





The proximal aspect of the dorsal condylar sagittal ridge and the adjacent soft tissues in the fetlock joint of the Warmblood horse:

Morphology and relationship with cartilage degeneration

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List of abbreviations

CDI Cartilage degeneration index

CT Computed tomography

DICOM Digital imaging and communications in medicine

MCIII Third metacarpal bone
MCP Metacarpophalangeal

MRI Magnetic resonance imaging

MTIII Third metatarsal boneMTP MetarsophalangealP1 Proximal phalanx

PDW Proton density weighted

s.d. Standard deviation

SE Spin echo

STIR Short T1-inversion recovery

T Tesla

T1W T1 weighted
T2W T2 weighted
TE Echo time

TR Repetition time
US Ultrasonography

Abbreviations of the radiographic projections

D125°Di-PaPrO Dorsal 125° distal-palmaroproximal oblique

D35°Di-PaPrO Dorsal 35° distal-palmaroproximal oblique

D45°L-Pa(Pl)MO Dorsal 45° lateral-palmaro(plantaro)medial oblique D45°M-Pa(Pl)LO Dorsal 45° medial-palmaro(plantaro)lateral oblique

DPa(PI) Dorsopalmar(plantar)

DPr-DDiO Dorsoproximal-dorsodistal oblique

LM Lateromedial

Preface

Today, a pre-purchase examination is recognised as one of the most important services offered by an equine practitioner, assessing the risk of buying a horse. This examination is performed to identify any abnormalities or potential problems that would make the horse unsuitable for its intended use.

Radiography has become an integral part of this pre-purchase examination to detect any potential or actual orthopaedic problems. Skeletal lesions can be present during a radiographic screening even when the horse is clinically sound. Some detected lesions should not interfere with future performance; others may limit the horse's ability to work or cause lameness. Therefore, it is important to identify any abnormal radiographic findings and to try to predict if they will correlate with future lameness. In case the veterinarian makes a mistake in interpreting the clinical relevance of the detected lesions, the economic and legal consequences may be important.

Variation in the radiographic appearance of the proximal aspect of the dorsal condylar sagittal ridge in the equine MCP/MTP joint is detected in Thoroughbreds. These do not interfere with their future sports career. However, due to the difference in type and length of sports career, a simple extrapolation of the conclusions drawn for Thoroughbreds to Warmbloods is not possible. This makes the assessment of the importance of these variations challenging when detected during a pre-purchase examination of a Warmblood horse.

Therefore, the need for an improved knowledge of the morphological appearance of these variations at the level of the proximal aspect of the dorsal condylar sagittal ridge in Warmbloods, as well as the possible interaction with the surrounding soft tissues and detrimental effects at the level of the joint is essential.

Chapter 1 The equine metacarpo/metatarsophalangeal joint

Chapter 1.1 Anatomy of the equine metacarpo/metatarsophalangeal joint

Adapted from:

Hauspie S., Declercq J., Martens A., Zani D.D., Bergman E.H.J., Saunders J.H. (2011) Anatomy and imaging of the equine metacarpophalangeal/metatarsophalangeal joint. *Vlaams Diergeneeskundig Tijdschrift* 80, 263-270.

The anatomic terminology used in italic between brackets herein conforms to that listed in the Nomina Anatomica Veterinaria (international Committee on Veterinary Gross Anatomical Nomenclature and General Assembly of the World Associoation of Veterinary Anatomists, 2012).

The MCP/MTP joint (articulation metacarpophalangea et metatarsophalangea) comprises of four bones: the MCIII (os metacarpale III) or MTIII (os metatarsale III) bone, P1 (phalanx proximale) and the paired proximal sesamoid bones (ossa sesamoidea proximalia) (Fig. 1). The MTIII is longer; stronger and slightly more flattened in a dorsoplantar direction in its distal third compared to the MCIII. The length of the lateral cortex of the cannon bone is longer than the length of the medial one, resulting in a slight oblique orientation of the distal articular surface of the cannon bone compared to the proximal articular surface. The distal epiphysis of the MCIII/MTIII has two unequal convex condyles and a sagittal ridge that separates them. The medial condyle is slightly bigger compared to the lateral one. This distal epiphysis articulates with P1 distally and with the proximal sesamoid bones palmarly/plantarly (Barone 1986; Alrtib et al. 2012).

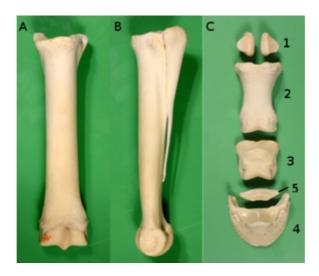


Figure 1. Illustration of the bones of the distal front limb: A) Dorsal view of the third metacarpal bone, B) Lateral view of the third metacarpal bone with at its palmar aspect a splint bone, C) dorsal view of 1: the proximal sesamoid bones, 2: the proximal phalanx, 3: the middle phalanx, 4: the distal phalanx, 5: the distal sesamoid bone or navicular bone (Vakgroep morfologie, faculteit Diergeneeskunde).

An articular capsule *(capsula articulares)* and multiple ligaments reinforce the MCP/MTP joint (Fig. 2). The ligaments can be divided into three main groups: the MCP/MTP ligaments, the sesamoidean ligaments and the palmar/plantar (intersesamoidean) ligament.

The MCP/MTP ligaments are subdivided into the collateral ligaments (*ligg. collateralia*), the dorsal reinforcement of the articular capsule and the suspensory ligament (*m. interosseus*). Each collateral ligament has a superficial and a deep part. The superficial part is the longest and strongest, running approximately in a vertical direction. Its origin is located at the lateral or medial aspect of the MCIII/MTIII, just distal to the distal tip of the splint bones and it is attaching at the proximal aspect of P1. The deep part is more triangular shaped, having its origin at the abaxial condylar fossa, running in a distopalmar/plantar direction and inserts on P1. The deep part of the collateral ligaments is covered by synovium at its deepest border. The dorsal fibrous reinforcement of the articular capsule has fibres running in different directions. At the lateral and medial aspect of the MCP/MTP joint it fuses with the respective collateral ligament. The lateral and medial branch of the suspensory ligament inserts at the apical and abaxial border of the proximal sesamoid bones. At the level of their attachment, they form a small "extensor" tendon that runs in a dorsodistal direction and fuses dorsally with the common (front limb) or long (hind limb) extensor tendon at the level of P1 (Barone 1989; Weaver *et al.* 1992; Vanderperren *et al.* 2008).



Figure 2. Overview of the ligaments and tendons surrounding the metacarpophalangeal joint. 1: the common extensor tendon, 2: the lateral digital extensor tendon, 3: the lateral collateral ligament, 4: the suspensory ligament, 5: the "extensor" tendon of the suspensory ligament, 6: the superficial digital flexor tendon, 7: the deep digital flexor tendon, 8: the manica flexoria, 9: the oblique sesamoidean ligament (Vakgroep morfologie, faculteit Diergeneeskunde).

The sesamoidean ligaments are subdivided into the collateral sesamoidean ligaments (ligg. sesamoidea collateralia) (Fig. 2) and the distal sesamoidean ligaments (Fig. 3). The

(lateral and medial) collateral sesamoidean ligaments course from the abaxial surface of the proximal sesamoid bones to MCIII/MTIII and the tuberosity of P1 (Barone 1989; Vanderperren et al. 2008). The distal sesamoidean ligaments are organised in multiple layers. The most superficial located straight sesamoidean ligament (lig. sesamoideum rectum) originates from the base of the proximal sesamoid bones and the palmar intersesamoidean ligament and inserts on the second phalanx (phalanx media). The intermediate located oblique sesamoidean ligament (lig. sesamoidea oblique) originates just dorsal to the straight sesamoidean ligament at the base of the proximal sesamoid bones (medial and lateral bundle) and the palmar intersesamoidean ligament (thin sagittal part), running distally and inserting on the palmar surface of P1. The most deeply located are the short and cruciate sesamoidean ligaments (ligg.sesamoidea brevia et cruciata). These latter are crossed, originating at the axial part of the base of the proximal sesamoid bones to the contralateral axial aspect of P1. The short distal sesamoidean ligaments extend from the dorsal aspect of the base of the proximal sesamoid bones to the palmar margin of the articular surface of P1. The short and cruciate distal sesamoidean ligaments form the palmar/plantar wall of the MCP/MTP joint (Barone 1989; Vanderperren et al. 2008).



Figure 3. Overview of the sesamoidean ligaments and suspensory ligament. 1: the straight sesamoidean ligament, 2: the oblique sesamoidean ligament, 3: the cruciate sesamoidean ligaments (after removal of the straight sesamoidean ligament), 4: the palmar intersesamoidean ligament, 5: the 2 branches of the suspensory ligament, 6: collateral sesamoidean ligament (Vakgroep morfologie, faculteit Diergeneeskunde).

The palmar intersesamoidean ligament (*lig. palmaria*) (Fig. 3) is thicker and forms at its dorsal aspect the groove in between both proximal sesamoid bones. At its palmar/plantar aspect, it covers almost completely the axial margins of both proximal sesamoid bones, forming the proximal scutum (*scutum proximale*), over which the flexor tendons slide (Barone 1989).

The articular capsule is formed by an outer stratum fibrosum, strengthened by the above-mentioned ligaments, and an inner stratum synoviale, responsible for the homeostasis of the synovial fluid. The MCP/MTP joint has a small dorsal recess (recessus dorsales) and a large palmar/plantar recess (recessus palmares/plantares). In the dorsoproximal recess of the MCP/MTP joint, the synovium and fibrous connective tissue forms a fold (plica) (plica synovialis), projecting distally from the dorsoproximal attachment of the joint capsule and tapering to a thin edge. This covers the transition zone between the condylar cartilage and the attachment of the joint capsule (Fig. 4) (Dabareiner et al. 1996). This synovial plica has a fibrous structure with a linear arrangement of fibrous connective tissue containing a small number of blood vessels. The edges are covered by squamous to low-cuboidal cells up to three cells thick, which represent the synovium (Steyn et al. 1989; White 1990).

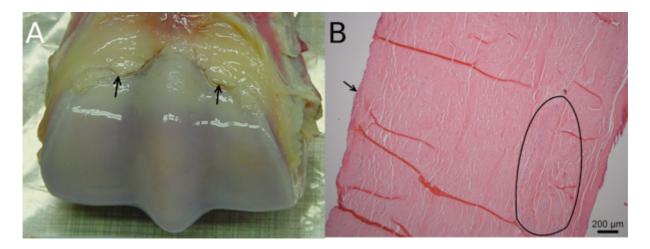


Figure 4. Illustration of the synovial plica: A) The synovial plica in situ at the dorsoproximal aspect of the distal metacarpal condyle (arrows), B) Histological detail illustrating the fibrous structure with a linear arrangement of fibrous connective tissue containing a small number of blood vessels (black oval) and covered by synovium (arrow).

The extensor tendons are located at the dorsal aspect of the MCP/MTP joint (Fig. 2). In the front limb, both the common and lateral digital extensor tendons (m. extensor digitorum communis; m. extensor digitorum lateralis) are present, whereas in the hind limb, the long

and lateral digital extensor tendon (m. extensor digitorum longus; m. extensor digitorum lateralis) are already fused at the level of the mid MTIII, resulting in only one tendon at the dorsal aspect of the MTP joint. At the palmar/plantar aspect of the MCP/MTP joint the superficial and deep digital flexor tendon (m. flexor digitorum superficialis, m. flexor digitorum profundi) run in the digital sheath (vag. synovialis tendinum digitorum manus/pedis) (Fig. 2). The superficial digital flexor tendon is more flattened and just proximally to the proximal sesamoid bones it forms the manica flexoria (manica flexoria), which surrounds the deep digital flexor tendon. Distal to the MCP/MTP joint, the superficial digital flexor tendon splits in a medial and lateral branch, inserting just medial and lateral to the straight sesamoidean ligament on the second phalanx. The deep digital flexor tendon, located just dorsal to the superficial digital flexor tendon, is more oval shaped proximal to the MCP/MTP joint, whereas it has a more bilobed appearance distally.

Chapter 1.2

The use of different imaging modalities in a pre-purchase examination

Adapted from:

Hauspie S., Declercq J., Martens A., Zani D.D., Bergman E.H.J., Saunders J.H. (2011) Anatomy and imaging of the equine metacarpophalangeal/metatarsophalangeal joint. *Vlaams Diergeneeskundig Tijdschrift* 80, 263-270.

Introduction

The MCP/MTP joint of the horse is prone to injury. It has a relatively small surface area to transmit the body weight of the horse and it has the highest range of motion of any of the limb joints, and therefore sustains the greatest forces of acceleration of any of the joints (Pool and Meagher 1990). Lameness attributable to the MCP/MTP joint is a frequent cause of early retirement from athletic career in horses and should therefore be detected as early as possible (Rossdale *et al.* 1985; Santschi 2008).

Jumpers and dressage horses are often considered as an investment rather than an avocation and owners have great expectations of the performance and physical condition of their horse. On the other hand, they have less understanding of the uncertainties and the limitations of a pre-purchase examination (Marks 1999). To address this, good communication with the (potential) horse owner is essential and a thorough and standardized pre-purchase examination is needed (Suslak-Brown 2004; Mitchell 2009). The basis of this pre-purchase examination is a thorough clinical and physical examination (Marks 1999). This clinical examination is completed with a radiographic examination for screening purposes. Additional imaging techniques can be used if abnormalities are detected either during the clinical or radiographic examination. The decision of what additional imaging technique to use needs to be faced upon the professional assessment of the horse by the veterinarian and sound economic considerations (Mitchell 2009).

Radiography has presently become an integral part of the pre-purchase examination (Suslak-Brown 2004). The radiographic examination can reveal findings inconsistent with the clinical examination, advocating further radiographic images or other imaging techniques such as ultrasound, scintigraphy or magnetic resonance imaging. These techniques can be used to gain more information about the significance of a lesion detected on radiographs (Van Hoogmoed *et al.* 2003; Mitchell 2009). Computed tomography is as additional imaging technique valuable however, due to the need for general anaesthesia, this technique is less commonly used in a pre-purchase examination.

