fengur, an international database containing Icelandic horses worldwide, which can be traced back to Iceland (Lorange, 2011).

The tarsus of horses has a complex structure (Raes *et al*., 2011; Vanderperren *et al*., 2009a) and osteoarthritis (OA) in the distal tarsus (bone spavin) is considered to be the most common disease to give rise to lameness in this area (Baxter & Adams 2011). Heritability concerning radiological signs of OA at a specific age in horses has been valued as a medium-high trait in Icelandic horses (Arnáson & Björnsdóttir, 2009). Therefore, in order to reduce culling rate, pain, as well as minimizing the cost for owners, a cost-effective, readily available and minimally invasive diagnostic method for diagnosing early signs of osteoarthritis in the distal tarsus is of great interest for the individual horse as well as for the selection of horses used for breeding.

Radiography has been considered to be unable to identify early signs of OA (Palmer *et al.*, 2013) due to its difficulties in visualizing soft tissues and cartilage. However, mineralization front defects and central osteophytes in the centrodistal joint (CD) of young Icelandic horses has been identified using radiography. These horses were 2.5 years old i.e close to skeletal maturity. According to a study by Strand *et al*. (2007), most appendicular growth plates of Icelandic horses close around 3 years old. The level of sensitivity and specificity was at the same level or higher than that of low-field magnetic resonance imaging (MRI) (Ley *et al.*, 2016). These radiological signs could, if proven to be related to OA, be a biomarker of great value in identifying early stage OA. The aims of the study were to examine the occurrence of mineralization front defects with and without OA in skeletally mature Icelandic horses using a previously collected material of tarsal radiographs. An additional aim was to document how two observers graded the radiographs whether the defects was present or not and radiographic signs related to OA.

The three hypothesis of this study is:

- The subchondral bone defect can be identified on analogue radiographs of adult skeletally mature horses.
- The subchondral bone defect can be identified as the only pathological change and in combination with various radiographical signs of OA
- There will be a difference between observers in the number of joints that is evaluated as having the subchondral defect.

LITERATURE REVIEW

Anatomy of the tarsus in horses

Bones

The equine tarsus has a complex anatomy (Raes *et al.,* 2011; Vanderperren *et al.,* 2009a). It consists of seven to eight bones according to Adams & Stashak (2011). A review article by Jackman (2006) claims that the tarsus is divided into ten different bones and Kawcak (2016) mentions eleven bone structures involved in the tarsus. The reason why the number of bones included in the tarsal region differs between authors might depend on whether the first and second tarsal bone can be counted as one or two separate bones, and if all the bones at the proximal and distal end are included. The bones mentioned by Kawcak (2016) are the tibia, calcaneus, talus, central, first and second tarsal bones which are commonly fused in adult horses, third and fourth tarsal bones and lastly, the second, third and fourth metatarsal bones (Figure 1). The first and second tarsal bones are, according to Shelley and Dyson (1984) most commonly fused in adult horses, although some horses either have unilaterally or bilaterally unfused first and second tarsal bones.

The tarsus consists of four different joints, these are, from a proximal to distal direction, the tarsocrural joint or proximal tibiotarsal joint, talocalcaneal-centroquartal or proximal intertarsal joint, the distal intertarsal joint (CD) and the tarsometatarsal (TMT) joint (Jackman, 2006).

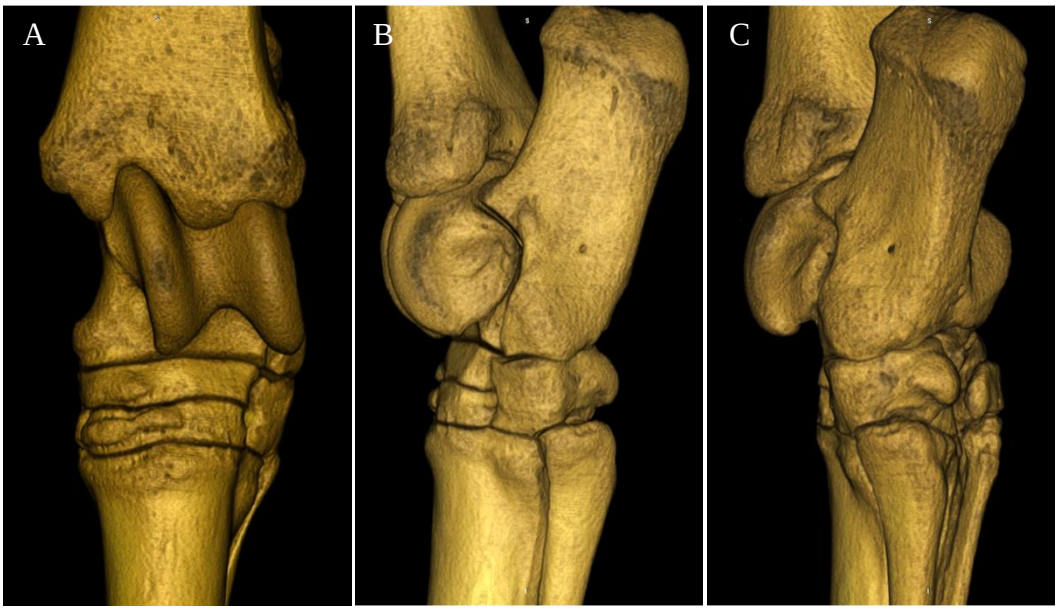

Figure 1. *Three-dimensional reconstructions of computerized tomography of the tarsus of Icelandic horses. (A). Image from a dorsolateral angle. (B). Image from a lateromedial angle. (C). Image from a plantarolateral angle. Source: SLU, Diagnostic Imaging.*

Ligaments

Ligaments have an important role in maintaining normal joint structure and in limiting too much or abnormal movement in the joint. Consequently the ligaments are required to tolerate a great deal of tensile force (Rumian *et al.,* 2007). The tarsal region has eight collateral ligaments.

There are three short collateral ligaments on each side and one longer collateral ligament spanning the lateral and medial aspect of the tarsus (Updike, 1984). According to Kümmerle and Kummer (2013), the long ligaments get stretched when the tarsocrural joint extends and loosens when the joint flexes. The short ligaments mostly behave in an opposite manner, which is stretched when the joint is flexed and loose when it extends (Sisson, 1975).

On the dorsal aspect of the joint region, the dorsal tarsal ligament (talocentrodistalmetatarsal ligament) is proximally attached to the medial aspect of talus, and its distal attachment is on the central and third tarsal bones as well as on the third and second metatarsal bones. The plantar tarsal ligament runs along the plantarolateral aspect of the tarsus and is attached to the calcaneus, fourth tarsal bone and the fourth metatarsal bone. Apart from collateral and other tarsal ligaments there are several smaller ligaments, such as the interosseus ligaments, which connects different bone structures in the tarsus (Sisson, 1975).

Joint capsule and synovia

The fibrous joint capsule spans from the distal part of tibia to the proximal aspect of the metatarsal bones. The external part of the joint capsule surrounding the joint region is fibrous, has a stabilizing function and contains nerve endings with proprioceptive and pain registering receptors. The synovium, which is made of connective tissue lines the synovial cavity and has small villi projecting into the cavity which expands the surface area of the synovium (Dyce *et al*., 2011). The synovium is further divided into a subintimal and intimal layer. The subintimal layer is vascularized and contains nerve endings. The intimal layer lines the surface of the joint cavity. It contains a thin layer of cells and lacks a basal membrane, which facilitates the filtration of blood plasma into the joint cavity. The cells in the intimal layer is thought to be constituted by two different type of cells, the macrophage-like synoviocytes and the fibroblast-like synoviocytes. The macrophage-like synoviocytes primarily has a phagocytic function, while the fibroblast-like synoviocytes produce and excrete components such proteins and hyaluronan into the synovia. These cell types is also capable of producing cytokines, inflammatory mediators and growth factors and therefore plays an important role in maintaining joint homeostasis. The synovia contains blood plasma, hyaluronan and white blood cells (van Weeren (2016). The amount of synovia, which has both lubricating and nourishing properties, varies between joints. The lubricant is thought to reduce the friction in the articular cartilage and therefore minimize attrition. The nutrients in the synovia supply the articular cartilage (Dyce *et al.*, 2011).