Radiography

Radiography is a relative low cost, widely available technique and very effective for the evaluation of bony structures. Radiography is based on the principle that x-rays, produced by accelerating electrons onto a tungsten target, penetrate an object placed in the path of the x-ray beam. The obtained image is dependent on the total number of x-rays produced, the distance from the focal spot to the film and the ability of the x-rays to penetrate the tissue (Butler *et al.* 2000b).

Over the past decade, digital radiography has largely replaced conventional radiography. Digital radiography represents on the one hand a higher investment cost than an analogue system, but on the other hand, it has several distinct advantages: less film waste, lesser films per examination due to the extra possibilities for image post-processing and improvement of the image quality due to the almost instantaneous acquisition time (images can be reviewed on site). For the correct interpretation of digital radiographs, recognition of the specific artefacts associated with digital radiography is required (Dalla Palma 2000; Mcknight 2004; Mattoon 2006; Jimenez *et al.* 2008; Pilsworth and Head 2010).

The regions that are imaged during a pre-purchase examination as well as the obtained projections of that region are depending on the breed and the purpose of the horse. The standard projection of the MCP/MTP during a pre-purchase examination of a Warmblood horse is often limited to a LM projection (Verwilghen *et al.* 2009). However, up to 4 projections of the MCP/MTP joint can be advocated: LM, D45L-Pa(Pl)MO, D45M-Pa(Pl)MO, DPa(Pl) (Fig. 5) (Poulos 1992; Richard and Alexander 2007).

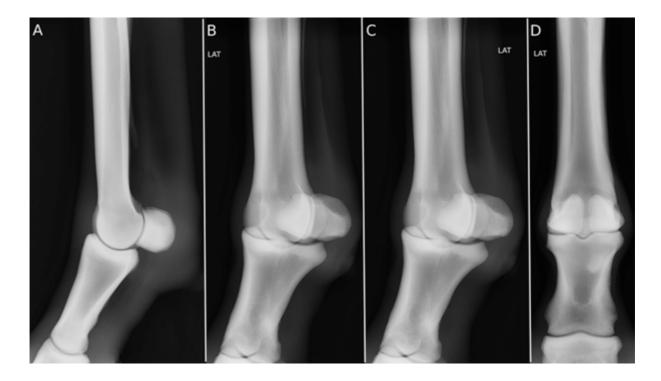


Figure 5. The normal radiographic appearance of the metacarpo-/metatarsophalangeal joint: A) Conventional LM projection, B) D45L-Pa(Pl)MO projection, C) D45M-Pa(Pl)LO projection, D) DPa(Pl) projection.

The LM projection is performed with the horse weight-bearing and a horizontal x-ray beam parallel to the heel bulbs. However, if there is some rotation of the distal limb, the position of the MCP/MTP joint relative to the foot should be evaluated, sometimes necessitating to angle the line 5° towards palmar/plantar (Edwards 1984; Butler et al. 2000a). The condyles of the MCIII/MTIII bone and proximal sesamoid bones should be superimposed on each other and the MCP/MTP joint space should be identifiable (Fig. 6) (Park 2000). The standard oblique projections are made with the primary beam orientated at an angle of 45° (medial or lateral) to the sagittal plane. This angle can be adapted depending on the lesion identified or suspected on the initial LM projection (Edwards 1984). dorsomedial/dorsolateral aspect of P1 and the borders of the proximal sesamoid bones, superimposed on the distal aspect of the MCIII/MTIII bone, should be identifiable on the oblique projections. Superimposition of the base of the sesamoid bones over the proximal palmar/plantar process of the P1 should be avoided (Fig. 7) (Park 2000). The DPa/DPl projection is performed with a horizontal x-ray beam, centred on the joint. The superimposition of the proximal sesamoid bones over the joint space can be avoided by angling the x-ray beam proximodistally (approximately 10° for the DPa projection; 15° for the DPI projection) (Fig. 8) (Butler et al. 2000a).



Figure 6. Illustration of: A) A LM projection with superimpostion of the distal third metacarpal condyles on each other, allowing evaluation of the dorsal aspect of the sagittal ridge and B) A slight oblique projection, prohibiting evaluation of the dorsal aspect of the sagittal ridge.

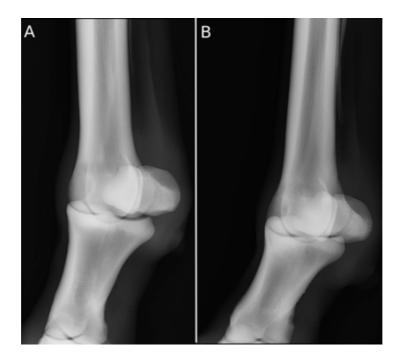


Figure 7. Illustration of: A) A D45L-PaMO projection with no superimposition of the base of the proximal sesamoid bone on the proximal palmar process of the proximal phalanx, B) A D45L-PaMO projection where the base of the proximal sesamoid bone is superimposed onto the proximal palmar process of the proximal phalanx.



Figure 8. Illustration of the contrast in visualization of the joint space between: A) Normal DPa(Pl) projection and B) Angled DPr/Pa(Pl)DiO projection, by avoiding the superimposition of the proximal sesamoid bones onto the joint space.

In case of detected pathology, additional projections, like a flexed LM or D125°Di-PaPrO or flexed D35°Di-PaPrO or flexed DPr-DDiO (Fig. 9), can be obtained to highlight specific areas of the joint, respectively the distal aspect of the sagittal ridge (flexed LM) and palmar (DDi-PaPrO), central (D35°Di-PaPrO), dorsodistal (DPr-DDiO) articular surface of the MCIII/MTIII bone (Richard and Alexander 2007). The decision on which additional projection is the most suitable depends on the problem suspected in the individual case. However, these projections are technically challenging and represent an additional exposure risk. Therefore, more advanced imaging techniques can be used, such as ultrasound, scintigraphy or MRI, to evaluate the above-mentioned regions, thus obliterating the extra radiation hazard for the practitioner (Mcdiarmid 1995; Denoix *et al.* 1996).

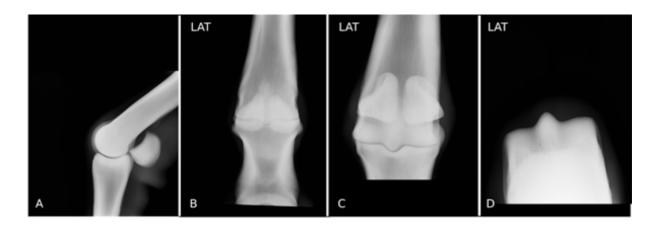


Figure 9. Illustration of additional projections, highlighting the different regions of the metacarpal condyle: A) Flexed LM, B) D125°Di-PaPrO, C) Flexed D35°Di-PaPrO, D) Flexed DPr-DDiO.

Additional positive and negative contrast media can be used to delineate soft tissue structures, not readily identified on survey radiography. The negative contrast agent most commonly used is air. Positive contrast agents for intra-synovial use are most often water-soluble iodinated media. Arthrography is useful in defining the margins of the joint capsule and articular cartilage, possibly highlighting synovial proliferation, articular cartilage defects, traumatic ruptures of the articular capsule or abnormal communication or herniation of the joint capsule. Tendinography or tenography can be used to diagnose tendon adhesions, tendon sheath communication with a joint, tendon rupture and inflammatory lesions (Lamb 1991; Watson and Selcer 1996).

On normal LM radiographs, the joint surface of the distal MCIII/MTIII bone describes a smooth curve, with a mild flattening in its palmarodistal aspect. In some horses, the distal metaphysis of the MCIII/MTIII bone shows some irregularity at the level of the fused physis. A mild remodelling (osteophytosis and/or enthesiophytosis) of the dorsoproximal aspect of P1 is a common finding in older horses, but can also be an early sign of degenerative joint disease (Butler *et al.* 2000a). On a DPa/DP1 projection, the joint should be approximately symmetrical, with the medial condyle being slightly wider than the lateral. The joint space should be approximately perpendicular to the long axis of the MCIII/MTIII. A clear demarcation between the subchondral bone plate of P1 and the underlying cancellous bone should be present. On the oblique projections the proximal sesamoid bones have a smooth outline of their palmar/plantar aspect. Their axial and abaxial surfaces may show some unevenness on a DPa(Pl) projection due to the insertion of ligaments. However, a marked roughening at that level is abnormal (Butler *et al.* 2000a).

Opinions differ about the interobserver and intraoberserver agreement of interpretation of radiographic images. Some state an acceptable to excellent interobserver agreement (Weller *et al.* 2001; White *et al.* 2008), while others state the opposite (Labens *et al.* 2007). A good to excellent intraobserver agreement has been mentioned (Labens *et al.* 2007; White *et al.* 2008). However it appears that this agreement is both for intra- as well as for interobserver agreement depending on the evaluated parameter on the radiograph (Groth *et al.* 2009).

Ultrasonography

Ultrasonography is a useful imaging modality for the investigation of joint abnormalities as it enables the evaluation of soft tissue components of the joint and provides information on the regularity of the bony contours (Redding 2001a; Smith 2008).

This technique uses high frequency waves produced by a transducer. The transducer converts electrical signals into ultrasound waves, and vice versa for the reflected ultrasound waves. When placing the transducer on the skin, pulses of ultrasound are sent into the tissues. Based on the different tissue interfaces, echoes are reflected back to the transducer. These echoes are processed into an electric signal, which is converted to an image. The time that an echo needs to return to the transducer determines the distance from the probe. In the resulting image, this echo is represented by a dot, creating the anatomical echo-generated image. The brightness of the dot depends on the strength of the echo. The physical interactions of sound with the tissues determine the appearance of the ultrasound images. At the boundary of 2 materials with different acoustic impedances some of the energy of the ultrasound waves will be reflected back to the transducer while the remainder of the energy is transmitted through the second tissue type. At soft tissue to soft tissue interfaces, most of the energy of the ultrasound wave is transmitted deeper; an interface between bone and soft tissue reflects approximately 50% of the energy while between soft tissues and air, almost 99.9% of the energy is reflected. This necessitates the removal of air between the transducer and the patient (Martin and Ramnarine 2003).

For optimal ultrasonographic examination, the joint should be clipped, cleaned and coated with conducting gel (Redding 2001a). The MCP/MTP joint can be examined with a high frequency (7.5-10 Mhz) linear transducer. The use of a standoff pad is helpful to increase the contact between the probe and the skin and therefore to enlarge the acoustic window as

well as to better evaluate the profile of the skin. The MCP/MTP joint can be approached in 6 steps.

With the dorsal approach the dorsal compartment of the MCP/MTP joint can be evaluated: the tendon of the dorsal extensor of the phalanx, articular capsule, proximal synovial plica, dorsal recess of the joint, the joint space, articular cartilage and the bony structures (Fig. 10). The dorsal approach with the limb in flexion allows evaluation of the articular cartilage and subchondral bone surface of the most distal part of the MCIII/MTIII, which cannot be evaluated with the limb weight bearing. The subchondral bone should be smooth and the thickness of the proximal synovial plica should be less than 5 mm. The articular cartilage is seen as a hypoechoic image between the echoic joint capsule and subchondral bone. In normal circumstances there should be a distinct soft tissue-cartilage interface. However, in case of absence of synovial fluid between joint capsule and articular cartilage, this interface can be difficult to appreciate (Redding 2001b; Smith and Smith 2008).

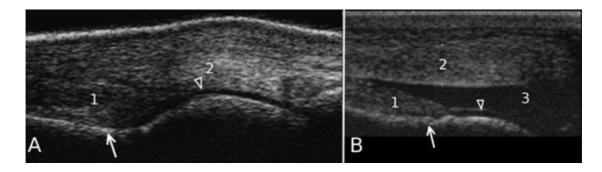


Figure 10. Illustration of the dorsal approach of the metacarpophalangeal joint: A) No distension of the joint, B) Distension of the joint, illustrating the better visualisation of the proximal synovial plica and interface of the articular cartilage when the joint is distended. 1: the synovial plica, 2: the articular capsule, 3: synovial fluid. The arrowhead is illustrating the soft tissue-articular cartilage interface, the arrow is highlighting the suhchondral bone.

With a lateral and medial approach a part of the dorsal joint capsule, the collateral ligaments, the collateral sesamoidean ligaments and the surrounding bony structures can be evaluated. The collateral ligaments should be comparable in thickness, with a parallel fibre patterns and uniform echogenicity (Fig. 11).

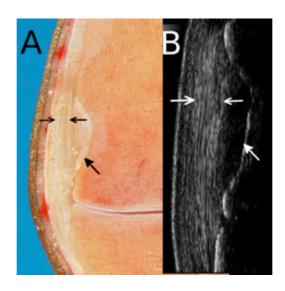


Figure 11. Illustration of the lateral approach of the metacarpophalangeal joint: A) Anatomical specimen, B) Corresponding ultrasound image. The superficial part of the collateral ligament is present between the arrows. The arrow with filled arrowhead is located at the level of the abaxial condylar fossa, the origin of the deep part of the collateral ligament.

Using a plantaro/palmaro-medial/lateral approach, the sesamoidean ligaments, branches of the suspensory ligament, proximal sesamoid bones, medial and lateral part of the oblique sesamoidean ligament, plantaro/palmaro-medial/lateral aspect of the MCIII/MTIII and P1 can be evaluated. These ligaments should present a parallel fibre pattern and uniform echogenicity.

With a palmar/plantar approach the proximal palmar/plantar recess of the joint, the palmar/plantar aspect of the MCIII/MTIII, the deep en superficial digital flexor tendon, annular ligament, proximal sesamoid bones, digital sheath and intersesamoidean ligament can be evaluated proximal to the ergot (Fig. 12); distal to the ergot, at the level of the pastern, the palmar/plantar aspect of the joints space, the straight sesamoidean ligament, medial and lateral parts of the oblique sesamoidean ligaments and cruciate ligaments can be imaged (Fig. 13). The short sesamoidean ligament cannot be evaluated. (Denoix *et al.* 1996; Busoni 2001; Smith and Smith 2008; Smith 2008).