Articular cartilage and subchondral bone

The articular cartilage is a flexible, shiny and translucent structure which has a macroscopically smooth surface. The articular cartilage lacks vascularization and nerves. Nutrients diffuse into the cartilage through the synovia, blood vessels in close approximation of the cartilage as well as from blood vessels in the bone marrow. The nutrients and oxygen are pressed into the porous cartilage during compression of the joints (Dyce *et al.*, 2011). The articular cartilage of the synovial joint can histologically be divided into three different layers; the superficial, intermediate and deep zone (Decker *et al*., 2017). The superficial zone is the outer most part of the articular cartilage and is in contact with the synovial cavity. This zone has flat-shaped

chondrocytes and these cells produce lubricin and hyaluronan which enables smooth movement against other articular surfaces (Jay *et al*., 2001). Chondrocytes in the articular cartilage only constitute a minority of the volume (2-5%). The vast majority of the articular cartilage consists of extracellular matrix (ECM) which in turn consists of proteoglycans (i.e. aggrecans) and collagens (Findlay & Atkins, 2014). Chondrocytes in the intermediate zone tend to be more rounded in shape in contrast to the superficial layer. In the deep zone, the chondrocytes are larger than in the intermediate zone. In the space between the cells, the ECM is made up of collagen fibrils. The fibrils are organized in basket-like manners in close perimeter to the chondrocytes but are otherwise organized in a parallel matter between the chondrocytes. Moreover, the ECM also contains precipitated proteoglycans which has small microvilli processes (Hunziker *et al*., 2007). Together with collagen fibrils, a macromolecule called aggrecan, provide tensile strength and resilience to the articular cartilage (Decker *et al*., 2015). Between the hyaline articular cartilage and the calcified cartilage lies the tidemark. It is seen as a well defined line between calcified and noncalcified cartilage (Lyons *et al*., 2005). The tidemark is proposed to be the result of debris from chondrocytes which have undergone apoptosis (Simkin, 2012). Beyond the tidemark lies the calcified cartilage. It's constituted by a mineralized matrix and lacunae in which spherical chondrocytes are situated. There are less chondrocytes present in the calcified cartilage in comparison to the noncalcified cartilage (Martinelli *et al*., 2002). The subchondral bone, which is composed of the subchondral bone plate and the subarticular spongiosa, is situated beneath the calcified cartilage. These two structures are separated by the so-called cement line. The subchondral bone plate is included in the periarticular bone along with subchondral trabecular bone as well as bone which are situated at the joint margins.

Function of the joint compartments

When examining the movement and function of the joint regions, the tarsocrural joint, which is considered a hinge joint (ginglymus), is the most moveable of the four joints. The tarsocrural joint can flex, extend, abduct, adduct and rotate in- and outwards. The three distal joint regions are planar joints with restricted rotational and translational movement (Lanovaz *et al*., 2002). The distal tarsal joint region also has a shock absorbing function (Back *et al*., 1995).

Communication between joint compartments

The communication between the distal joints of tarsus has been investigated in several different studies (Bell *et al*., 1993; Dyson & Romero, 1993; Kraus Hansen *et al*., 1992; Seabaugh *et al*., 2017; Gehm et al., 2019). Communication between the CD joint and TMT joint ranged between 20 - 45.7% in the aforementioned studies when the results were combined. The tarsocrural joint and the proximal intertarsal joint always communicate (Gehm *et al*., 2019). Bell *et al*. and Gehm *et al*. saw communication between the CD joint and the tarsocrural joint in 3/73 (4.1%) and 8/70 (11.4%) cases respectively.

Osteoarthritis

Osteoarthritis (OA) is considered to be a degenerative joint disease (Boyde *et al*., 2014; Findlay & Atkins, 2014; Schlueter & Orth 2004). Recent knowledge has suggested that the key factor for further development of the disease is the inflammation that arises (Robinson *et al*., 2016). The disease affects all structures of the joint, with time resulting in loss of articular cartilage due to degradation. The main features of OA (apart from the aforementioned degradation of articular cartilage), involves alterations in the subchondral bone, the tendons, ligaments, inflammatory responses in the synovium and joint capsule, (Findlay & Atkins, 2014).

The Osteoarthritis Research Society International (OARSI) has established a definition of OA:

> "Osteoarthritis is a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness (OARSI, 2019)."

Pathogenesis

The disease is yet to be fully understood and research continues in order to gain more knowledge about the pathogenesis of OA (Martel-Pelletier *et al*., 2016). The risk factors for developing OA are of complex nature, each factor interacting in various degrees in promoting further development of the disease. The risk factors in humans include person bound features such as weight, age, gender, genetic predisposition and diet as well as more joint-localized factors, such as injury, overloading and failure of alignment in the diarthrodial joint (Palazzo *et al*., 2016). Felson (2013) argues that the progression of OA most likely is due to the inflammatory response which occurs as a result of mechanical injury to the joint.

Inflammatory mediators are thought to have an important role in the initiation and progression of OA. In the case of OA, synoviocytes and chondrocytes produce various inflammatory mediators such as cytokines, nitric oxide, reactive oxygen species and matrix degrading enzymes (Rahmati *et al*., 2016). When abnormal loading or damage in a diarthrodial joint occurs, it is hypothesized that chondrocytes produce enzymes that causes the ECM between the chondrocytes to be broken down (Xu *et al*., 2011). In a different study by the aforementioned authors, the temporomandibular joints in mice revealed that the first observable pathological changes in OA were increased staining with safranin O of proteoglycans in the cartilage due to their initial overproduction during OA. However, the proteoglycans decrease in the articular cartilage as the disease progresses (Xu *et al*., 2009). Proteoglycans, such as aggrecan, serve to enable the cartilage to withstand compressive force by providing an osmotic resistance (Knudson & Knudson, 2001).

The superficial zone protein (SZP), also known as lubricin, keeps the articular cartilage frictionless (Sakata *et al*., 2015). When the secretion of SZP is impaired, the diarthrodial joints suffer further damage as a result of increased friction (Elsaid *et al*., 2005). When friction in the joint increases, proteoglycans on the surface of the joint start diminishing. Furthermore, collagen II particles at the articular cartilage becomes exposed and damaged. Moreover, on the

superficial part of the articular cartilage, the chondrocytes gets rearranged into chondrocyte clusters (Madry *et al*., 2016). Since the damage on the collagen network can't be repaired, it represents a key feature in further development in early OA (Goldring & Berenbaum, 2015). This damage to the collagen eventually leads to release of collagen particles into the synovial cavity. Because of the erosion of the cartilage, chondrocytes are exposed, leading to expression of Toll-Like Receptors (TLRs). When TLRs bind with so-called ligands, it produces an inflammatory response which ultimately leads to inflammation in the synovial fluid and synovial membrane (synovitis), further contributing to the development of OA in the joint (Scanzello & Goldring, 2012). All these changes of the joint tissues give rise to a low-grade inflammation with chronic character which has an important role in the pathogenesis of OA (Robinson *et al*., 2016).

During OA, changes to the periarticular bone involves increased subchondral bone thickening, modified structure of the trabecular bone, osteophyte formation and also the development of so called bone-cysts (Goldring & Goldring, 2010). Pritzker *et al*. (2006) proposed a grading system in order to readily assess the different pathological stages of OA in the cartilage in the human population. Using histological samples stained with Safranin O tissues were viewed in microscopes to evaluate the cartilage. The system consists of six stages going from grade 1 to grade 6. Grade 1 involves intact superficial chondrocytes, but with certain amount of fibrillation of the articular surface. The superficial matrix can also undergo swelling; chondrocyte can form clusters, or die through programmed apoptosis or undergo necrosis. At grade 2, in addition to the changes in grade 1, deeper fibrillation and discontinuity of the superficial cartilage is observed, where some of the tissue is released into the synovial cavity. Grade 3 involves vertical fissures which extend down into the intermediate zone. These fissures may become branched. At grade 4, which is characterised by erosion, the branches from the vertical fissures merge together and create loose fragments of cartilage at the superficial and intermediate cartilage zone. At grade 5, the bone surface is exposed in some areas. The bone appears denser due to a fibrocartilaginous repairing process in the bone grade 6 show deformation of the joint. This is due to microfractures in the articular bone plate which with the process of repairing results in central and marginal osteophytes. Palmer *et al*. (2013), stated that by using the grading system proposed by Pritzker *et al*. (2006), radiology would be able to identify pathological changes at grade 4, while morphological MRI would be able to visualize abnormalities at grade 3.

In order to identify what has been termed "early OA", Luyten *et al*. (2012) established criteria's using radiology, arthroscopy and MRI to determine the threshold for when the stage of OA is considered early. These criteria's involved episodes of pain, and a certain amount of pathological development in the human knee. By using a modified version of the Kellgren & Lawrence scoring which in contrast to the original version did not evaluate any potential narrowing of the joint space (Felson *et al.*, 2011). Moreover, the patients had to have had a history of at least two periods with pain with duration of a minimum of ten days, along with either arthroscopic findings and/or using a combination of grading systems in order to evaluate the bone marrow, meniscus and cartilage.

Osteoarthritis in the distal equine tarsus

Osteoarthritis in the distal portion of the tarsus, is the most frequently occurring reason for lameness in the equine tarsus (Baxter & Adams, 2011) and pain in these regions with osteoarthritis, also known as "bone spavin", occurs frequently (Branch *et al*., 2005; Sparkman *et al*., 2015). The pain originates, in different degree, from the subchondral bone, synovial membrane, joint capsule and the marginal periosteum (van Weeren & de Grauw, 2010). Bone spavin usually involves the CD joint and the TMT joint, but in some cases the proximal intertarsal joint can also be affected (Auer & Stick, 2012; Baxter & Adams, 2011). A lot of research is being done in order to investigate the cause of bone spavin in horses. A study by da Costa Barcelos *et al*. (2016) investigated the connection between the tarsal angle between the cranial aspect of the tibia and the dorsal aspect of the third metatarsal with tarsal joint disease in Mangalarga Marchador horses. Horses (particularly male horses) with an angle of 140º or greater were more prone to have radiological changes in the joint. The most frequently occurring disease was OA in the CD and TMT joint.

The conformation of distal tarsal bones and their correlation with development of OA has been investigated. Sprackman *et al*. (2015) found that 'wedging' of the central tarsal bones and the third tarsal bone correlated with OA. Another study by Skelly-Smith *et al*. (2016) saw an association between abnormalities such as less visible interosseus space where the interosseus ligament is located in the CD joint and occurrence of OA using radiology.

In Icelandic horses, the age, height at the croup, decreased tarsal angle between the cranial aspect of the tibia and dorsal aspect of the third metatarsal bone angles as well as place of birth were considered significant risk factors for developing osteoarthritis in the distal tarsal joints (Axelsson *et al*., 2001).

Osteoarthritis in the distal tarsus of Icelandic Horses

In a study performed by Björnsdóttir *et al*. (2000a), Icelandic horses from selected sires were examined both radiologically and through flexor test of the tarsal joint in order to quantify the level of heritability in the breed. The combination of radiological signs and lameness after flexor test in the horse were considered to be of relevance and therefore would be of value in order to lower the prevalence of OA. A study presented by Arnáson & Björnsdóttir (2003) later concluded that the level of heritability coupled to radiological signs from the aforementioned study was undervalued. Therefore, heritability with respect to radiological signs at a specific age was considered to be a medium-high level trait. The prevalence of bone spavin in Icelandic horses was investigated by use of radiological signs and was estimated to be 33.0% in horses between 4 and 8 years old (Eksell *et al*., 1998). In a later study by Björnsdóttir *et al*. (2000b), horses between 6-12 years of age were investigated in order to estimate the prevalence of bone spavin using flexor tests and radiography. Radiological signs were found in 30.3% of the Icelandic horses where the majority of the horses showed signs of changes in both hind limbs. The increased prevalence of bone spavin between 6 year old and (18.4%) and 12 year old (54.2%) horses support the notion that OA, in many cases, behaves as a chronic and progressive disease. A study was performed in order to investigate the rate of culling of Icelandic horses (Björnsdóttir *et al*., 2003). They found that, out of 508 horses included in the study, 98 horses

had been culled five years later. The reason of culling was also accounted for, and 42 horses (42.9%) were culled due to hind limb lameness. Using radiography and histological samples, Björnsdóttir *et al*. (2004) examined CD joints from young Icelandic horses aged between 6 months and 6 years. In Iceland, most horses start getting ridden when they become 4-5 years old. From the radiological investigation 60% of the horses showed signs of subchondral bone sclerosis, and 27% had defects in the subchondral bone plate. Radiographic findings related to OA were seen in horses as early as 6 month old. The study therefore indicates that the signs associated with OA starts at an early age and doesn't relate to the fact that the horse has been ridden.

Diagnostic imaging of osteoarthritis in horses

Radiology

Radiology is one of the most common diagnostic techniques used to examine the tarsal region in horses. It is non-invasive and cost efficient. A radiological image is a two-dimensional image of a three-dimensional object. A two-dimensional projection of a three-dimensional object as a result creates summations of the different structures within this object (O'Brien *et al*., 2011). Hence it is appropriate to produce several projections to achieve a more complete presentation of the object being examined. Moreover, radiological imaging, while efficient at identifying calcified tissues can't distinguish between soft tissues such as tendons, ligaments, muscles, cartilage and synovial tissues if there's no air, fat or calcified tissues between them due to their corresponding opacity (Loeuille & Chary-valckenaere, 2012). In assessing OA it is proposed that radiology fails to identify the early signs of disease and radiological signs are distinguishable only when the disease has reached an irreversible stage (Palmer *et al*., 2013).

A radiological image is produced by high energy ionizing electromagnetic radiation. In an X-ray tube there is a negatively charged cathode and a positively charged anode which are enclosed inside a glass envelope. At the cathode there is a filament which with increased current causes a rise in temperature. This in turn creates to a cloud of free negatively charged electrons and the size of this cloud is determined by how great the current inside the filament is and is measured by milliampere (mA). At the opposite end of the glass envelope, the positively charged anode resides. When inducing a difference in voltage (kVp) between the anode and the cathode, the negatively charged electrons passes through the x-ray tube and hits the anode. The greater the voltage difference between the cathode and the anode, the higher the velocity of the electrons that passes through the tube. Both the size of the electron cloud and the velocity of the electrons are determined through a control panel. The X-ray tube is completely sealed, and the space within is a vacuum. When electrons are released from the cathode, they collide with the anode (which is made out of tungsten) on an area called the focus. In order to endure the amount of heat produced from the electrons the anode is shaped as a spinning disc, which allows the energy to be spread out on a wider area. As the electrons collide with the anode they interact with atoms in the anode to form an X-ray beam. From the focus the beam travels down from the X-ray tube, through the tissue being examined and onto a cassette/film in an analogue system or image plate/detector in a digital system. Depending on the tissue, there is varying degree of so called attenuation. The level of X-ray attenuation depends on the density, thickness and the atomic number of the tissues being examined. When examining the thorax, most of the

electrons will travel through the tissue because of it mostly being filled with air. This makes the image appear dark (radio-lucent). When examining calcified tissues, fewer electrons pass through the tissue, resulting in a whiter (radio-opaque) image. There are in general four tissues with different degree of density to take into consideration when evaluating a radiological image. These are, in order from least to the most radio-opaque, air, fat, soft tissue and calcified tissues.

Radiographic examination of the tarsus in horses

When examining the tarsus using radiography there are generally four different projections considered in order to get a full evaluation of the tarsal region (Figure 2). These are the lateromedial (LM), dorsolateral-plantaromedial oblique (DLPlM-O), plantarolateral-dorsomedial oblique (PlLDM-O) and dorsoplantar (DPl) view (Busoni & Audigié, 2018). Eksell *et al*. (1999) examined the sensitivity and specificity of these projections when evaluating radiographic signs of OA in the distal tarsus of Icelandic horses. It was determined that the PlLDM-O projection had the highest sensitivity and specificity in identifying signs of OA. In the study, the combination of the PlLDM-O and the DLPlM-O managed to detect all the changes associated with OA. However, the authors of the study recommend a minimum of three different projections in order to get a satisfactory coverage of the structures being examined in the distal tarsal region. The LM-projection provided more information than the DPl-projection and should according to the authors be prioritized to be used in combination with the PlLDM-O and DLPlM-O-projections.

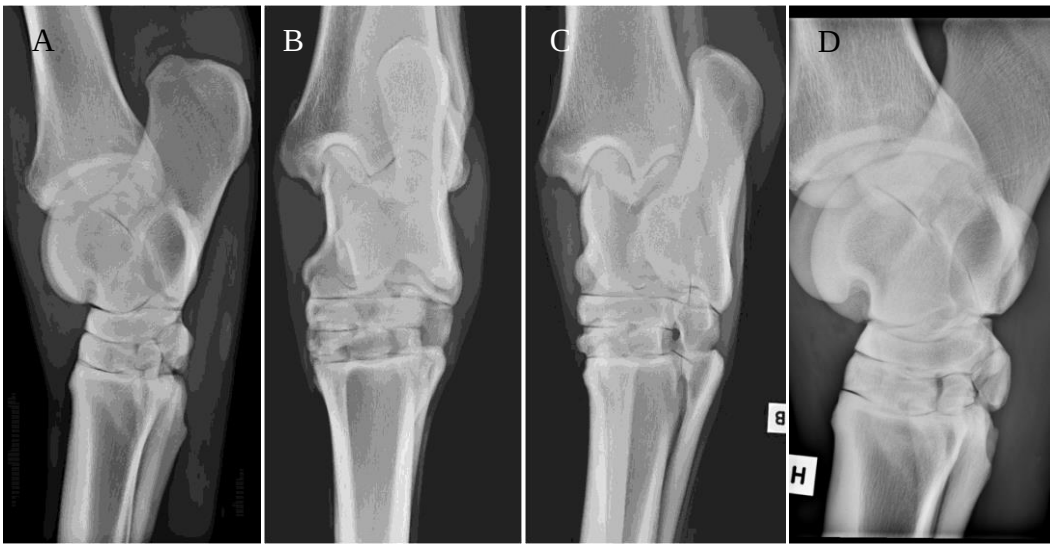

Figure 2. *The four different standard projections for radiographic imaging of the distal tarsal joints. These include the (A) lateromedial (LM). (B), dorsoplantar (DPl). (C). dorsolateral-plantaromedial-oblique (DLPlM-O) (D). Plantarolateral-dorsomedial-oblique (PlLDM-O). Source: SLU, Diagnostic Imaging.*

Osteoarthritic lesions of the equine distal tarsus detectable with radiology

As stated before, OA is a progressive disease which involves the entire joint (Findlay & Atkins, 2014). However, radiology of the distal tarsus itself is limited to pathological findings such as formation of periarticular osteophytes and entheseophytes, narrowing of the joint space, subchondral and trabecular bone sclerosis, lysis and ankylosis (Kawcak, 2016; Laverty *et al*., 1991) (Figure 3). Osteophytes are a common feature in OA and are seen as a protrusion in

diarthrodial joints. The ways in which osteophytes are formed are yet to be fully understood (Junker *et al*., 2016; Kaneko *et al*., 2013; van der Kraan & van der Berg, 2007). The outer most layer of an osteophyte has fibroblast-like structure as it grows. Inside this layer, the osteophyte undergoes chondrogenesis; the most central chondrocytes in a growing osteophyte continue to grow in both size and numbers. Eventually these chondrocytes develop into ossified tissue (van der Kraan & van der Berg, 2007).