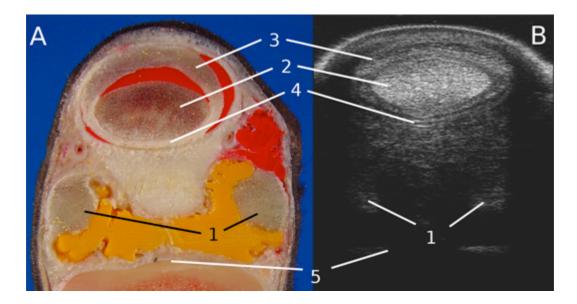


Figure 12. Illustration of the palmar approach of the metacarpophalangeal joint, just proximal to the proximal sesamoid bones: A) Anatomical specimen, B) Corresponding ultrasound image. 1: the branches of the suspensory ligament, 2: the deep digital flexor tendon, 3: the superficial digital flexor tendon, 4: the manica flexoria, 5: the palmar cortex of the third metacarpal bone.

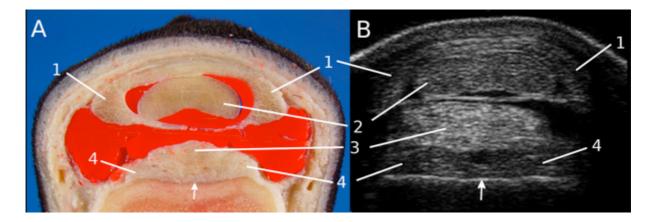


Figure 13. Illustration of the palmar approach of the metacarpophalangeal joint, at the mid aspect of the proximal phalanx: A) Anatomical specimen, B) Corresponding ultrasound image. 1: the medial and lateral branch of the superficial digital flexor tendon, 2: the more bilobed shape of the deep digital flexor tendon, 3: the straight sesamoidean ligament, 4: the medial and lateral part of the oblique sesamoidean ligament. The arrow is highlighting the palmar cortex of the proximal phalanx.

Dynamic ultrasonographic examination of the MCP/MTP joint allows a better evaluation of the joint capsule by eliminating hypoechoic relaxation artefacts. Flexion and extension can also be helpful in demonstrating the mobility of an osteochondral fragment and in evaluating fluid movement (Modransky *et al.* 1983; Denoix 1996; Reef 1998; Redding 2001a; Vanderperren *et al.* 2009). The comparison of the same structure with the contralateral

limb improves the sensitivity and specificity of the ultrasonographic diagnosis (Denoix and Audigie 2001; Redding 2001a).

Ultrasound is known to be a very operator depended technique. It is demonstrated that significant differences are present for the evaluation of a lesion between operators (Pickersgill *et al.* 2001). Therefore, if a difficult problem is expected, it is sometimes better to ask a more experienced colleague to help (Mitchell 2009).

Ultrasound can be used to complement a pre-purchase examination. However, it is best to inform the owner of the technical limitations before the exam and have a clear understanding of the expectations of the owner. Performing ultrasonography during a pre-purchase examination can pose technical difficulties if the horse is presented with long hair. Only in fine haired horses, it is possible to perform the examination without clipping. If the owner is unwilling to allow the horse to be clipped when needed, it is better not to perform the examination. With an ultrasound examination, suspected joint problems can be evaluated more completely if suspicious findings occur during the clinical or radiographic examination (Mitchell 2009).

Scintigraphy

The basic principle of nuclear scintigraphy is the detection of gamma-rays, emitted during the decay of a radionuclide, by a gamma camera. This radionuclide is attached to a tracer, together called a radiopharmaceutical, which is most commonly injected IV. Other, less commonly used methods are subcutaneous injections or inhalation. The most commonly used radioisotope in equine is technetium 99m. Technetium 99m has a short physical half-life of approximately 6 hours. This together with the low energy of the gamma rays, results in a low radiation dose for the patient. On the other hand, the energy value of 140 keV of technetium 99m allows sufficient gamma-radiation to escape the patient. The choice of tracer depends on the targeted organ to be examined. For bone imaging, technetium 99m is usually methylene diphosphonate or hydroxymethylene diphosphonate. bound to diphosphonate salts binds to hydroxyapatite in the bone and their accumulation in a specific area is relative to blood flow to the bone and metabolic activity of the bone. The gamma camera consists of a lead collimator, a gamma sensitive sodium iodide crystal and a photomultiplier tube. The collimator allows only those rays moving parallel to its holes to reach the crystal, which are only a fraction of the radiation leaving the horse. By eliminating

the scatter radiation, the origin of the gamma radiation can be determined and positioned correctly in the resulting image. The gamma rays interact with the sodium iodide crystal and their energy is converted into light. This emitted light is detected and converted to electrical pulses by an array of photomultiplier tubes. These electrical pulses are converted into an image in terms of where in the crystal the light is formed and, indirectly, where the radiopharmaceutical is located in the patient (Driver 2003; Twardock 2003). Nuclear medicine can be divided into 3 phases. The vascular phase (or phase I) images are acquired immediately after injection and highlight the radiopharmaceutical as it courses through the blood vessels. Pool-phase (or phase II) images are acquired within fifteen to twenty minutes after injection, while most of the radiopharmaceutical is in the soft tissues. Bone-phase (or phase III) images are acquired two hours after injection to allow the radiopharmaceutical to bind to the bone and clear from the soft tissues (Chambers et al. 1995). It is important to realize that an area of increased radiopharmaceutical uptake, reflecting an area with increased blood flow or osteoblastic activity, does not necessarily reflect bone pathology, as it can also represent bone remodelling due to biomechanical loading or development (Fig. 14). A standard pattern of radiopharmaceutical uptake in the MCP/MTP joint has been established in non-lame horses without a clear variation over age (Fig. 14) (Weekes et al. 2004) and significant differences are present compared to lame horses (Biggi et al. 2009). Scintigraphy is a highly sensitive method to localize a region with a potential problem, and allows to detected remodelling or lesions before they are radiographically evident (Chambers et al. 1995). However, because of the low specificity, the result must always be interpreted together with the result of the clinical examination and other imaging modalities in order to avoid misinterpretation (Weekes et al. 2004). There is an excellent agreement between observers for assessing relevant increased radiopharmaceutical uptake (Weller et al. 2001).

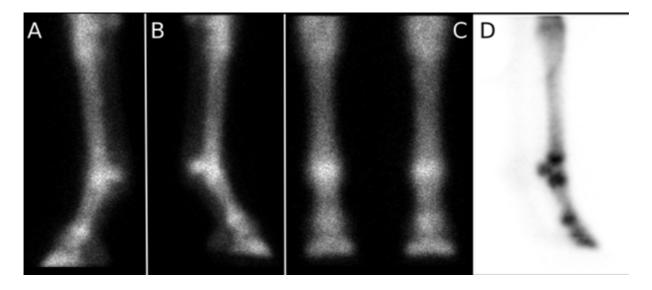


Figure 14. Illustration of pattern of normal radiopharmaceutical uptake at the level of the metacarpophalangeal joint: A) Left lateral view, B) Right lateral view, C) Dorsal view of a normal uptake, D) Pattern of uptake in a young foal with a normal increased radiopharmaceutical uptake at the level of the physis. (A, B, C) Courtesy of Strömsholm Equine Referral Hospital, Strömsholm, Sweden.

The use of scintigraphy during a pre-purchase examination is mainly advocated when worrisome bony findings in the absence of lameness are detected. In such cases, scintigraphy can be helpful in evaluating the significance of the detected findings (Martinelli 2006; Mitchell 2009).

Magnetic resonance imaging

The basic principle of MRI is that in the presence of a static magnetic field, nuclei become sensitive to oscillating magnetic fields and resonate in a synchronized manner. A nucleus is the dense centre of an atom and consists out of protons (positive electrical charge) and neutrons (neutral charge). Atoms who have a nucleus with an odd number of protons will have a spin, and this spin will create a detectable magnetic field. Hydrogen is the most important atom for magnetic resonance imaging, because the nucleus of hydrogen consists of an odd number of protons and hydrogen is abundantly present in organic tissues. Because of its spin and thus magnetic field an atom will interact with an externally applied magnetic field. However, due to its own spin, the nucleus is forced to move at right angles of the applied force (external magnetic field) like a gyroscope, oscillating at a certain frequency ("precession"). If a second external magnetic field is applied at right angles to the first with the same "precession" as the nucleus, this latter will also interact with the second magnetic

field, which causes the magnetization to tip over. If this second magnetic field is stopped, the nucleus will go back to it first state, releasing its excess energy as a radiofrequency signal, which can be detected and converted into an image (Bolas 2011).

The time constant for the nucleus to go back to its original alignment with the main external field is called T1, the process is called T1 relaxation or spin-lattice relaxation. During this relaxation the nuclei will release their excessive energy in the surrounding environment or lattice. This T1 constant is faster in fat, slower in fluid (water). On a T1 weighted image, water will appear hypointens (or black); while fat will appear hyperintens (or white) (Fig. 15). During the application of the second magnetic field, individual nuclei are also "precessing" together. If the second magnetic field stops, they will gradually lose synchronization. This is called spin-spin relaxation and makes the main contribution to the relaxation time T2. This T2 relaxation time is much longer in mobile fluids, making that there is an increased T2 time in tissues with increased water content. On a T2 weighted image water will appear hyperintens (white) (Fig. 15) (Bolas 2011). Signal intensity varies widely in different tissues, due to differences in proton density. This determines the tissue's signal intensity (Kraft and Gavin 2001).

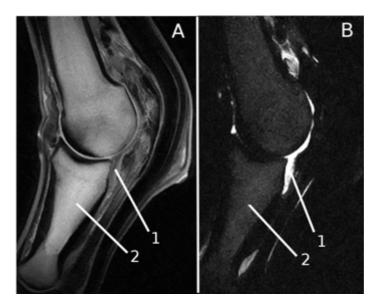


Figure 15. Illustration of a sagittal slice of the metacarpophalangeal joint on MRI illustrating the different appearances of fat and water on a: A) T1 weighted image, B) T2 weighted image. 1: joint fluid, 2: medullary fat

The magnetic field strength is measured in Tesla. In equine medicine, several systems are used, ranging from low field (0,2T) to high field (1,5T). The high field systems enable

faster scanning times and have a better image quality (Tucker and Sande 2001). Both low-field and high-field MRI systems give comparable data about abnormal structures, enabling the detection of small and subtle lesions without the presence of gross structural changes (Kraft and Gavin 2001). However, lesions are more detailed with a high field MRI, therefore, high field MRI is superior in the detection of articular surface abnormalities (Murray *et al.* 2009). Contrast enhanced MRI with IV gadolinium is possible in the horse and can improve lesion detection. This contrast enhanced MRI provides additional anatomic and physiologic assessment of pathologic change (Saveraid and Judy 2012). However this technique is still in its infancy, but is promising to allow evaluation of articular cartilage pathology (Pease 2012).

Several studies concluded that the inter- and intraobserver agreement is good with MRI, with a low intra- and interobserver variability (Barrett *et al.* 2009; De Decker *et al.* 2011; Wucherer *et al.* 2012). However, one study mentions only a moderate intra- and interobserver agreement for evaluation of osteoarthritis status if the equine MCP joint (Olive *et al.* 2010).

A standing low field MRI system has been developed for horses to avoid the risk of general anaesthesia, to ease patient handling and to reduce the operating costs. Its purchase and maintenance are considerably cheaper, but it provides a poorer magnetic field homogeneity, which can result in image degradation and artefacts. Due to the lower magnetic field used in the standing MRI system, longer imaging times are needed. This increases the risk of movement of the horse and thus the use of motion correction software is necessary (Tucker and Sande 2001; Mair *et al.* 2005; Murray and Mair 2005). It generally provides lower quality images compared to a high field system (Mitchell 2009). On the other hand, at the level of the MCP/MTP joint, the low field system has enough resolution to detected pathology at the level of the bone, tendons and ligaments. At the level of the articular cartilage, a high field system is better to evaluate possible lesions (Murray *et al.* 2009)

The main advantage of MRI over radiography and diagnostic ultrasound is that it provides both anatomical and physiological information in multiple planes. Most of the soft tissues surrounding the MCP/MTP joint can readily be identified even with a low field system (Martinelli *et al.* 1997).

Magnetic resonance imaging has its place in a pre-purchase examination to asses a specific area know to have been previously affected with pathology (Mitchell 2009). However, often multiple abnormalities or abnormalities on a lame-free limb are detected.

During a pre-purchase examination, it is difficult to decide what the significance of these findings is or what these could mean for the specific horse (Schulze 2010).

Conclusions

A thorough and comprehensive clinical examination remains the basis for every prepurchase examination, completed with a radiographic examination for screening purposes. Some findings during this clinical and radiographic examination may require a further examination with additional imaging modalities. The decision of which imaging technique to use, needs to be based on the professional assessment of the horse by the veterinarian and sound economic considerations.

Chapter 1.3

The evaluation of the equine metacarpo-/metatarsophalangeal joint during a prepurchase examination

Adapted from:

Hauspie S., Declercq J., Martens A., Zani D.D., Bergman E.H.J., Saunders J.H. (2011) Anatomy and imaging of the equine metacarpophalangeal/metatarsophalangeal joint. *Vlaams Diergeneeskundig Tijdschrift* 80, 263-270.

During a pre-purchase examination, several variations can be detected at the level of the MCP/MTP joint. Some will have an effect on the future performance of the horse, others not.

In Thoroughbreds and Warmblood horses several radiographic findings are seen at the level of the MCP/MTP joint (Becht and Park 2000; Kane *et al.* 2003b; Verwilghen *et al.* 2009). In Thoroughbreds, flattening of the sagittal ridge, flattening of the distal palmar/plantar articular surface of the MCIII/MTIII, variations in size and visibility of the transverse ridge, the medial proximal sesamoid bone being more cuboidal than the lateral one, a separate centre of ossification at the proximal aspect of the proximal sesamoid bones or the distal border of the hind proximal sesamoid bones being more flatter than the distal border of the fore proximal sesamoid bones are considered normal radiographic variations at the level of the MCP/MTP joint (Becht and Park 2000). Other radiographic findings like palmar supracondylar lysis, enthesophyte formation on the fore proximal sesamoid bones and proximal dorsal fragmentation of P1 in the hind MTP joint and enthesophyte formation on the hind proximal sesamoid bones, have been associated with reduced performance in Thoroughbred horses (Kane *et al.* 2003a).

The detected radiographic finding at the level of the MCP/MTP joint in Warmblood horses includes remodelling of the proximal border of P1, mild surface irregularity or osteochondral defect of the proximal border of the sagittal ridge of the MCIII/MTIII, dorsal osteochondral fragments originating from the MCIII/MTIII, plica synovialis or the dorsoproximal border of P1 to palmar or plantar fragments of P1 (Stock *et al.* 2005; Declercq *et al.* 2008; Declercq *et al.* 2009; Verwilghen *et al.* 2009). The clinical relevance of several of these findings is still unclear (Declercq *et al.* 2008; Martens *et al.* 2008).