The subchondral bone is as previously mentioned, divided into the subchondral bone plate and the trabecular bone. In subchondral bone, both resorption and formation of bone occurs separately through the activity of osteoclasts and osteoblasts respectively. To some extent, the subchondral bone is able to withstand increased forces, however when the forces become too great pathological changes occur (Stewart & Kawcak, 2018). Excessive loading or abnormal conformation of the joint can cause microfractures in the subchondral bone. The healing process for the subchondral bone can in turn cause a more dense sclerotic tissue, which makes it stiffer and therefore partially loses its shock-absorbing ability (Boyde, 2003). Radiographically, sclerotic areas in affected subchondral bone will be regarded as a tissue with increased opacity (Smith *et al*., 2016). Sclerosis can also be seen in trabecular bone (Laverty *et al*., 1991). A common feature of OA is narrowing of the joint space. This pathological change can be seen due to the cartilage degradation that occurs within the affected joint (Kawcak & Barret, 2016). When Ley *et al*. (2016) examined the prevalence of early signs of OA using radiography and MRI in Icelandic horses they found that out of 42 centrodistal joints examined, 28 had mineralization front defects (subchondral bone defects) (Figure 4). The defect was described as follows:

"Focal concave mineralisation front (the interface between the hyaline articular cartilage and the articular calcified cartilage or subchondral bone) regions that resulted in a focal region of increased distance between the mineralisation fronts."

This is believed to be a potential biomarker for further development of OA in horses. However, the defect has yet to be investigated in a longitudinal study in order to evaluate potential progression or regression of the lesion. A biomarker is used to, in early stages; predict the outcome of a disease in patients (Strimbu & Tavel, 2010).

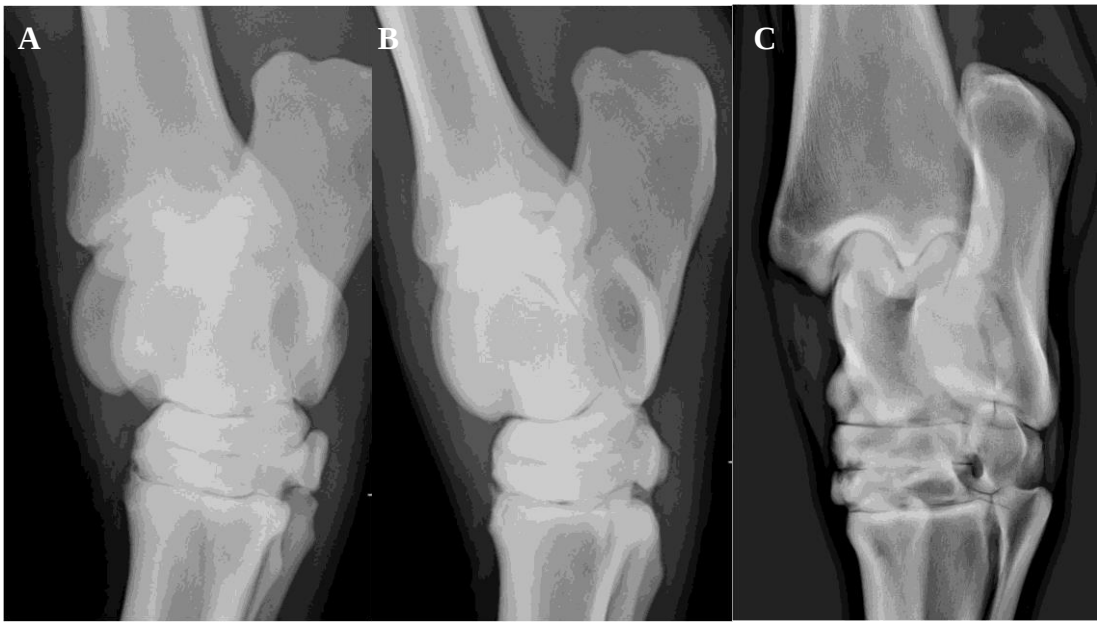

Figure 3. *Images of plantarolateral-dorsomedial-oblique (A), lateromedial (B) and dorsolateral-plantaromedial-oblique (C). Projection with osteoarthritic lesions in two horses not included in the study. (A, B) The images are from the same horse and shows periarticular osteophytes at the dorsolateral aspect of the centrodistal (CD) and tarsometatarsal (TMT) joint. The CD joint also shows signs of decreased joint space width. (C), another horse with cyst-like lesions on the dorsomedial aspect of the CD joint and decreased joint space width.*

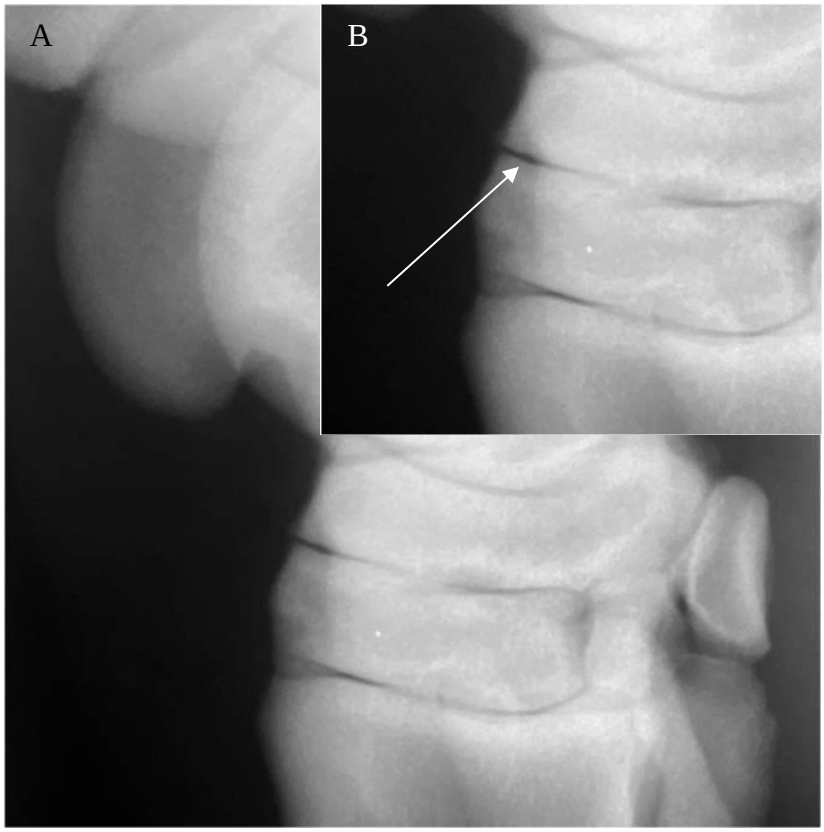

Figure 4. *(A) Plantarolateral-dorsomedial-oblique projection of a horse in the study (joint V77). Note the subchondral bone defect (mineralization front defect) at the dorsolateral aspect of the centrodistal joint. (B). Same image of the distal tarsal joints with defect in the centrodistal joint (arrow). Source: SLU, Diagnostic Imaging.*

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) has an increasingly important role in identifying pathological changes through the possibility to visualize soft tissues. Magnetic resonance imaging is in general established from protons, which are abundant in water and fluid filled tissues throughout the body. Protons, which are positively charged, are randomly oriented in tissues, spin around their own axis and create a small magnetic field of their own. Because of the random arrangement of these protons, the protons individual magnetic fields cancel out. When placed in a strong external magnetic field, the protons align in a parallel or antiparallel fashion to that field. As a small majority of protons align in a parallel, it creates a net magnetization in the tissue being evaluated. Radio wave pulses cause protons to absorb energy through resonance. When protons absorb this energy it gives rise to excitation that flips the proton from its longitudinal axis. The angle of which the proton flips from the axis depends on the magnitude and length of the pulse. When the pulse ends, the protons will go back to their previous orientation. As this happens, energy from protons is released. This aforementioned excitation that is created in the targeted tissue is recognized by a receiving coil which perceives the signals as "echoes". The images produced by MRI are built up by so called voxels which have a depth, height and a width. The smaller the voxels, the more detailed the image. However the signals produced by the protons in each voxel known as the signal-to-noise ratio also influences the quality of the resolution. Smaller voxels contain fewer signals than larger voxels, so even if the resolution is greater with smaller voxels, the image can appear blurry due to a large proportion of "noise" (d'Anjou, 2018).

In veterinary practice low-field MRI is commonly used most likely due its lower cost. These are in the field strength of 0.2-0.4 Tesla. The major disadvantage with low-field MR is the lower signal-to- noise ratio (SNR) which produces less detailed images compared to high-field MR. This can be somewhat compensated by using longer scanning times (Konar & Lang, 2011).