In Warmbloods and Thoroughbreds variation in radiographic appearance of the proximal aspect of the dorsal condylar sagittal ridge is detected (Kane *et al.* 2003b; Verwilghen *et al.* 2009). In Warmbloods the described variations were a mild surface irregularity of the proximal border of the sagittal ridge of the metacarpus/tarsus. In Thoroughbreds the detected variations at that level were a well defined semicircular notch, lucency or a fragment/loose body. In Thoroughbreds, these variations were not associated with reduced performance (Kane *et al.* 2003a). However, due to the difference in type and length of the sport career for both breeds (short sports career in Thoroughbreds; long lasting career in Warmbloods) (Hinchcliff and Hamlin 2004; O'Sullivan and Lumsden 2004), and the

difference in described appearance between both breeds of the proximal aspect of the sagittal ridge, the same conclusion cannot just be extrapolated to Warmblood horses.

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Chapter 2 Scientific Aims

A veterinary pre-purchase examination is an important service offered by the veterinarian to clients who wish to sell or buy a horse. With this examination, the responsible veterinarian tries to identify abnormalities or potential problems that could make the horse unsuitable for the intended use. In a pre-purchase examination, radiography is an important aid to detect actual or potential orthopaedic problems.

During this pre-purchase examination, radiographic changes are frequently detected at the level of the MCP/MTP joint. It is up to the veterinarian to decide if these changes will have an influence on the future sport career of the horse. This decision is best taken on the basis of scientific data, however this information is often not available, resulting in different opinions of veterinarians on the same case. In Thoroughbreds, it has been shown that there is some variation in the radiographic appearance of the proximal aspect of the dorsal condylar sagittal ridge, but without influence on the sports career of the horse. Extrapolation of the relevance of these appearances to Warmbloods is however not appropriate because of the large difference in type and duration of sports career.

The general aim of this research project was therefore to describe the variation in radiographic appearance of the proximal aspect of the dorsal condylar sagittal ridge in Warmblood horses, to evaluate their histological basis, as well as to assess the influence of these variations on the joint cartilage and their interaction with the surrounding soft tissues.

More specific by:

- 1. Describing the prevalence of variation in the radiographic appearance of the dorsoproximal aspect of the condylar sagittal ridge in a population of Warmblood stallions.
- 2. Evaluating the histological appearance of these variations at the dorsoproximal aspect of the sagittal ridge.
- 3. Assessing the possible predisposition of these variations in appearance of the dorsoproximal aspect of the sagittal ridge to articular cartilage degeneration.
- 4. Describing the influence of hyperextension of the MCP/MTP joint on the position of the synovial plica surrounding the proximal aspect of the dorsal condylar sagittal ridge.

Chapter 3

Radiographic features of the dorsoproximal aspect of the sagittal ridge of the third metacarpal and metatarsal bones in young Warmblood stallions

Adapted from:

Hauspie S., Martens A., Declercq J., Busoni V., Vanderperren K., van Bree H., Saunders J.H. (2010) Radiographic features of the dorsal condylar sagittal ridge of the third metacarpal and metatarsal bone in young Warmblood horses. *Veterinary and Comparative Orthopaedics and Traumatology* 23, 411-416.

Summary

Radiography is a standard practice during a pre-purchase examination of a horse. During this examination, several variations can be detected. The objective of this study is to describe the prevalence of variation in radiographic appearance of the dorsoproximal aspect of the condylar sagittal ridge of the MCIII/MTIII in young Warmblood stallions.

The LM radiographic projections of the MCP/MTP joints performed on horses as a part of stallion selection were used. The radiographic appearance of the bone surface at the dorsoproximal aspect of the condylar sagittal ridge was classified as "smooth", "irregular", "cam", "indentation" and "lucency".

The radiographic appearance of the proximal aspect of the sagittal ridge ranged from "smooth" in 51.5% of the joint, 19.3% was "irregular", 8.9% presented a "cam", 8.1% had a "lucency" and 12.2% had an "indentation". In 1.2% of the horses a fragment was present at the level of the dorsoproximal aspect of the sagittal ridge and in 1.7% a fragment was suspected superimposed on the dorsoproximal aspect of the sagittal ridge.

Radiographic variation is present at the dorsal aspect of the MCP/MTP joint in young Warmblood stallions. These various aspects should be recognized and described in horses presented for pre-purchase examination. However, their clinical relevance in the individual horse is unclear and needs further investigation.

Introduction

The dorsoproximal aspect of the equine MCP/MTP joint is composed of a thick joint capsule including a synovial plica, a layer of cartilage, subchondral bone, a synovial membrane and synovial fluid (Dabareiner *et al.* 1996; Denoix *et al.* 1996; McIlwraith 2001). Although the exact function of the synovial plica has not been studied, its location and structure suggest that it acts as a contact interface or cushion between the proximal dorsal rim of P1 and the dorsal surface of the distal MCIII/MTIII during full extension of the MCP/MTP joint (McIlwraith *et al.* 2005). This anatomical region can be affected by specific disorders (osteochondrosis, chronic proliferative synovitis in the MCP joint), or it can be involved in a generalised joint disorder (capsulitis/synovitis, osteoarthritis, infectious or traumatic arthritis) (Vanderperren and Saunders 2009a, 2009b).

An examination is frequently performed prior to the sale of a horse in order to assess the suitability of the animal for the purpose for which it is required. Depending on the intended use and value of the animal, a radiographic examination may be part of the prepurchase examination. If radiographs are taken, projections of the MCP/MTP joints will be included (Van Hoogmoed *et al.* 2003). Should the veterinarian make a mistake in interpreting the radiographic images, the economic and legal consequences may be important (Van Hoogmoed *et al.* 2003). However, there is a general lack of published information regarding the clinical significance of many radiographic findings, as well as the common anatomical variations (McIlwraith *et al.* 2003). Moreover, in addition to the horse's function, the owner's expectations and intended use for the horse will largely determine their significance (Becht and Park 2000; Bladon and Main 2003; Kane *et al.* 2003b). Because of the difference in type and duration of their sports career, great caution should be exercised when using the conclusions drawn from Thoroughbred horses to interpret the radiographs of a Warmblood horse (Kane *et al.* 2003a; Kane *et al.* 2003b; McIlwraith *et al.* 2003; Spike-Pierce and Bramlage 2003; Van Hoogmoed *et al.* 2003).

The objective of this study is to describe the prevalence of variation in radiographic appearance of the dorsoproximal aspect of the condylar sagittal ridge of the MCIII/MTIII in young Warmblood stallions.

Materials and methods

The LM radiographic projections of MCP/MTP joints performed on horses presented at our Institution (Ghent University's large animal teaching hospital) as a part of stallion selection between April 2007 and March 2009 were used. Only horses younger than 6 years were used in this study. A short lameness examination was performed: horses were evaluated trotting in a straight line and lunging (hard and soft surface). However, due to the nature of the stallion selection, an in-depth lameness examination, with flexion tests, was not performed. Using a computed radiography imaging system (Regius model 190, Konica Minolta, Tokyo, Japan), radiographs were made with a horizontal X-ray beam and with the horse bearing weight. The radiographs were evaluated using commercially available software (Osirix, Geneva, Switzerland). If the projection was excessively oblique - defined as superimposition of the distal condyles of the MCIII/MTIII on the sagittal ridge, preventing a thorough radiographic interpretation - the radiograph was not used in the study.

Two readers - a Board-certified radiologist (JHS) and a PhD-student (SH) - reviewed all of the examinations together, and each decision was made consensually. When there was disagreement, consensus was sought between senior radiologists. Special attention was given to the dorsoproximal aspect of the condylar sagittal ridge of the MCIII/MTIII (Fig. 1).

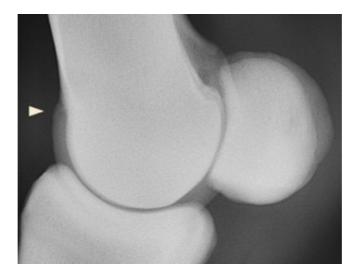


Figure 1. LM radiograph of the metacarpo-/metatarsophalangeal joint. The arrow is highlighting the proximal third of the visible dorsal aspect of the sagittal ridge, at the level of the synovial plica.

The dorsoproximal aspect of the condylar sagittal ridge corresponded to the proximal third of the visible dorsal aspect of the sagittal ridge, at the level of the synovial plica. The

appearance of this area was classified according to these 5 categories: "smooth" (defined as flat or sharp and smoothly delineated; Fig. 2), "irregular", small and well-defined bony prominence or "cam", irregularly shaped "lucency" or a sharply delineated "indentation" (Fig. 3) (Kane *et al.* 2003b; Cohen *et al.* 2006). The presence of a fragment or a suspected fragment was also noted (a suspected fragment was defined as an ill defined and ill delineated bony opacity). If possible, the surface area of the fragment was measured directly from the LM radiographic projection without allowance for magnification. A crude indication of the surface area was obtained by drawing a closed polygon around the outer edges of the fragment and then calculating the surface area with commercially available imaging software (Osirix, Geneva, Switzerland).

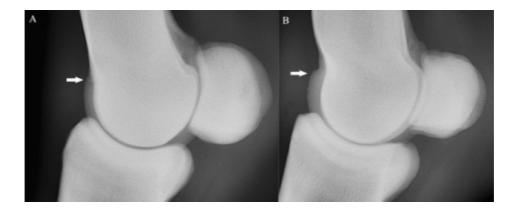


Figure 2. LM radiograph of the metacarpo-/metatarsophalangeal joint showing the difference in shape of the proximal aspect of the sagittal ridge in the "smooth" category: A) Flat and smoothly delineated, B) Sharp and smoothly delineated (arrow).

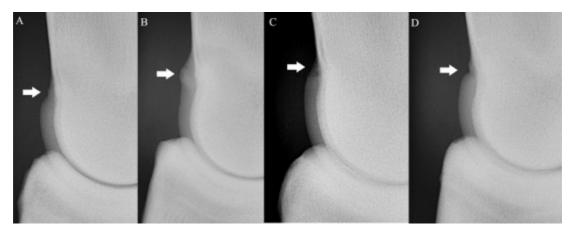


Figure 3. LM radiograph of the metacarpo-/metatarsophalangeal joint demonstrating the other appearances recorded at the proximal aspect of the sagittal ridge: A) "irregular", B) "cam", C) "lucency", D) "indentation".

Statistical analysis was performed with SAS version 9.2 (SAS Institute Inc., Cary, NC) to determine if there was a difference between left and right MCP/MTP joints and between the front and hind limbs. Statistical analysis was performed using the chi-square test (or the Fisher exact test if sample sizes were smaller than 10).

Results

Animals

A total of 1232 radiographs of MCP/MTP joints of 308 Warmblood stallions were available for this retrospective evaluation. The mean age of the population was 2.22 years (s.d. 0.035), the median age was 2 (Fig. 4). None of the horses showed lameness. Twenty-eight (28/1232 = 2.3%) radiographs were excluded from the study because of excessive obliqueness. In total, 1204 radiographs were used for interpretation: 594 were front limbs and 610 were hind limbs.

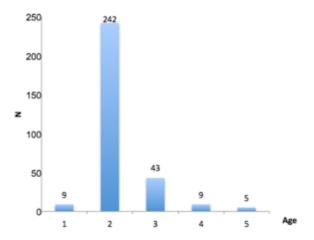


Figure 4. Age (years) distribution of the horses.

Radiographic findings at the dorsoproximal aspect of the sagittal ridge

The radiographic findings are summarised in Table 1.

category	left	front	right front		left hind		right hind		total	
	n	%	n	%	n	%	n	%	n	%
smooth	159	53.5	175	58.9	137	44.9	149	48.9	620	51.5
irregular	61	20.5	60	20.2	54	17.7	57	18.7	232	19.3
cam	28	9.4	24	8.1	30	9.8	25	8.2	107	8.9
lucency	25	8.4	21	7.1	26	8.5	26	8.5	98	8.1
indentation	24	8.1	17	5.7	58	19.0	48	15.7	147	12.2

Table 1. Radiographic findings in the metacarpo-/metatarsophalangeal joints in young Warmblood horses.

The radiographic appearance of the dorsoproximal aspect of the condylar sagittal ridge of the MCIII/MTIII had a "smooth" appearance in 51.5% (620/1204) of the joints, 19.3% (232/1204) were "irregular", 8.9% (107/1204) had a "cam", 8.1% (98/1204) had a "lucent" area and 12.2% (147/1204) had a well-defined "indentation" (Fig. 3). No significant differences were found between left and right MCP/MTP joints. A significant difference (P < 0.0001) was found between front limbs and hind limbs: an "indentation" was more present in the hind limbs than in the front limbs. In 1.2% (15/1204) of the joints, a fragment was present; always dorsal to, or partially superimposed on the dorsoproximal aspect of the sagittal ridge. The appearance of the sagittal ridge was evenly distributed over the 5 categories. The shape of the fragment varied from linear to oval, with a mean surface of 0.093 cm² (ranging from 0.011 cm² to 0.472 cm²) (Fig. 5A). In 1.7% (20/1204) of the joints, the presence of a fragment was suspected; always completely superimposed on the sagittal ridge (Fig. 5B). In this case the appearance of the sagittal ridge was mostly "normal" to "irregular". One horse had a fragment in both front limbs and 1 horse had a fragment in the left front limb and hind limb.

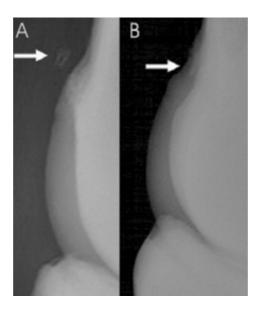


Figure 5. LM radiograph of the metacarpo-/metatarsophalangeal joint showing: A) The presence or B) the suspected presence of a fragment at the dorsoproximal aspect of the sagittal ridge.

Discussion

In our study, there was a lot of variation in the radiographic appearance of the dorsoproximal aspect of the sagittal ridge of the MCIII/MTII. Variation in appearance at this level of the sagittal ridge is also described in Thoroughbreds (notch, lucency and fragment/loose body) (Kane *et al.* 2003b) and in an other population of Warmbloods (mild surface irregularity or well defined osteochondral defect) (Verwilghen *et al.* 2009). In Thoroughbreds these variations had no implications for future performance in sport (Kane *et al.* 2003a). It is tempting to extrapolate these conclusions. However, in contrast to the population of Thoroughbred horses used for racing (Kane *et al.* 2003a), our population consisted of Warmblood horses used primarily for jumping or dressage. Compressive strains at the dorsal aspect of the MCII/MTIII have been shown to be more prevalent in racehorses working at high speed, whereas dressage or jumping horses - which usually work at a lower level of impact - have a different direction of strain, a longer sports career, and possibly another conformation as well (Davies *et al.* 1993; Bennell *et al.* 1997; Davies and Merritt 2004).

In our study, 55% of the front limbs and 46% of the hind limbs showed a "smooth" appearance of the dorsoproximal aspect of the sagittal ridge, compared to 72% and 75%,

respectively (categorized as no detected changes) in a previous study of Thoroughbreds (Kane *et al.* 2003b). Besides the difference in breed, this difference in percentages can be explained by the use of additional parameters ("irregular" and "cam") in the present study. If the MCP/MTP joints with an "irregular" outlining are considered to be normal, 77% of the front limbs and 65% of the hind limbs fall into this category, which corresponds more closely to the previous study (Kane *et al.* 2003b).

The dorsoproximal aspect of the sagittal ridge was "irregular" outlined in a total of 19,3% of the joints in this study, which is more than the 6.8% described in an other population of Warmblood stallions (Verwilghen et al. 2009). The presence of a small and well-defined "cam" was a bilateral finding in 55% of the front limbs and 46% of the hind limbs. The presence of a small and well-defined "cam" was previously not described as a possible radiographic appearance of the proximal aspect of the sagittal ridge in Thoroughbreds, nor in Warmbloods (Kane et al. 2003b; Verwilghen et al. 2009). The aetiology of this cam or the reason why this was only detected in this population of Warmblood stallions is unknown. A "lucency" was present in 47 front limbs and 53 hind limbs and was a bilateral finding in only 2 horses and 5 horses, respectively. This is in agreement with the study of Kane et al. 2003 in which a "lucency" was most often a unilateral finding. However, this "lucency" was much more detected in our study: approximately 8% for each joint, where this only occurred in only 1% of the joints in Thoroughbreds (Kane et al. 2003b). The presence of an "indentation" was a bilateral finding in 17,1% (6 out of 35 horses) of the front limbs and is in contrast to Thoroughbreds were this was a bilateral finding in 65% of the front limbs. In 26% (24 out of 92 horses) of the hind limbs the "indentation" was a bilateral finding, which is more in agreement with the number (27%) found in the latter study (Kane et al. 2003b). This "indentation" was present in a total of 12.2% of joints, which is much more than the total of 1% of the joints affected in another Warmblood population (Verwilghen et al. 2009).

A reason for the difference in occurrence of certain variations at the dorsoproximal aspect of the sagittal ridge ("irregular" and "indentation") or the lack of presence of certain variations ("cam" and "lucency") between our Warmblood population and the Warmblood population used in the study of Verwilghen *et al.* 2009 is not known (Verwilghen *et al.* 2009).

A bone fragment was more frequently suspected than confirmed. The difficulty of detecting a fragment can be due to poor fragment opacification, superimposition on other

bony structures, and/or variation in soft tissues (Widmer and Blevins 1994). In addition, a fragment without a corresponding contour defect or lucency may also represent (synovial) calcification, e.g. synovial chondromatosis (Smith *et al.* 1995). Additional projections can partially address the problem of detecting these abnormalities, but they are not part of the standard radiographic protocol for the stallion selection at this institute. Ultrasonography has been shown to be superior to radiography for detecting and characterising dorsal bone fragments (Vanderperren *et al.* 2009). However, performing an ultrasonographic examination in stallions for screening purposes is unrealistic. Other techniques such as arthroscopy, computed tomography or MRI may also enable detection of bone fragments (Kawcak *et al.* 2000; Schneider *et al.* 2005; Vanderperren *et al.* 2009). However, due to their cost and invasiveness, the use of these techniques is even less realistic in equine selection. As MCP/MTP joint fragments may have an influence on a horse's future lameness, it is important to detect them early (Declercq *et al.* 2009). Radiography remains the diagnostic method of choice for the practitioner, because of its low cost and high availability compared to the other imaging modalities (Horstmann *et al.* 2003).

In conclusion, there is variation in radiographic appearance of the dorsoproximal aspect of the condylar sagittal ridge of the MCIII/MTIII in young Warmblood stallions. The histological background and clinical relevance of these variations needs further investigation.

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Chapter 4

The histological appearance of the dorsoproximal aspect of the condylar sagittal ridge of the third metacarpal and metatarsal bone in young Warmblood horses and the correlation with detected radiographic variations

Adapted from:

Hauspie S., Forsyth R., Vanderperren K., Declercq J., Martens A., Saunders J.H. (2012) The histological appearance of the proximal aspect of the dorsal condylar sagittal ridge of the third metacarpal and metatarsal bone in young Warmblood horses: Normal appearance and correlation with detected radiographic variations. *Anatomia, Histologia, Embryologia* doi: 10.1111/ahe.12006.

Summary

Is has been shown in previous research that variation in radiographic appearance of the dorsoproximal aspect of the condylar sagittal ridge is present in Warmblood horses, ranging from "smooth", "irregular", "cam", "lucency" to "indentation". However, no information is available on their histological appearance. The objective of this study was to describe the histological appearance of the variations detected at the dorsoproximal aspect of the condylar sagittal ridge of the MCIII/MTIII Warmblood horses.

A total of 25 MCP/MTP joints of 12 Warmblood horses were used. Five samples of each radiographically described group were selected for histological processing. The histological appearance of each group and differences in appearance between each group were described.

Each group showed a bone cortex, covered by hyaline cartilage distally and a transition to longitudinally aligned collagen fibres proximally, at that level covered by loosely organized connective tissue. No clear difference in appearance of the cortex was detected between the "smooth" and "irregular" group. The "cam" group presented an expansion of the cortex. In the "indentation" and "lucency" group, a depression in the cortex was detected. The collagen fibres and loosely organized connective tissue were located in the depression in the "indentation" group whereas their location varied in the "lucency" and "cam" groups.

The detected differences in appearance of the dorsoproximal aspect of the sagittal ridge on radiography are a representation of anatomical variations resulting from detectable histological differences. Further research is warranted to determine whether these variations are developmental or congenital and to evaluate their potential influence on the joint and surrounding soft tissues during hyperextension.

Introduction

A radiographic examination of Warmblood horses is an important part of the prepurchase examination. The decision of which anatomic regions to be included is mostly based on the discipline in which the horse is to function. Show jumpers and dressage horses commonly have front feet, MCP/MTP joint, hock and stifle related lameness and projections of the latter are therefore frequently included. Veterinarians are asked to assess the radiographs and to make a judgement on the importance of detected morphological variations at the level of the MCP/MTP joint (Van Hoogmoed *et al.* 2003; Mitchell 2009). In Warmbloods, radiographic variation of the dorsoproximal aspect of the condylar sagittal ridge of the MCIII/MTIII has been described ("smooth", "irregular", "cam", "lucency" and "indentation") (Hauspie *et al.* 2010).

The origin of these detected variations is not known. These variations can either be congenital, or be the result of remodelling of the bone in adaptation to sustained workload. In case of this latter, a reactive process would be expected since joint pattern, bone mass and turnover are likely to vary, depending on the type and intensity of exercise (Bennell *et al.* 1997; Davies and Merritt 2004).

The histological background of these radiographically detected variations is until know not described. The objective of this study is to describe the histological findings of these consistent and characteristic radiographic features of the dorsoproximal aspect of the condylar sagittal ridge in young Warmblood horses.

Materials and methods

Collection and preparation of specimen

Thoracic and pelvic limbs of Warmblood horses, aged younger than 6 years were collected immediately after slaughter. The age and breed of the horses was verified by means of the chip and passport. The reason for slaughter, level of exercise and possible lameness was unknown. The limbs were sectioned in the carpal and tarsal joints and after collection the limbs were stored immediately at -20°C until processing. A LM radiographic projection of the MCP/MTP joint was obtained post mortem, without the removal of skin and subcutis. The LM projection was defined as superimposition of the MCIII/MTIII condyles, not prohibiting a

thorough evaluation of the sagittal ridge. Joints showing radiographic abnormalities or variations, other than the previously described radiographic variations at the level of the sagittal ridge (Hauspie *et al.* 2010) on the LM projection were excluded from the study. Encountered abnormalities included osteochondral fragments, peri-articular new bone formation and abnormalities at the level of the proximal sesamoid bones (fractures, apical fragments). Based on the appearance of the proximal aspect of the dorsal condylar sagittal ridge on the LM projection, the joints were categorized as described in a previous study: "smooth", "irregular", "cam", "lucency" or "indentation" (Fig. 2-7) (Hauspie *et al.* 2010).

Sampling

The limbs were thawed at 7°C two days before histological sampling was performed and were opened by a circumferential incision of skin, collateral ligaments and joint capsule. A macroscopic inspection of the joint was performed to ensure a normal appearance. If any macroscopic abnormalities were detected, except for cartilage damage and the previously described variations at the dorsoproximal aspect of the sagittal ridge, the limb was excluded from the study. Encountered abnormalities were fragments not detected on radiography, fracture of the proximal sesamoid bones, ununited palmar/plantar eminence.

To facilitate the collection of the samples, the remainder of the joint capsule and proximal synovial plica were removed. The proximal aspect of the dorsal condylar sagittal ridge was collected using a band saw, resulting in blocks of approximately 2x1x2 cm (height x depth x width) in size (Fig. 1). The samples were fixed in a 4% buffered paraformaldehyde solution for 24 hours. Samples were rinsed with tap water and placed in a decalcifying solution (DC2, Labonord SAS, Temblemars, France). The solution was changed every 2 days until samples were suited for histological processing. This was evaluated with test cuts made by using a #10 scalpel blade on the most distal edge of the sample. All samples underwent routine processing in paraffin under vacuum followed by embedding in paraffin with the sample orientated to create sections in a sagittal plane (Fig. 1). All sections were cut at 5µm thickness and stained with haematoxylin-eosin. Multiple slices were made, located from abaxial to axial.

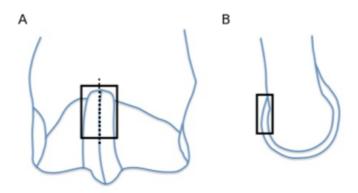


Figure 1. Diagram illustrating the exact relation of the bone block to the entire bone (square = bone block); the dotted line shows the plane in which the samples were cut: A) Dorsal view, B) Lateral view.

Histological scoring

Descriptive examination was performed by use of a light microscope. The histological appearance of the cortex, cartilage, the transition zone from cartilage to joint capsule and loose connective tissue was evaluated. The 5 samples of the group "smooth" radiographic appearance were considered as the control group, representing the most frequently encountered variation based on radiography (Hauspie *et al.* 2010). The amount of overlying connective tissue in the different groups was evaluated semi-quantitatively and varied from mild, moderate to pronounced, compared to the amount detected in the "smooth" group, were it was defined as being moderate. The histological differences observed in the 4 remaining groups, compared to the control "smooth" group were recorded.

Results

A total of 25 joints (MCP joint = 12; MTP joint = 13) originating from a total of 12 horses (median age of 3 years; max 5 year and min 2 year old), were included in this study.

The control "smooth" group (Fig. 2) showed a smooth contour of the cortex, consisting of lamellar bone. Distally, the cortex was covered with normal hyaline cartilage. Proximally, longitudinal aligned collagen fibres were detected (Fig. 3). No clear proximal border of the cartilage could be observed here. At the level of the transition cartilage to longitudinal aligned collagen fibres some overlying loosely organised connective tissue was

present. This transition of cartilage to longitudinal aligned collagen fibres, covered with loose connective tissue was called "interface". No synovial lining was detected in any of the samples.

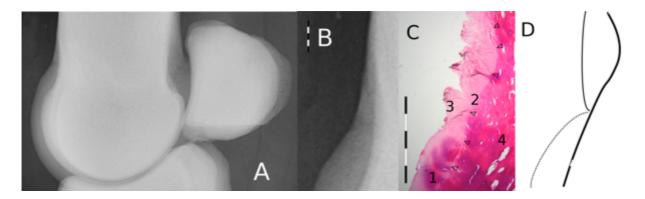


Figure 2. A) LM radiograph of the metacarpophalangeal joint of the "smooth" group, B) Radiographic detail of the dorsoproximal aspect of the sagittal ridge corresponding to the sampled region (bar = 1 mm), C) Corresponding histological sample (bar = 1 mm). 1: proximal margin of hyaline cartilage, 2: aligned collagen fibres, 3: loosely organised connective tissue, 4: cortex, unfilled arrowheads: surface of mineralized tissue, D) Diagram demonstrating the appearance of the surface of mineralized tissue (thick line), the proximal margin of hyaline cartilage (dotted line) and the orientation of the longitudinal aligned collagen fibres (thin line).

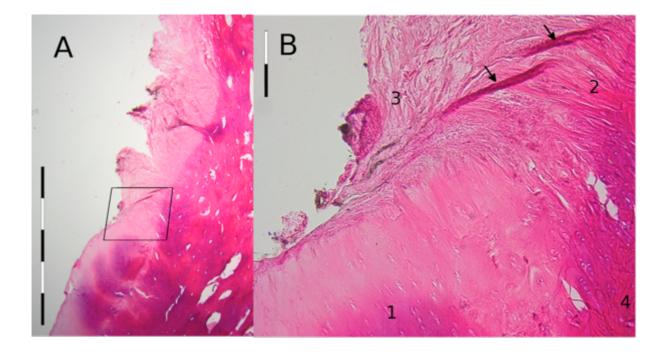


Figure 3. A) Histological sample of the "smooth" group (bar = 1 mm), and B) Detail taken at the level of the transition cartilage to aligned collagen fibres, represented by the square in A (bar = 1 μ m). 1: proximal margin of hyaline cartilage, 2: aligned collagen fibres, 3: loosely organised connective tissue, 4: cortex, arrows: artefacts due to processing of the sample.