In equine medicine high-field MR requires horses to be under general anesthesia in order to perform the examination due to its closed configuration, where a cylindrical tube is used. Low-field MR in equine practices are available in an open configuration, which has a c-shaped magnet This enables the horse to be examined standing and allows the procedure to be performed under sedation instead of general anesthesia which in turn lowers the risk of complications (Porter & Werpy, 2014). In a study by Ley *et al*. (2016) radiography and low-field MR was used in order to identify early sign of OA in centrodistal joints of Icelandic horses. The result suggested that conventional radiography had an equal or better ability to identify the early changes which is suspected to be related to OA.

Computed Tomography

As with MRI, computed tomography (CT) uses images made up of voxels. In CT imaging, a voxel with high amount of attenuation will have a whiter appearance and a voxel with low grade of attenuation will appear darker. The CT equipment is made up of a gantry with a rotating X-ray emitting tube, detectors, table for the patient and a computer system for analyzing and altering the images taken. The images from a CT can only be created in a transverse angle relative to the patient. However, due to the large amount of images taken, they can later be

processed to create a multiplanar picture as well as the ability to rearrange the images into a 3D-format (d'Anjou, 2018).

In equine practice, standing CT is an available modality in a scarce number of clinics. Most CT examinations are performed under general anesthesia which, as mentioned with MRI, is related to increased risk for complications during examination. Standing CT allows the equine patient to be examined under sedation, where the distal limbs and head region is possible to scan. Compared to standing MRI, an examination with standing CT takes less time which entails shorter duration of sedation of the horses. It is also a more price friendly modality compared to MRI in regards to the lower set-up and maintenance costs. A major disadvantage concerning CT scanning as well as conventional radiography is the exposure of radiation (Porter & Werpy, 2014).

A study by Desbrosse *et al*. (2008) used a standing CT (peripheral Quantitative Computerized Tomography) to evaluate the hooves of horses. The technique was considered to be of possible use as an alternative to the conventional CT regards to evaluating calcified tissue in the hoof, but was not considered useful at analyzing soft tissues. A study performed by Raes *et al*. (2010) concluded that CT under general anesthesia showed superior ability in recognizing lesions in the distal tarsal region compared to radiography. They proposed CT to be considered an alternative when radiographs were ambiguous or showed no signs.

Ultrasonography

Ultrasonography is a technique that makes use of high frequency sound waves. These sound waves are created when electricity causes vibration in piezo electric crystals inside the transducer. The emitted sound waves move through the targeted tissues. Some of these waves (if it hits a tissue in a perpendicular manner) are reflected back to the crystals and converted into an electrical signal which results in an image. Reflection occurs as the sound waves travel between tissues with different acoustic impedance. As the sound waves interact with different tissues, it may get attenuated in several various ways. Reflection and other types of attenuating interactions with sound waves is what give rise to ultrasonographic image. Echogenicity is the term used to describe the look of different tissues when using ultrasonography. A structure which appears darker is termed hypoechoic and a tissue with brighter appearance as hyperechoic. However the echogenicity of different tissues must be evaluated in relation to each other due to their difference in acoustic impedance.

Because of the complex anatomic structure of the tarsus, a solid understanding of the various anatomic tissues is essential in order to successfully differentiate between different tissues in the joint region. It is the most cost effective imaging tool for soft tissues, and is helpful for diagnosing specific pathologic changes in tendons, ligaments and synovial structures (Whitcomb, 2006). Ultrasonography is also effective at visualizing pathological changes such as periarticular osteophytes, entheseophytes, cartilage degeneration, thinning of the cartilage at loaded joint areas as well as joint capsule thickening (Vanderperren *et al*., 2009b). However, a major disadvantage in examining the distal tarsal joints is the inability to visualize the articular cartilage due to the close alignment between the joint margins (Tomlinson *et al*., 2008).

Scintigraphy

Nuclear scintigraphy involves using radionuclides which emit energy in the form of gamma-rays (γ-rays). The radionuclides are often coupled with a pharmaceutical to form a radio-pharmaceutical drug that can localize the tissue that is going to be examined. Diphosphonate salts is used as radiopharmaceutical when evaluating osseous tissues due to the increased absorption in osseous tissues with increased osteoblastic activity, increased metabolism and increased blood flow to an area. The γ-rays that are being emitted from the radionuclides can be visualized by using a gamma camera. These cameras have collimators which absorb γ-photons. The collimator is created so that only the photons that travel in a perpendicular direction towards the camera can travel through the hexagonal holes in the collimator. The smaller and deeper the holes, the better the resolution. However, smaller and deeper holes mean that less photons reach the camera. When the γ-photons reach the camera, they can create photoelectric interactions with a sodium iodide crystal. Later, they are transferred through photomultiplier tubes that absorb light photons and emit electrons which accelerate towards the positively charged anode at the end of the tube. When the electrons reach the anode they are processed into a digital image.

The image information that is acquired is good in order to localize the anatomical position where abnormal physiological activity is occurring, but lacks detail to assess the anatomical structure itself in comparison with other imaging modalities such as MRI and CT (Dyson *et al.*, 2003).

Murray *et al*. (2005) performed a study to investigate the radiopharmaceutical uptake (RU) using ^{99m}Technetium methylene diphosphonate in horses with pain in the distal tarsal region. In relation to an earlier study by Murray *et al*. (2004), horses without distal tarsal pain were examined using the same technique. In relation to the first study, horses with distal tarsal pain had increased RU compared to normal horses. A study by Daniel *et al*. (2012) compared radiography, MRI and scintigraphy when identifying and describing pathological changes in the distal tarsal region of horses. Scintigraphy proved very useful in order to identify and localize pathological changes, but lacked the ability to further assess the anatomical detail of the pathological findings in comparison to MRI.

MATERIALS AND METHODS

Material

The study is retrospective and observational. The material consists of analogue radiographic projections taken on Iceland as a part of a screening program. The exact time span when the radiographs were taken is unknown as this information is lacking on the radiographs. The radiographs were stored in boxes marked April 1996 so they are assumed to be from that year. All radiographs were taken by the same person (Sigridúr Björnsdóttir) and it is possible that the radiographs originated from a study published by Björnsdóttir *et al.* (2000a). The screening program was used to investigate the presence of OA in the distal tarsus of Icelandic horses in order to prevent affected horses from further breeding. Information on sex and age of the radiographed horses was not available. In the screening program, LM, PlLDM-O and DLPlM-O projections were used to evaluate the tarsus in the horses. All radiographs were taken using Kodak X-Omatic with Lanex Regular intensifying screens. The film used was of a latitude type (FUJI HRL) and processed using a developing machine of un-known type. All images were marked with lead numbers and the letters "H" (höger, Swedish for right leg) or "V" (vänster, Swedish for left leg) in order to clarify which leg the projection concerns. The radiographs have been archived at the Swedish University of Agricultural Science. The number of archived material consisted of projections from 788 joints of adult and skeletally mature horses.

Methods

Out of the initial 788 joints, all joints with less than three projections were excluded. Further exclusion criteria were; if the series of three projections included any view that could not clearly visualize the dorsal aspect of the joint margins in the CD and the TMT joint, projections with motion artefacts as well as artefacts that originate from the development of the analogue projections, radiographs showing open physes in distal tibia or proximal metatarsus and joints with advanced stages of OA that complicates evaluation of the joint with regards to the mineralization front defect. After screening of these 788 joints, 130 joints from 117 different horses were selected for further evaluation.

The joints selected for further evaluation were then, with regards to their order, randomly assigned, and were graded by the student and the supervisor (KH) in the same order.

Each included projection was graded according to a predefined grading table and there was also a summary assessment of all included projections regarding OA (see below for grading details). Before grading the images in the study the grading table was tested on six joints of different Icelandic horses randomly selected by the supervisor. These joints were not included in the study material. The student sent the grading of these test images to the supervisor. After evaluating the students grading, the supervisor selected 14 of the 108 data points that needed discussion and clarification with the student. These data points were selected to make sure the student was looking at the correct structures and was using the correct terminology for the various lesions.

In the grading table the structures that were graded were periarticular osteophytes, entheseophytes, joint space width, subchondral sclerosis, subchondral cyst-like lesions and subchondral bone defect. If no changes was found or the structure was regarded as normal the grade was 0 and presence of changes was graded as 1 (mild), 2 (moderate) or 3 (severe). Furthermore the changes were recorded as focal or generalized and the number of lesions as few or multiple. For joint space width the grades was 0 (normal), 1 (mildly narrowed), 2 (clearly narrowed) or 3 (no joint space can be seen). Each of the three available projections for the joints were assessed separately. The assessment of the subchondral bone defect and joint margin width could be termed "not assessable" whenever the projection and/or the conformation of the joints obscured or made it hard to visualize the structures of interest. All grades were subjectively given and there was no pre-grading consensus discussion on severity of grading between the author and the supervisor. The joints were also given a subjective grading (0 - 4) as to what degree of OA were seen when assessing the joint. For this grading all three projections were used. There was no consensus discussion before regarding criteria's that would result in a certain grade.