The remaining groups were compared with the histological appearance of the control group ("smooth") in mind. In all samples normal hyaline cartilage could be observed. Compared to the control group differences were observed in appearance of the cortex, location and appearance of the "interface" and the amount of loose connective tissue at the level of the "interface" (Table 1).

Radiography	histology			
Group	Appearance of mineralized tissue	<u>Location</u>	Amount of connective tissue	
Smooth	Smooth	n/a	Moderate (n = 5)	
	Smooth	n/a	Mild (n = 2)	
Irregular			Moderate (n = 2)	
			Pronounced $(n = 1)$	
	Expansion of cortical bone	Interface proximal to expansion $(n = 2)$	Moderate (n = 1)	
		interface proximar to expansion (n = 2)	Pronounced (n = 1)	
Cam		Interface at level of expansion $(n = 2)$	Mild (n = 1)	
		interface at level of expansion (n – 2)	Moderate (n=1)	
		Interface distal to expansion (n = 1)	Pronounced (n = 1)	
	Impression of cortical	Interface distal to pit $(n = 3)$	Mild (n = 1)	
Lucency	bone only detected in a	Interface distar to pit (n = 3)	Moderate (n = 2)	
	part of sample	Interface at level of pit $(n = 2)$	Moderate $(n = 2)$	
	Impression of cortical bone detected in		Mild (n = 2)	
Indentation		Interface in pit (n = 5)	Moderate (n = 2)	
	complete sample		Pronounced (n = 1)	

Table 1. Overview of the histological characteristics observed at the level of the surface of the mineralized tissue, the location of the transition of cartilage to longitudinal aligned collagen fibres ("interface") and the amount of loose connective tissue at that level, grouped by radiographic category.

In the "irregular" group (Fig. 4), no clear differences in appearance of the cortex, cartilage or connective tissue could be detected when compared to the control group.

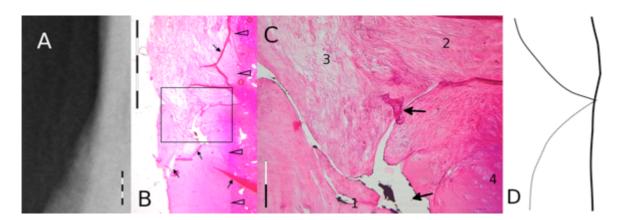


Figure 4. A) Radiographic detail of the dorsoproximal aspect of the sagittal ridge of the "irregular" group (bar = 1 mm), and B) Histological sample (bar = 1 mm), and C) Detail taken at the level of the transition cartilage to aligned collagen fibres, represented by the square in B (bar = 1 μ m). 1: proximal margin of hyaline cartilage, 2: aligned collagen fibres, 3: loosely organised connective tissue, 4: cortex, arrows: artefacts due to processing of the sample, unfilled arrowheads: surface of mineralized tissue, D) Diagram demonstrating the appearance of the surface of mineralized tissue (thick line), the proximal margin of hyaline cartilage (dotted line) and the orientation of the longitudinal aligned collagen fibres (thin line).

An expansion of cortical bone was observed in the "cam" group (Fig. 5). Depending on the sample, the "interface" was located distal to, at the level of or more proximal to the cortical proliferation. The cortical expansion was best identified on the most medial or lateral sagittal slices rather than on the central sagittal slices.

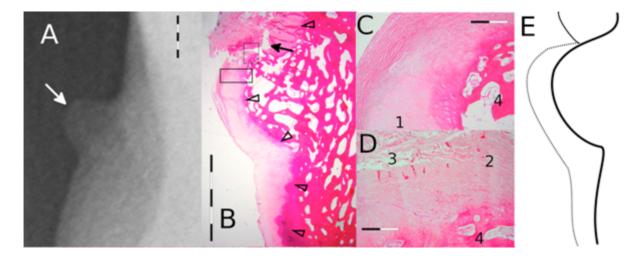


Figure 5. A) Radiographic detail of the dorsoproximal aspect of the sagittal ridge of the "cam" group (bar = 1mm, white arrow is representing the expansion of cortical bone), and B) Histological sample (bar = 1 mm), and (C, D) Details taken at the level of the transition cartilage to aligned collagen fibres, represented by the squares in B (bar = 1 μ m), C) Is representing a detail taken at the level of the full line square, D) Is representing a detail taken at the level of the dotted line square. 1: proximal margin of hyaline cartilage, 2: aligned collagen fibres, 3: loosely organised connective tissue, 4: cortex, black arrow: artefacts due to processing of the sample, unfilled arrowheads: surface of mineralized tissue, E) Diagram demonstrating the appearance of the surface of mineralized tissue (thick line), the proximal margin of hyaline cartilage (dotted line) and the orientation of the longitudinal aligned collagen fibres (thin line).

A smooth impression in the cortex was observed in the "lucency" group (Fig. 6). This impression was present in all samples, but was only detected in a limited amount of slices of each sample. The remaining part of the sample had a histological appearance comparable with the control group. The "interface" was located distal to the pit in 3 samples, whereas this was located at the level of the pit in 2 samples.

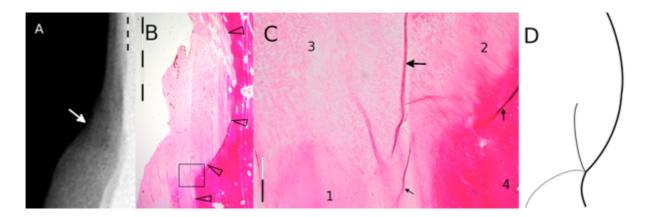


Figure 6. A) Radiographic detail of the dorsoproximal aspect of the sagittal ridge of the "lucency" group (bar = 1mm, white arrow is representing the more lucent area), and B) Histological sample (bar = 1 mm), and C) Detail taken at the level of the transition cartilage to aligned collagen fibres, represented by the square in B (bar = 1 μm). 1: proximal margin of hyaline cartilage, 2: aligned collagen fibres, 3: loosely organised connective tissue, 4: cortex, black arrows: artefacts due to processing of the sample, unfilled arrowheads: surface of mineralized tissue, D) Diagram demonstrating the appearance of the surface of mineralized tissue (thick line), the proximal margin of hyaline cartilage (dotted line) and the orientation of the longitudinal aligned collagen fibres (thin line).

A smooth impression in the cortex was also observed in the "indentation" group, (Fig. 7). This pit was present throughout the sample but appeared to be more abruptly compared to the impression in the "lucency" group. The "interface" was located at the level of this pit in all 5 samples.

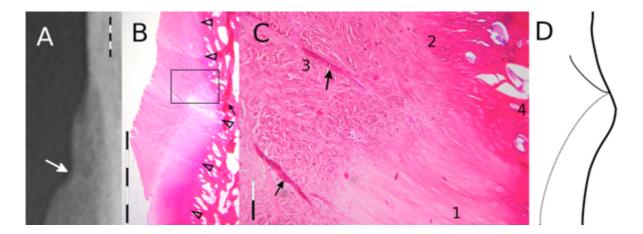


Figure 7. A) Radiographic detail of the dorsoproximal aspect of the sagittal ridge of the "indentation" group (bar = 1mm, white arrow is representing the indentation), and B) Histological sample (bar = 1 mm), and C) Detail taken at the level of the transition cartilage to aligned collagen fibres, represented by the square in B (bar = 1 μm) 1: proximal margin of hyaline cartilage, 2: aligned collagen fibres, 3: loosely organised connective tissue, 4: cortex, black arrows: artefacts due to processing of the sample, unfilled arrowheads: surface of mineralized tissue, D) Diagram demonstrating the appearance of the surface of mineralized tissue (thick line), the proximal margin of hyaline cartilage (dotted line) and the orientation of the longitudinal aligned collagen fibres (thin line).

In 3 samples a blood vessel was detected, located proximal to the interface cartilage-longitudinal aligned collagen fibres.

Discussion

Radiographic variations are present at the dorsoproximal aspect of the sagittal ridge of the MCIII/MTIII bone of young Warmblood stallions (Hauspie *et al.* 2010). These variations are to some extent in accordance with the ones that are described in a Thoroughbred population ("lucency", well defined semicircular notch or "indentation"), in which no statistically significant association could be demonstrated with reduced performance (Kane *et al.* 2003a; Kane *et al.* 2003b). These conclusions, however, cannot be extrapolated to a Warmblood population because of the important differences in type and duration of sport career and additional variations detected in Warmbloods.

Bone

In our study, there was no clear microscopic difference between the "irregular" group and the "smooth" group. The similar aspect between these two groups can be related to the sectioning of the samples and the quality of the samples. In this study the samples were only sectioned in a sagittal plane. Sectioning in other planes might be required to detected changes

in the "irregular" group. The suboptimal quality of the samples due to difficulties with decalcification en subsequent staining might be the reason that subtle alterations in the surface of the mineralized tissue could have been missed.

Based on histology, a similar type of reaction as observed in the "cam" group is described at the level of the femoral neck in humans (Keogh and Batt 2008; Emary 2010). This reaction area appears macroscopically as a circular to oval elevation with a roughened shiny surface and histology reveals inactive reactive new bone covered with hyaline cartilage and collagenous tissue (Pitt et al. 1982). The aetiology of this reaction area in the human hip joint is an impingement or chronic abutment of the acetabular rim on the femoral head-neck junction or vice versa (Ganz et al. 2003). Macroscopically and microscopically, this type of lesion shows close resemblance with the "cam" group in our study. In horses, no information is available on the origin of this "cam". The equine MCP/MTP joint, however, is a high motion joint and it has been demonstrated that cartilage degeneration in this joint is most frequently initiated at the dorsoproximal aspect of P1 in comparison to the more central regions of the articular surface of P1 (Brommer et al. 2004). It is therefore possible that, comparable to the human reaction area at the level of the femoral neck, this "cam" is the result of chronic abutment of the dorsoproximal aspect of P1 against the dorsoproximal aspect of the distal MCIII/MTIII condyle during hyperextension of the joint. During hyperextension, the lateral and medial dorsoproximal aspect of P1 will contact the dorsodistal aspect of MCIII/MTIII before the bones contact in the midline (Brama et al. 2001). This is in accordance with the more medial or lateral sagittal location of the cortical expansion in this study.

Other reactions at the femoral neck in the human hip joint may result from "synovial" herniation. The radiographic appearance of this herniation is an oval to rounded lucent area, corresponding to a herniation of soft tissue (most likely synovium) through defects of the cortex (Pitt *et al.* 1982). In the "indentation" group a cortical impression was detected and the "interface" or transition of cartilage to longitudinal collagen fibres was located in the impression in all 5 samples. It is possible that this cortical impression can evolve to a herniation-like lesion in horse as a herniation pit in humans can increase in size (Pitt *et al.* 1982). In horses, a possible similar lesion has recently been described with MRI in the medial condyle of the distal MCIII bone (Gonzalez *et al.* 2010). A correlation with the "lucency" group is less plausible because of the variable location of the "interface" and the smooth impression that is only detected in a limited amount of slices while the remaining part of the

sample has a "normal" appearance. To come to a more definitive conclusion, histological comparison with a comparable group of older horses is needed.

In 3 samples a blood vessel was detected, resembling with a nutrient channel. This is a normal finding at the level of the junction of the MCIII/MTIII condyles with the MCIII/MTIII shaft (Denoix 1996).

The radiographically detected variations at the dorsoproximal aspect of the condylar sagittal ridge of the MCIII/MTIII are the representation of detectable histological variation of the cortex. Question is whether the observed histological variation is the result of remodelling, for example as a reaction on the abutment of the dorsoproximal aspect of P1 against the distal MCIII/MTIII condyle during hyperextension (= developmental joint adaptation to workload), or whether this variation is already present at birth or shortly after birth with no major changes during life (congenital). It has been demonstrated that subchondral bone density, bone mass and bone turnover are likely to vary in structure depending on the type and intensity of exercise that is undertaken (Bennell et al. 1997; Kawcak et al. 2000; Davies and Merritt 2004). A reactive process would be expected on histology if the observed variations were a reaction to the abutment of the dorsoproximal aspect of P1 against the MCIII/MTIII condyle. This was not the case in this study. However, a slow ongoing process still remains possible. On the other hand, if the variations are already present at birth, this could mean that they largely remain unchanged during life. Regardless of their origin, these variations still can influence the function of the dorsoproximal aspect of the joint during hyperextension and therefore possibly have an influence on the future sport career.

Interface/transition bone-soft tissues

The present study focused on the histological appearance of the bony structures and bone interface. Due to the removal of the articular capsule and proximal synovial plica, the location of the plica and capsule in relation to the collagen fibres could not be established.

In diarthrodial joints, al non-articular surfaces within the joint cavity are covered with synovium (Van Der Kraan and Van Den Berg 2007) and fibrocartilage is expected at the transition of cartilage to joint capsule. In all samples of this study, aligned collagen fibres were observed proximal to the articular cartilage without the presence of synovial lining. These parallel collagen fibres could resemble Sharpey's fibres, described at this level

(McIlwraith 2002). However based on the strict definition of these fibres, stated as being accompanied by an arteriole and one ore more nerve fibres (Retzlaff *et al.* 1982) we were not able to identify these collagen fibres as true Sharpey's fibres. The lack of synovium is possibly explained by the procedure of collecting the samples. However there is a lack of published data about the normal histologic appearance of the transition cartilage to joint capsule at this level, making it difficult to formulate a clear conclusion.

The discrete changes in morphology at the interface between articular cartilage and collagen fibres, and the variation between samples, may indicate an adaptation to local forces at this level. It has been demonstrated that the dorsoproximal aspect of P1 sustains high pressure during full extension and is in contact with the distal MCIII/MTIII condyle (Brama *et al.* 2001). The longitudinal alignment of the fibres could therefore indicate a damper or cushion function at this level. However the exact place of contact of P1 on the distal MCIII/MTIII condyle and interaction of the proximal synovial plica during hyperextension is unknown.

In conclusion, the difference in radiological appearance of the proximal aspect of the dorsal condylar sagittal ridge of the third metacarpal and metatarsal bone results from detectable histological differences. Further research is warranted to determine whether these variations are developmental or congenital and to evaluate their potential influence on the function of the joint during hyperextension.