After individual grading, the student and the supervisor had a discussion to decide which type of lesion, if recognized as the only lesion in the joint, that would result in OA grade >0. These lesions were periarticular osteophytes, narrow joint space and subchondral cyst-like lesions. If a subchondral bone defect was the only lesion detected the OA grade was 0 in this study. Furthermore it was decided that joints assessed with either entheseophytes or sclerosis as single lesions would not generate a subjective OA of >0. All grading was done using the same light cabinet and light was possible to partially block using a thick paper envelope; no hot light was used. The results from the grading would later be analyzed and presented with tables and descriptive statistics.

RESULTS

Out of the 130 joint that were further evaluated, the student recognized the subchondral bone defect in 38 (29.2%) different joints. In 24 (55.3%) of these 38 joints, the defect was seen as the only abnormality. The remaining 14 joints with the defect were assessed to also have grade 1 – 3 subjective OA alterations. Altogether there were 57 (43.8%) joints that the student graded as 0 in regards to if there were a defect or OA changes (Table 1).

Table 1. *Distribution of joints with and without (w/o) subchondral bone defect and subjective grade of osteoarthritis (OA) graded by the student (SW)*

	Defect w/o OA	**Defect with OA**	**No defect w/o OA**	**No defect with OA**	
Grade 0	24	0	57	0	**81**
Grade 1	0	11	0	25	**36**
Grade 2	0	2	0	8	**10**
Grade 3	0	1	0	2	**3**
Grade 4	0	0	0	0	**0**
Total	24	14	57	35	**130**

The supervisor of the study recognized the defect in 35 joints (Table 2). Of the 35 joints that were assessed to have the defect, 14 (40.0 %) were perceived to have only the defect. The other 21 joints were perceived to have grade 1 – 3 subjective OA grade. There were 80 (61.5%) joints that the supervisor graded as 0 in regards to if there were a defect or OA changes (Table 2).

Table 2. *Distribution of joints with and without (w/o) subchondral bone defect and subjective grade of osteoarthritis (OA) graded by the supervisor (KH)*

	Defect w/o OA	**Defect with OA**	**No defect w/o OA**	**No defect with OA**	
Grade 0	14	0	80	0	**94**
Grade 1	0	16	0	14	**30**
Grade 2	0	5	0	1	**6**
Grade 3	0	0	0	0	**0**
Grade 4	0	0	0	0	**0**
Total	14	21	80	15	**130**

The number of joints with subchondral bone defects that was recognized by the student and the supervisor was 38 and 35 respectively (Table 1 & Table 2). However, the supervisor graded far more joints with the subchondral bone defect in combination with subjective OA, while the student graded more joints with the subchondral bone defect without OA (Table 1 & Table 2). If the column with "defect with OA" and the column with "no defect with OA" where compared in table 1 and table 2, the difference between the student and the supervisor in regards to number of joints with OA is 49 and 36 respectively. The subjective degree of OA differed in the higher range as the student gave 10 joints a subjective OA grade of 2 and 3 of the joints a grade of 3 (Table 1). The supervisor graded 6 joints to have a subjective OA grade 2, and 0 joints were graded as 3.

The subchondral defect was seen in 55 different joints when evaluating the joints. In 18 of these 55 joints (32.7%) the supervisor's and the student's assessments corresponded in which joints the defect was seen in (Figure 5). In 75 out of 130 joints (57.7%), both the supervisor and the student agreed that there was no defect detectable. When further comparing the student's and the supervisor's assessments, table 3 shows the joints where agreement and disagreement occurred between the student and supervisor with regards to the occurrence of the defect in relation to OA. In 20 different joints, the student and the supervisor disagreed as to the occurrence of the defect without OA findings. This means that either the student or the supervisor assessed the joint to have only the defect without any OA findings, while the other assessor did not recognise the joint to have the defect or recognised the defect in combination with OA findings. The student recognised the defect without any OA findings in 17 joints while the supervisor assessed the same 17 joints to have either no defect, or the defect in combinations with OA findings. The supervisor recognised the defect with no OA findings in 3 different joints, where the student did not recognise the defect (Table 3). The supervisor's and student's assessments with regards to the defect in joints that was subjectively graded as OA corresponded in 7 out of 18 (38.9%) of the joints, and in 7 out of 18 (38.9%) joints where the defect was seen as the only abnormality (Table 3). In 4 out of 18 joints (22.2%), both assessors agreed that the defect was present, but differed in as to if there was presence of OA or not.

Table 3. *Agreement and disagreement between the student (SW) and the supervisor (KH) in joints with only the defect and joints with the defect and osteoarthritis (OA)*

	Agreed	**Disagreed**		
		SW and KH	SW	KH
Joints with only defect	7	20	17	3
Joints with defect and OA	7	17	3	14
Total	**14**	**37**	**20**	**17**

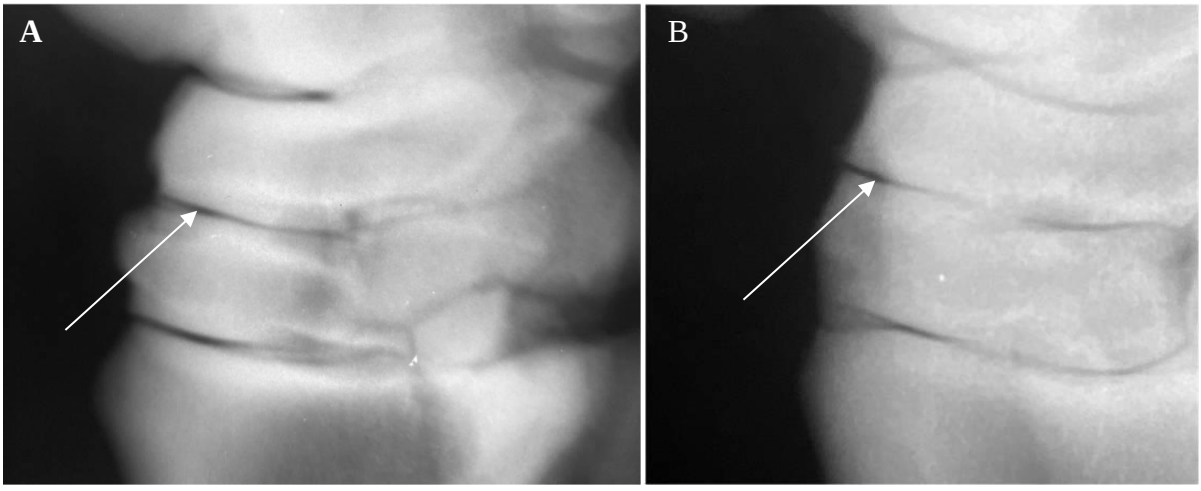

Figure 5. *Radiographs included in the material. (A). LM projection from joint V391 showing a centrodistal (CD) joint with what could be perceived as a subchondral bone defect (arrow). However, this appearance is caused by two parts of the joint compartments being at different levels and not a subchondral bone defect. The student assessed this joint to have a subchondral bone defect in the centrodistal joint, while the supervisor did not. (B). PlLDM-O projection from joint V77 showing a subchondral bone defect in the CD joint (arrow). Both the student and the supervisor assessed this to be a subchondral bone defect. Source: SLU, Diagnostic Imaging.*

With regards to OA, the student and the supervisor agreed upon the occurrence of OA in 8 joints. There were disagreement about the occurrence of OA in 17 different joints, 14 of which the student recognised OA in while the supervisor did not (Table 4).

Table 4. *Agreement and disagreement between the student (SW) and the supervisor (KH) in joints without the defect with or without osteoarthritis (OA)*

	Agreed	**Disagreed**		
		SW and KH	SW	KH
Joints with OA	8	17	14	3
Joints with no OA	50	16	3	13
Total	**58**	**33**	**17**	**16**

Both the student and the supervisor saw the highest number of defects in the PlLDM-O projection. The student saw the lowest number of defects in the DLPlM-O in difference to the supervisor who saw the lowest number of defects in the LM projection. Both the student and the supervisor saw the majority of the defects in the CD joint (Table 5 and Table 6).

Table 5. *Distribution of defects per joint region, centrodistal (CD) and/or tarsometatarsal (TMT) in the lateromedial (LM), dorsolateral-plantaromedial-oblique (DLPlM-O) and plantarolateral-dorsomedial-oblique (PlLDM-O) projections. Note that the same defect can be seen on multiple projections. Students (SW) assessment*

Joints	**LM**	**DLPlM-O**	**PlLDM-O**	**Total**
CD and TMT	1	0	0	**1**
TMT	6	0	3	**9**
CD	13	15	19	**47**
Total	**20**	**15**	**22**	**57**

Table 6. *Distribution of defects per joint region, centrodistal (CD) and/or tarsometatarsal (TMT) in the lateromedial (LM), dorsolateral-plantaromedial-oblique (DLPlM-O) and plantarolateral-dorsomedial-oblique (PlLDM-O) projections. Note that the same defect can be seen on multiple projections. Supervisors (KH) assessment*

Joints	**LM**	**DLPlM-O**	**PlLDM-O**	**Total**
CD and TMT	1	2	2	**5**
TMT	5	2	1	**8**
CD	8	12	17	**37**
Total	**14**	**16**	**20**	**50**

The number of periarticular osteophytes and joints with decreased joint space width recognized in combination with the subchondral bone defect was larger in the assessment by the supervisor (Table 7).