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Chapter 5

Radiographic variation of the proximal aspect of the dorsal condylar sagittal ridge of the metacarpo-/metatarsophalangeal joint in Warmblood horses versus the risk of cartilage degeneration

Adapted from:

Hauspie S., Declercq J., Brown B., Duchateau L., Vanderperren K., Saunders J.H. Radiographic variation of the proximal aspect of the dorsal condylar sagittal ridge of the metacarpo-metatarsophalangeal joint in Warmblood horses versus the risk of cartilage degeneration (submitted).

Summary

It has been shown in previous research that variation in radiographic appearance of the dorsoproximal aspect of the sagittal ridge is present in Warmblood horses. These can either be congenital or developmental. The objective of this study was to investigate whether an association could be found between the variable morphological appearance of the proximal aspect of the dorsal condylar sagittal ridge and the degree of cartilage degeneration, determined by the CDI at the level of the MCP/MTP joint, distal metacarpal/metatarsal condyle or proximal articular surface of the P1.

For this study, the distal limbs of 86 cadavers of Warmblood horses were used. After radiographic evaluation, each joint was opened. The distal metacarpal/metatarsal condyles and P1 were freed from the surrounding tissues. The articular surface was stained with Indian ink to evaluate cartilage degeneration. A stereo triangulation scanner was used to calculate the CDI.

No significant association was found between the CDI of the complete MCP/MTP joint, distal metacarpal/metatarsal condyle or P1 and the covariates, which included the radiographic variations at the level of the sagittal ridge, and the age and position of the limb (left/right or front/hind).

There was no association between the variable morphological appearance of the proximal aspect of the sagittal ridge and the degree cartilage degeneration.

Introduction

Lameness is an important factor affecting the future of a horse (Rossdale *et al.* 1985), therefore, a radiographic survey is frequently performed during the pre-purchase examination to identify problems or abnormalities that could make the horse unfit for its intended purpose (Van Hoogmoed *et al.* 2003). In most cases, the clinical relevance of any radiographic variations is clear, but not always, which leaves the relevance open to debate and may be controversial (McIlwraith *et al.* 2003; Van Hoogmoed *et al.* 2003).

The MCP/MTP joint is a high motion joint that is frequently injured in athletic horses (Pool 1996). Radiographic variations have been described at the proximal aspect of the dorsal condylar sagittal ridge in both Warmblood (Hauspie *et al.* 2010) and Thoroughbred horses (Kane *et al.* 2003b). In Thoroughbreds, they may be seen as a lucency or a well-defined semicircular notch at the proximal aspect of the dorsal condylar sagittal ridge. In Warmblood horses, a sagittal ridge with a smooth or an irregular outline, the presence of a cam, lucency, or indentation at the level of the ridge may be seen. In Thoroughbreds, no statistically significant association could be demonstrated with reduced performance in young horses (Kane *et al.* 2003a; Kane *et al.* 2003b). However, in Warmblood horses, who have a different type and duration of sportive career compared to Thoroughbreds (Hinchcliff and Hamlin 2004; O'Sullivan and Lumsden 2004), the clinical relevance may be different.

A technique was recently described that allows in vitro quantitative assessment of articular cartilage degeneration of the metacarpal/metatarsal condyle and P1 by objectively calculating of a CDI (Brommer *et al.* 2003a; Declercq *et al.* 2011). The technique is based on the fact that Indian ink particles have a high affinity for articular cartilage with surface fibrillation and depletion of proteoglycans, whereas normal intact articular cartilage with a high proteoglycan-rich matrix will prevent the particles from entering. Depending on the degree of uptake of Indian ink, the particles will deflect and absorb light, which can be measured quantitatively to calculate the CDI. This technique can be used to detect degenerative changes at the level of the cartilage of the MCP/MTP joint at an early stage (Brommer *et al.* 2003a).

So far the clinical relevance of the difference in morphological aspect of the proximal aspect of the dorsal condylar sagittal ridge is unknown. If an influence were present changes would be expected at the level of the joint, for example degeneration of the articular cartilage.

The objective of this study was therefore to determine whether an association could be found between the morphological aspect of the proximal aspect of the dorsal condylar sagittal ridge (Hauspie *et al.* 2010) and the degree of cartilage degeneration, determined by the CDI, at the level of the entire MCP/MTP joint, distal metacarpal/metatarsal condyle or P1.

Materials and methods

Collection and preparation of specimen

Three hundred forty-four MCP/MTP joints were harvested from 86 Warmblood horses immediately after normal slaughter and stored at -20° C, wrapped in plastic bags until processing. The horses were euthanized with a captive bolt, followed by exsanguination. Age and breed were recorded from their chips and passports, but no other clinical data were available. This study was conducted in accordance with the Belgian Law of 14 August 1986, and the European Directive 86/609/EEC.

Lateromedial projections of the joints were obtained without removing the skin and subcutis. A perfect LM projection was defined as a superposition of the condyles that did not prohibit a thorough evaluation of the proximal aspect of the dorsal condylar sagittal ridge. Joints showing radiographic abnormalities or variations, other than those previously described at the level of the sagittal ridge (Hauspie *et al.* 2010) were excluded from the study.

The limbs were thawed at 7°C two days before taking any measurements. The joints were opened by circumferential incision of the joint capsule, and the proximal two-thirds of the canon bone cut off with a band-saw. The metacarpal/metatarsal bones and P1 were freed from the surrounding soft tissues and the cartilage surface was kept moist with isotonic saline (0.9% NaCl).

Measurements

The metacarpal/metatarsal and P1 specimen were turned upside down for five minutes in a bath containing Indian ink. Excess ink was removed by gentle rinsing with 0.5 L of isotonic saline solution (Brommer *et al.* 2003a).

A stereo triangulation scanner, based on the hand-held scanner design (Weise *et al.* 2007), was used to acquire full 3D models of each joint before and after staining. The scanner system consisted of a calibrated Digital Light Processing projector and two black and white

cameras that the operator used to reconstruct the shape of the specimen, using principles of stereo triangulation. A colour camera provided enough colour for the operator to identify the macroscopically visible cartilage changes on the stored images, but all calculations were made using the higher quality black and white data (Declercq *et al.* 2011).

All metacarpal/metatarsal and P1 specimen were clamped and mounted vertically on a computer-controlled turntable and scanned at 5-degree intervals, making 72 scans that were automatically assembled using the method described by Brown *et al.* 2008 (Brown *et al.* 2008). The scanner was mounted at the angle that allowed the camera to get images of both the top and sides of the joint so that a complete model of the cartilage surface could be created without repositioning metacarpal/metatarsal condyles or P1 on the turntable. This model also eliminated any outlying bright values that resulted from the reflection of light.

Once the 3D models were generated, an operator (the second author) selected the cartilage region of interest (the articular surface of the distal metacarpal/metatarsal condyle and proximal articular surface of P1) using a paintbrush metaphor, and this surface area was measured based on the 3D geometry (Figs. 1 & 2). The CDI (percentage of darkening) was calculated as the ratio between stained and unstained grey values of the selected cartilage surface. The ratio was computed for every facet of the model and averaged (Declercq *et al.* 2011).

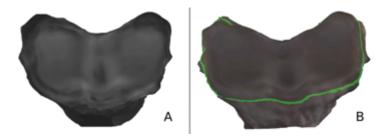


Figure 1. A) Proximal phalanx after staining with Indian ink; B) Proximal phalanx: cartilage selection on non-stained colour 3D image.

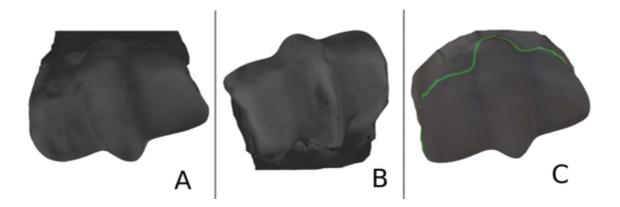


Figure 2. A) Metacarpal condyle: dorsal aspect after staining with Indian ink; B) Metacarpal condyle: palmar aspect after staining with Indian ink; C) Metacarpal condyle: cartilage selection on non-stained colour 3D image.

Statistical analysis was performed to determine whether the radiographic categories, the age and positions of the limb differed with respect to the CDI of the entire MCP/MTP joint, the distal metacarpal/metatarsal condyles and the P1 using a mixed model with animal as random effect. F-tests at a global significance level of 5% were used. All analyses were done with SAS version 9.2 (SAS Institute Inc., Cary, NC).

Results

After radiographic screening, 317 MCP/MTP joints (from 86 horses) from the original 344 were included in this study. The horses ranged from 2 to 28 years of age, with a mean age of 12.68 and a median age of 12. One hundred and fifty-six were front limbs and 161 were hind limbs. All 4 limbs from 65 horses were included, as well as 3 limbs from 16 horses, 2 limbs from 4 horses and 1 limb from 1 horse. A smooth radiographic aspect was seen in 133 MCP/MTP joints and the proximal aspect of the dorsal condylar sagittal ridge was irregularly outlined in 89 joints. A cam, lucency or indentation was detected at the proximal aspect of the dorsal condylar sagittal ridge in 47, 35 and 13 joints, respectively (Fig. 3).

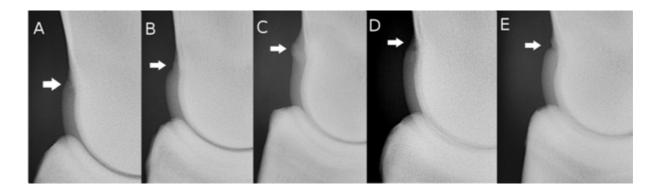


Figure 3. LM radiograph of the metacarpo-/metatarsophalangeal joint showing variations recorded at the proximal aspect of the sagittal ridge: A) "smooth", B) "irregular", C) "cam", D) "lucency", E) "indentation".

The ranges of mean (95% confidence interval) CDI value for the complete MCP/MTP joint, distal metacarpal/metatarsal condyle and P1 were 24.0 % (23.2-24.7), 22.4 % (21.7-23.0) and 27.0 % (26.1-27.8), respectively.

Table 1 summarizes the mean (95% confidence interval) CDI value for the complete MCP/MTP joint, distal metacarpal/metatarsal condyle and P1, subdivided by radiographic category and position of the limb. The increase of the CDI for each one-year increase in age was 0.16 % (SE 0.10; P = 0.11) for the complete MCP/MTP joint, 0.16 % (SE 0.09; P = 0.08) for the distal metacarpal/metatarsal condyle and 0.14 % (SE 0.12; P = 0.25) for P1.

		N	Complete Joint	Distal Condyle	Proximal Phalanx
Radiographic category	Smooth group	133	23.8 (22.8-24.8)	22.0 (21.0-23.0)	27.3 (25.9-28.6)
	Irregular group	89	24.3 (22.8-24.7)	22.7 (21.3-24.2)	27.0 (25.4-28.7)
	Cam group	47	23.0 (21.2-24.9)	21.5 (19.6-23.4)	25.9 (23.7-28.1)
	Lucency group	35	23.8 (21.8-25.8)	22.7 (20.8-24.5)	26.0 (23.4-28.6)
	Indentation group	13	27.3 (23.4-31.1)	25.7 (22.5-29.0)	30.0 (24.0-36.1)
	Front		23.8 (22.8-24.8)	22.3 (21.3-23.3)	26.6 (25.3-27.9)
Position of the limb	Hind		24.1 (23.2-25.1)	22.4 (21.5-23.4)	27.3 (26.2-28.4)
	Left		23.9 (22.9-24.9)	22.2 (21.3-23.2)	26.8 (25.6-28.0)
	Right		24.1 (23.1-25.0)	22.4 (21.5-23.3)	27.1 (25.9-28.3)

Table 1. Overview of cartilage degeneration index value (in percentage) (with 95% confidence interval) for the complete metacarpo-/metatarsophalangeal joint, distal metacarpal/metatarsal condyle and proximal phalanx subdivided per radiographic category and position of the limb.

There was no significant association between the CDI of the complete MCP/MTP joint, distal metacarpal/metatarsal condyle and P1 and the different covariates, such as radiographic categories, the age and the limb (left/right or front/hind).

Discussion

This study examined the relationship between the morphology of the proximal aspect of the dorsal condylar sagittal ridge, increase in age, the limb, and the presence of degenerative changes at the level of the articular cartilage in a random Warmblood horse population.

The Indian ink technique to stain cartilage surfaces and to determine the CDI, is very valuable, but reflects the cartilage surface damage only (Brommer *et al.* 2003a). No other features of osteoarthritis, such as changes in the subchondral bone (Kawcak *et al.* 2001) or joint capsule (Palmer and Bertone 1994) are taken into account. The strength of this technique is to have early detection of cartilage damage and of the whole cartilage surface, but on the other hand no information is gained on the status of the subchondral bone or the synovium. For evaluation of the structure, cellularity and tidemark, histological and histochemical grading as described by Mankin *et al.* 1971 is recommended for assessment of osteoarthrosis in horses (Mankin *et al.* 1971). This has the disadvantage that only the histological sample taken is evaluated; one sample does not always represents the status of the whole joint cartilage (McIlwraith *et al.* 2010).

The CDI technique developed by Brommer *et al.* 2003, which provides an objective evaluation of the quality of the articular cartilage over a large articular surface, was used (Brommer *et al.* 2003a). However, the technique described by Brommer *et al.* 2003, a manual method using digital photography, has been validated only for the proximal articular surface of the P1 and does not include area calculations on curved regions like the metacarpal/metatarsal condyles (Brommer *et al.* 2003a). Therefore a modified technique that created a complete 3D model of the articular surfaces of the distal metacarpal/metatarsal condyle and P1 and that allowed area calculations on curved surfaces was used (Declercq *et al.* 2011).

No significant differences were found in the mean CDI value of the front and hind limbs, which agrees with a study published by Riggs *et al.* 1999 that also found no differences

between the subchondral bone of corresponding regions of the distal metacarpal or metatarsal bone (Riggs *et al.* 1999). Changes at the level of the articular cartilage and adjacent subchondral bone are closely linked and adapt to loading in a coordinated way (Kawcak *et al.* 2001; Lewis *et al.* 2005; Lacourt *et al.* 2012). Therefore, if the subchondral bone architecture between metacarpal and metatarsal condyles is comparable, the cartilage status is also expected to be comparable. This was confirmed in our study.