Table 7. *Distribution of single lesions from joints graded to have both the subchondral bone defect and subjective osteoarthritis. Note that some joints may exhibit several lesions from either the same or different categories. PO = Periarticular osteophytes, JSW = Joint space width, SSC = Subchondral sclerosis, E = Entheseophytes, C = Subchondral cyst-like lesion*

	PO	**JSW**	**SSC**	**E**	**C**	**Total**
KH (21 joints)	26	17	3	3	2	51
SW (14 joints)	14	6	5	0	0	25
Total	**40**	**23**	**8**	**3**	**2**	**76**

The appearance of the skeletal structures showed that all horses were adult and skeletally mature when radiographed. During the assessment of the joints there were four occasions when the student assessed joints to be having moderate changes (2) coupled to subchondral sclerosis when the supervisor made the assessment that there was no detectable lesion (0) associated with subchondral sclerosis. In one joint, the student assessed the joint margin width to be clearly narrowed (2) while the supervisor did not detect any visible lesion associated with joint margin width (0). Also, in one case, the student graded a joint to have a moderate grade (2) of subchondral cyst-like lesions while the supervisor assessed the joint to have no subchondral cyst-like lesion. There was two occasions when the supervisor had graded the CD joints to be having clearly narrowed joint margin width, while the student had graded these joints to have no lesions associated with the joint margin width. At one occasion in one joint, a periarticular osteophyte was graded to be of severe (3) nature by the supervisor. This periarticular osteophyte was graded to be moderate (2) by the student. Two times during the assessment, the student identified cyst-like lesions in two joints and no subchondral defect. In the same joints, the supervisor graded the joints to have subchondral defects, but no cyst-like lesions.

When assessing the joint space width, the student and the supervisor had the option to grade either or both the CD and TMT joints to be "not assessable" when they could not with certainty evaluate the structures of interest. There are 260 joint compartments to be evaluated since there are two joint compartments (CD and TMT) of interest for every projection. There's when adding all projections 780 different joint compartments to assess. The student used the "not assessable"-option 167 times on joint compartments in the LM-projections, 215 in DLPlM-O projections and 165 times on the PlLDM-O. The supervisor only used the "not assessable"-option 3 out of 260 on the DLPlM-O projection and 1 out of 260 on the PlLDM-O projection. This option was also available when assessing the subchondral bone defect in each joint compartment. The student regarded 82 of the 260 joint compartments in the LM-projection, 101 out of 260 in the DLPlM-O-projections and 91 out of 260 in the PlLDM-O to be "not assessable" (Figure 6). The supervisor never termed any of the projections "not assessable" with regards to evaluating the presence of a subchondral bone defect.

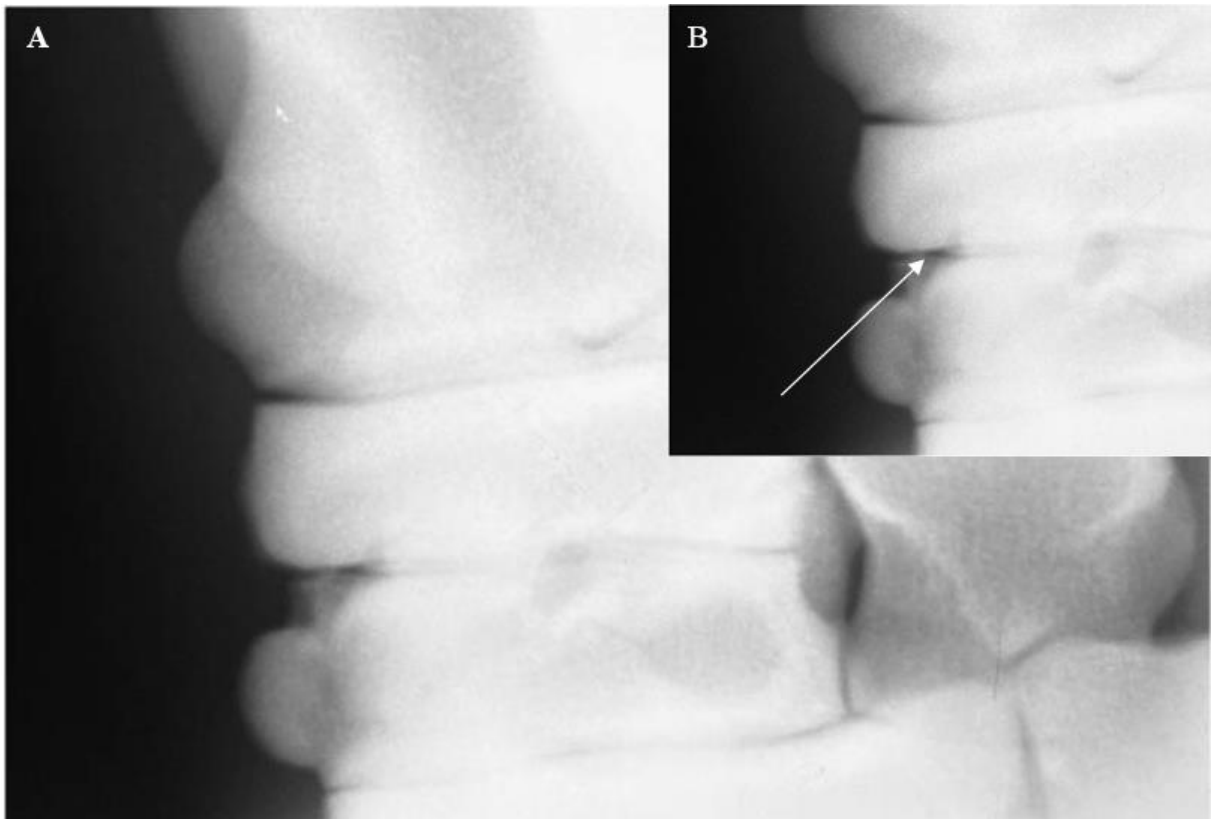

Figure 6. (A*). Radiograph of a DLPlM-O projection of a CD joint with an area at the dorsal aspect showing what could be mistaken for a subchondral bone defect. The distal joint margin in the joint is not completely straight which gives the appearance of what could be perceived as a subchondral bone defect. (B).Same image highlighting the area of interest (arrow).This joint (H247) was assessed not to have a defect by the supervisor while the student chose not to assess this joint compartment with regards to the defect. Source: SLU, Diagnostic Imaging.*

DISCUSSION

The result show that the mineralization front defect/subchondral bone defect can be visualized in the distal tarsal joints of Icelandic horses in a material different from what has been published by Ley *et al*. (2016). Despite the unknown age of the included horses the appearance of the skeletal structures shows that all horses were adult and skeletally mature when radiographed thus forming a different age cohort in comparison with the horses in the study by Ley *et al*. (2016). It is possible that the radiographs might have been used in a study published by Björnsdóttir *et al.* (2000a) where it is stated that the age range was 6–12 years.

The defect was identified both together with OA lesions and as a single finding. Earlier research has shown that the defect can be seen in the CD joint (Ley *et al*., 2016). In this study, both the supervisor and the student could identify the defect also in the TMT joint, however, to a less extent than in the CD joint.

Some joints had variations in the configuration of the CD and TMT joint due to differences in the shape of the third and central tarsal bones, possibly causing the projection being skewed in a distal, proximal, dorsal or plantar angle. This could to some degree have led to false negatives or false positives when evaluating the presence of individual OA signs and the subchondral bone defect. In 75 out of 130 joints (57.7%), both the supervisor and the student agreed that there was no defect detectable. In 55 joints either the student or the supervisor found a defect and in 18 of these joints both the student and the supervisor identified the defect in the same joints. In the remaining 37 other joints the defect were seen by either the student or the supervisor. This means that there was an actual difference in when the defect was graded as being present between the two observers. There are several possible explanations to this difference such as the quality of the images that were assessed and that the supervisor has several years of experience of looking at analogue radiographs.

One must also note that there was no predetermined description to follow with regards to the assessment. There were no description/illustration about how each lesion were to be graded which probably contributes to the variation in assessments between the student and the supervisor. A detailed description and/or illustration of each lesion would likely lower the amount of variation. The assessment was purely subjective and differences in evaluating presence of the defect, individual signs of OA and the subjective total OA grade were unlikely to correspond completely.

The supervisor recognized more periarticular osteophytes and more projections showing decreased joint space width than the student. However, the student opted not to assess a substantial number of joint compartments with regards to the joint space width. In order to visualize the joint margins separately in the distal tarsal joints the projections need to tangentially cross both the proximal and distal margins. If not, superimposition of both the margins will appear on the image. The joints conformation varies with each individual and the joint compartments are not straight horizontal. As for entheseophytes, they may appear in a joint without having any relation to OA. Therefore; joints would not be assessed with a subjective OA grade of 1 or higher if an entheseophyte was recognized as the only finding.

Sclerosis is hard to assess, when assessing one must be aware of the risk of superimposition of several structures. Because of this, subchondral sclerosis as the only finding did not result in a subjective OA grad of 1 or higher.

The student found it difficult to distinguishing what was superimposition and decreased/lack of joint space and therefore opted the choice of “not assessable”. This also applied to the assessment of images when considering the presence of the defect. The student termed a number of joint compartments in images to be "not assessable" also for this category. This also relates to the superimposition of the joint margins in both of the joint compartments which obscures and makes an evaluation more difficult. A more experienced assessor is perhaps more likely to evaluate the joint. As mentioned earlier in the results, the student assessed two joints to have cyst-like lesions and no subchondral defects while the supervisor assessed the same joints to have subchondral lesions and no cyst-like lesions. This highlights the problem with potential confusion between these changes in the joints. Their appearance may resemble each other, and an inexperienced assessor has trouble distinguishing between them.

The subchondral bone defect is as presented in figure 4 can be a subtle change. The angle of the projections and the conformation of the joints may give the appearance of a defect when it´s an optical illusion.

One of the limitations with the study was that the joints that were evaluated further consisted of only three different projections. Four different projections are recommended because of the complex architecture of the joint (Busoni & Audigié, 2018). However, according to Eksell *et al*. (1999), the PlLDM-O projection in combination with any other one of the three projections resulted in a sensitivity of at least 99% in regards to finding OA changes. The LM-projection was also included in this study, which was considered to be the third most informational projection with regards to evaluating the joint by the same authors. With material being retrospectively evaluated, it´s in this case not possible to retake projections to highlight the areas of interest in a more optimal way. Also, analogue images cannot be adjusted and processed afterwards which could make smaller structures harder to assess. Furthermore, there were lack of information on the age and sex of the horses that were included in this study.

Dik et *al*. (1999) studied early radiological signs of osteochondrosis longitudinally in the stifles and hocks of foals between 1 to 11 months of age in many cases, the lesions resolved as the foals grew older. As for the defect to be a potential early biomarker for OA, further research is needed in order to determine the possible longitudinal development of the defect into clinically significant OA. A longitudinal study is needed to determine whether or not the defect has a temporary occurrence and resolves or not. In these studies, knowledge about the age will be useful as well as radiographs taken with predetermined intervals.

POPULAR SCIENCE SUMMARY

Osteoarthritis (OA) in the hock (tarsus) is one of the most common reasons for pain and lameness in the horse. This leads to costs for the owners in order to diagnose and treat the disease, suffering and early culling of the horses. It is therefore of great importance to find a method that is cost effective, readily available and non invasive in order to detect OA at an early stage. If OA is detected at an early stage, owners can adjust the horses life to minimize future pain and also exclude the horse from breeding. The study aims at evaluating the presence of an earlier described and validated change (defect) in the bone tissue inside the tarsus of Icelandic horses. The subchondral bone defect is also evaluated in relation to presence of OA. The defect is a bone defect which is situated beneath the cartilage (subchondral) on the joint surface. The tarsus as a joint region is constituted by the four different joint compartments; the tarsocrural, talocalcaneal-centroquartal, the centrodistal (CD) and the tarsometatarsal (TMT) joints. This study concentrates on the CD and TMT joints due to the high frequency of OA and the defect in these joints. These two joint compartments are situated closest to the hoof in the tarsal region, and are therefore termed the "distal tarsal joints". This basically means that they are farther away from the body with respect to the tarsocrural and talocalcaneal-centroquartal joint compartments. In horses, tarsal pain occurs readily and is a frequent cause for veterinary costs, early culling and of course suffering for the affected horses. Osteoarthritis (OA), which is a disease that through chronic low-grade inflammation deteriorate the whole joint, is the most common reason for pain in the tarsus. Inflammation with thickening joint capsules, tendons, ligaments, degradation of cartilage, new bone formation and narrowed joint space are some of the joint changes associated with the progression of OA. The subchondral bone defect is believed to have a possible relation to OA and could be an early sign of the disease (a biomarker). As a possible biomarker, the subchondral bone defect OA could be identified at an early stage and can therefore exclude the horse from further breeding and adapt the workload for the horse in order to minimize complications which could arise if the disease would go unnoticed at an early stage.

In order to evaluate the presence of the defect in the CD and TMT joints of Icelandic horse´s radiographic images is used. In radiology, ionized electromagnetic radiation is used in order to create two-dimensional images of three-dimensional structures. The images are in black, white and different scales of grey depending on what structures are being examined. A structure, such as bone, will let fewer electrons pass through which makes the image of the bone appear whiter (radio-opaque). On the other end, if a projection was taken in order to evaluate the chest (thorax), the image would appear dark (radio-lucent) because the lungs in the thorax are mostly filled with air. In order to clearly visualize all parts of the structure of interest, one needs to take several images from different angles in order to get a proper presentation of, in this study, the CD and TMT joints.

The material used in this study was radiographic images from 788 joints from 394 horses. The age and sex of the horses were unknown. These images were taken on Iceland by Sigridur Björnsdóttir during a screening program for OA in Icelandic Horses. It was determined that three different projections (which is the angle from which the images are taken from) were required as a minimum for proper evaluation of the joints. The three projections that would be

required for the assessment were the dorsolateral-plantaromedial-oblique (DLPlM-O, taken from an angle slightly in front of and outside at the level of the tarsus), lateromedial (LM, taken from a position outside of the tarsus) and plantarolateral-dorsomedial-oblique (PlLDM-O, taken at an angle slightly behind and outside of the tarsus). The initial evaluation of the images concerned the image quality, if the projections were taken from a correct angle so that the joint could be properly visualised, if all three projections were included and if the joints that were to be assessed had advanced stages of OA or other pathological changes that would obscure the potential presence of the defect. After evaluating the joints with regards to the aforementioned criteria's, 130 joints from 117 different horses were selected for further evaluation by the supervisor (KH) and the student (SW).

The joints that were selected for further evaluation were scrutinized in more detail with respect to the presence of the subchondral bone defect, five different pathological changes related OA and the subjectively assessed level (0-4) of OA in general. Different pathological changes associated with OA were to be graded by both assessors as to if they were present and if so, how severe they were (graded 0-3 where 0 is normal, 1 is mild pathological change, 2 is moderate pathological change and 3 is seen as severe), in which joint compartments (CD or/and TMT), how many lesions of this kind and if they were distributed in a focal or generalized fashion (if they appeared in small area or are widespread throughout the joint). The pathological changes associated with OA that would be taken in consideration during the grading were periarticular osteophytes, decreased/lack of joint space width, subchondral sclerosis, entheseo-phytes and cyst-like lesions. The subchondral bone defect was assessed in the same manner as the OA lesions. Periarticular osteophytes and entheseophytes are new bone formation in the joint area, periarticular osteophytes is more often related to OA, but entheseophytes can also be present without being related to OA. Subchondral sclerosis is basically the increased density of the bone underneath the cartilage in the joint due to increased pressure in the joint, often due to less cushioning effect from the cartilage. With regards to the assessment of the joint space width, as the cartilage breaks down during OA the joint compartment will appear narrower and, in some cases, even lack joint space width. Cyst-like lesions appear as radio-lucent focal areas in the subchondral bone on a radiological image. The appearance of the lesions may be quite reminiscent of the subchondral bone defect, but the cyst-like lesions are situated further down into the subchondral bone and are more irregular in their shape compared to subchondral bone lesions. Both assessors had the option to choose not to evaluate the subchondral bone defect and/or the joint space width if the assessor found it hard to evaluate a joint compartment on a projection.

The results of the study show that the student recognised the subchondral bone defect in 38 out of the 130 joints (29.2%). The supervisor identified the subchondral bone defect in 35 out of 130 joints (26.9%). When comparing the results between the assessors, the subchondral defect was seen in 55 different joints. The supervisor and the student recognised subchondral defects in the same joints on 18 occasions out of these 55 joints (32.7%). In 7 joints out of 18 (38.9%) both the supervisor and student recognised the subchondral bone defect as the only finding. In 7 joints out of 18 (38.9%) both the supervisor and the student recognised the defect along with OA changes. In 4 out of 18 (22.2%) cases both assessors identified the defect, but had different assessments as to the presence of OA. Both the student and the supervisor identified most

subchondral bone defects in the PlLDM-O projections. The subchondral bone defect has previously been described in the CD joint. The result in this study suggests that the subchondral bone defect can be seen in the TMT joint as well.

The results show that the subchondral bone defect can be seen in the material evaluated. There was an actual difference in which joints the subchondral bone defect was observed by the assessors. There could be several reasons for this. One is that there was no consensus discussion before or after with regards to interpretation of projections/joints. There was also a lack of description of the different types of lesions being assessed which might have caused an increased variation. The student and the supervisor have different levels of experience in assessing analogue radiographic images which may have contributed to the varying assessments. This may have been reflected in that the student choose not to assess joint compartments with regards to the subchondral bone defect and the joint space width to a larger extent than the supervisor. The results show that the subchondral bone defect can be seen in the material evaluated. However, more research is needed in order to find out if the subchondral defect is a biomarker for OA.

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