The additional finding that the mean CDI value was not associated with the position of the limb (left or right) also agrees with the Riggs *et al.* 1999 study, where no significant difference was detected between distal metacarpal/metatarsal condyles from either the left or right limb (Riggs *et al.* 1999). Another study, however, showed a significantly higher glucosaminoglycan and water content of the cartilage of the right MCP joint, compared with the left (Brama *et al.* 2000). This higher glycosaminoglycan and water content is linked to an increase in stiffness of the cartilage (Palmer and Bertone 1996). Therefore a difference of the cartilage in response to loading conditions can be expected, possibly associated with a difference in the degree of cartilage damage, resulting in a different CDI value. This could however not be confirmed in our study at the level of the MCP joints.

In Thoroughbreds, no statistical significant association was detected between the radiographic appearance of the proximal aspect of the dorsal condylar sagittal ridge and reduced performance, determined as a decreased likelihood of starting a race, average earnings per start and money earned (Kane *et al.* 2003a). No significant association was detected in our study between the radiographic appearance of the proximal aspect of the dorsal condylar sagittal ridge and changes in the CDI of the MCP/MTP joint in Warmblood horses. This statement is valid not only for young horses but also older ones, as the age of horses in our study ranged from 2 to 28 years, with a median age of 12 years. However, the status of the articular cartilage is not the only factor with an influence on the joint function.

Local CDI values are known to increase from central to dorsal at the proximal articular surface of P1 (Brommer *et al.* 2003b) and significant differences have been detected between local CDI values of different points on the proximal articular surface of P1 (Brommer *et al.* 2005). A similar trend is likely for the distal metacarpal/metatarsal condyles, although they were not included in that study. The radiographic variations described in this study are located at the dorsal condylar sagittal ridge. If these variations influence the joint function, they probably have most effect at that level. Therefore, dividing the articular joint surfaces into

different regions could identify any differences in the CDI value at the level of the dorsal aspect of the joint between the different categories.

No significant effect of age was detected, neither on the mean CDI value of the entire joint, nor on the CDI of the distal metacarpal/metatarsal condyle or the proximal articular surface of P1. The latter result contrasts with a study published by Brommer *et al.* 2003 in which a moderate, but significant, association between the CDI value of the proximal articular surface of P1 and age was found (Brommer *et al.* 2003b). A possible explanation is that our population was larger compared to Brommer's (317 joints compared with 73), so our results more accurately represent the population. Finally, our study adjusted for the possible clustering effect of the limbs of the same horse by adding the horse as a random factor in our model.

In conclusion, the radiographic appearance of the proximal aspect of the dorsal condylar sagittal ridge has no significant association with an increase in the CDI at the level of the joint. This supports the study in Thoroughbreds where no association was detected between these variations and reduced performance at a young age (Kane *et al.* 2003a).

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Chapter 6

The position of the dorsal proximal synovial pad during hyperextension of the equine metacarpo-/metatarsophalangeal joint

Adapted from:

Hauspie S., Vanderperren K., Gielen I., Pardon B., Kromhout K. Martens A., Saunders J.H. The position of the dorsal proximal synovial plica during hyperextension of the equine metacarpo-metatarsophalangeal joint (submitted).

Summary

A synovial plica is present at the dorsoproximal aspect of the MCP/MTP joint. It is supposed to function as a cushioning surface or contact interface between P1 and the cannon bone where these are in contact during full extension of the joint. The objective of this study was to describe the location of the dorsoproximal synovial plica during hyperextension of the MCP/MTP joint using MRI, in order to get a better insight in its function.

For this study 20 cadaver limbs from 5 Warmblood horses were used. Of each joint, 2 MRI scans were performed. One with the limb fixed in a normal standing position of the MCP/MTP joint, a second scan with the joint fixed in a hyperextended position. Measurements were made of the dorsal and palmar/plantar length and the thickness of the plica in the normal position. During hyperextension, the position of the plica was described, the angle of contact between P1 and the condyle was measured, and the dorsal angle of extension was measured.

The dorsal length differed between front/hind limbs and between the medial/lateral aspects of the joint. The angle of contact between P1 and condyle differed between front/hind limbs and between the lateral and medial aspect of the joint and between different positions of the plica. Four different positions of the plica were observed during hyperextension: (1) shortened with the tip curved towards palmar/plantar; (2) projecting distally; (3) projecting towards dorsal and (4) projecting distally with the tip interposed between P1 and the condyle.

During hyperextension, a close relation is present between the proximal synovial plica, proximal aspect of P1 and the condyle with a variable position of the plica, suggestive for a contact interface between P1 and the cannon bone. However, due to the different positions of the plica, it does not seem to act consistently as a cushioning surface.

Introduction

The MCP/MTP joint in the horse comprises of the MCIII/MTIII, P1 and the proximal sesamoid bones (Barone 1986). An articular capsule and multiple ligaments reinforce this joint (Barone 1989; Weaver *et al.* 1992). As a result of this design, the MCP/MTP joint is limited in its motion mainly to the sagittal plane (Palmer and Bertone 1996; Les *et al.* 1997; Butcher and Shley-Ross 2002), resulting in either flexion or extension (Butcher and Shley-Ross 2002). During hyperextension, the proximal articular surface of P1 articulates with the dorsal aspect of the condyle of MCIII/MTIII (Palmer and Bertone 1996; Brama *et al.* 2001). At the dorsoproximal recess of the MCP/MTP joint, the joint capsule forms a pad or synovial plica, projecting distally from the dorsoproximal attachment of the joint capsule and tapering to a thin edge. This plica covers the transition zone between the cartilage and the attachment of the joint capsule (White 1990; Dabareiner *et al.* 1996). Several abnormalities are described at the level of this plica, ranging from synovial plica proliferation to the presence of fragments (White 1990; Declercq *et al.* 2008).

Radiographic variations in appearance of the dorsoproximal aspect of the sagittal ridge are described in the MCP/MTP joint of Warmbloods (Hauspie *et al.* 2010). Although the function of the synovial plica is unknown, its position and structure suggests that the plica acts as a cushioning surface or contact interface between P1 and the cannon bone where these are in contact during full extension of the joint (White 1990). Due to the location of the synovial plica and the different variations present at the dorsoproximal aspect of the sagittal ridge, these are possibly in close relation to each other. Therefore, it is possible that the variations at the dorsoproximal aspect of the sagittal ridge will interfere with the plica during hyperextension. However, the exact position of the plica during hyperextension is until know not described.

Magnetic resonance imaging is commonly being used for investigation of lameness in horses (Mair *et al.* 2005; Powell 2012; Smith *et al.* 2012). The high soft tissue contrast of MRI makes this modality particularly suited for the evaluation of soft tissues (Kraft and Gavin 2001). The normal MRI anatomy of the MCP/MTP joint has been described using both low- and high-field magnets (Park *et al.* 1987; Martinelli *et al.* 1996; Martinelli *et al.* 1997; Dyson and Murray 2007), showing the ability of outlining the dorsoproximal synovial plica on different sequences.

The objective of this article was to describe the location of the dorsoproximal synovial plica during hyperextension of the MCP/MTP joint using MRI, in order to get a better insight in its function.

Materials and methods

Collection and preparation of the limbs

Fresh cadaver front and hind limbs from Warmblood horses, aged younger than 5 years, were collected for this study. These horses were euthanized for reasons unrelated to this study and collected at the pathology department of our institute. The limbs, obtained less than 24 hours after euthanasia, were sectioned at the level of the carpometacarpal or tarsocrural joint. The proximal end of the limbs was covered with a latex glove to prevent blood loss during handling. The shoe was removed from each digit. After collection each limb was placed in a plastic bag and frozen at -25 °C. A perfect LM radiograph of each joint, defined as a superposition of the condyles that did not prohibit a thorough evaluation of the dorsal condylar sagittal ridge, was obtained without thawing the limb to ensure the normal appearance of the MCP/MTP joint. If radiographic abnormalities at the level of the joint were detected, such as bone fragmentation or peri-articular new bone formation, the horse was excluded from this study and new specimen were collected until a total of 20 normal joints were collected. Before the MRI examination, limbs were thawed at ambient temperature for 24 hours. It has been proven that this procedure has the least influence on image quality (Bolen *et al.* 2011). After thawing, the skin of the limb was clipped and the hoof was cleaned.

MR imaging examinations

Imaging sequences were obtained while the limb was fixed in a custom made loading frame (Fig. 1). Two scans were performed on each limb, one with the MCP/MTP joint fixed in normal standing position (dorsal angle between MCIII/MTIII and P1 of approximately 150°) and one with the joint fixed in hyperextension (dorsal angle between the MCIII/MTIII and P1 of approximately 120°) (Meershoek *et al.* 2001; Brown *et al.* 2003). The angle of extension was measured macroscopically with a protractor at the level of the dorsal aspect of the distal metacarpus/metatarsus and pastern region. To allow sufficient hyperextension of the MCP/MTP joint using a custom made wooden loading frame, the suspensory ligament and accessory ligament of the deep digital flexor tendon had to be sectioned at the proximal aspect

in front limbs, whereas in the hind limbs, the suspensory ligament, accessory ligament of the deep digital flexor tendon, the deep digital flexor tendon and superficial digital flexor tendon had to be sectioned at the proximal aspect. Without sectioning of these tendons, a metal loading frame was needed, making an MRI examination impossible. This methodology was based on the results of a preliminary study where 20 limbs were hyperextended twice using a custom made metal loading frame: a first time with the flexor tendons intact; a second time after selective sectioning of the tendons and/or ligaments as mentioned above. The position of the bones of the MCP/MTP joint was evaluated by means of radiography (LM, D45°M-Pa(Pl)LO and D45°L-Pa(Pl)MO projections) and showed that sectioning of these tendons and/or ligaments did not alter the position of the bones relative to each other during hyperextension.

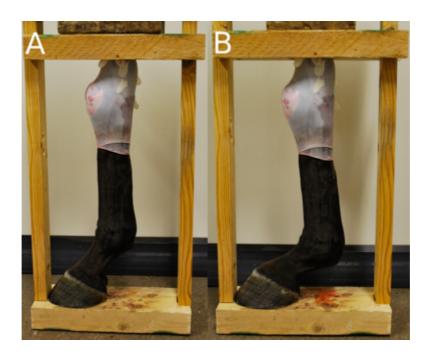


Figure 1. Illustration of the custom made loading frame used to facilitate extension of the metacarpo-/metatarsophalangeal joint: A) The limb is fixed in the normal standing position, B) The limb is fixed in a hyperextended position.

Magnetic resonance imaging was performed using a permanent 0.2 T magnet (Airismate, Hitachi, Japan). The limb was positioned in right lateral recumbence and placed in a human knee coil. Sagittal images were made with slice thickness of 3mm without interslice gap. The image matrix was 256×256 . In the normal standing position, T1W (TR = 520 ms, TE = 17 ms); T2W (TR = 4700 ms, TE = 125 ms) and a PDW SE sequence (TR = 4700 ms,

TE = 25 ms) were obtained. In the hyperextended position, the above-mentioned scans were repeated and an additional STIR sequence (TR = 6800 ms, TE = 30 ms) was obtained.

Measurements

The DICOM studies were retrieved and analysed on a diagnostic workstation using DICOM viewing software (Osirix, Geneva, Switzerland) for the following bone and soft tissue parameters.

With the limb in a normal standing position, the dorsal length (in cm), the palmar/plantar length (in cm) and the thickness (in cm) of the proximal synovial plica were measured at the level of the medial and lateral condyle, approximately in the centre of each condyle. The dorsal length was measured from its most proximal point (defined as the point where it was projecting distally originating from the articular capsule) till the tip of the plica. The palmar/plantar length was measured from the tip toward its attachment to the MCIII/MTIII. The thickness of the plica was measured at the point of maximal thickness of the "free floating" part of the plica (Fig. 2). The measurements were made on the sequence that allowed the best outlining of the plica.

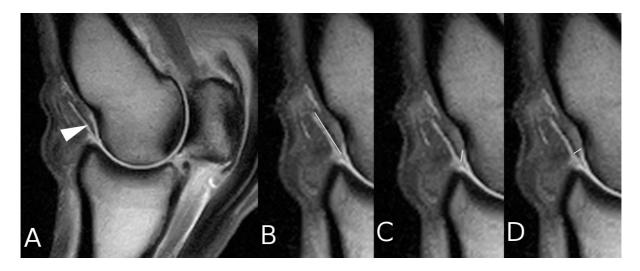


Figure 2. Illustration of the different measurement made at the level of the dorsoproximal synovial plica with the limb in a normal standing position: A) Synovial plica (arrowhead), B) Measurement of the dorsal length of the plica (white line), C) Measurement of the palmar/plantar length of the plica (white line), D) Measurement of the thickness of the plica (white line).

With the limb fixed in a hyperextended position the position of the plica was described for its medial and lateral part. The dorsal extension angle was measured at the level of the sagittal ridge by connecting the lines parallel to the dorsal cortex of the third metacarpal/metatarsal bone and P1. At the level of the medial and lateral dorsal eminence of P1, the place of contact between P1 and the third metacarpal/metatarsal condyle was determined. Any abnormal signal intensities in the adjacent bone structures at the place of the contact of P1 and condyles were recorded.

The place of contact between P1 and the third metacarpal/metatarsal condyle was defined as the place where the most proximal part of the dorsoproximal eminence of P1 was in contact with the distal third metacarpal/metatarsal condyle (Fig. 3). This place of contact was determined medial and lateral in the sagittal plane at the point where the most proximal part of the dorsal eminence of P1 was identified first when scrolling from proximal towards distal on a transverse reconstruction. It was determined as an angle using a previously described method (Vanderperren et al. 2009). In summary, the best fitting circle around the condyle was drawn. Next, a line parallel to the dorsal cortex of the third metacarpal/metatarsal bone was drawn from the centre of this circle towards P1. The distal crossing of this line with the circle was the reference point (0°) . Next a line was drawn from the most proximal aspect of the dorsomedial or dorsolateral proximal eminence of P1 towards the centre of the circle. The angle between these 2 lines was defined as the angle of the place of contact between P1 and the third metacarpal/metatarsal condyle (Fig. 3). All measurements were made in agreement by two observers (S.H., K.V.). The position of the plica with the limb fixed in a hyperextended position was defined as either shortened with the tip of the plica bending or curving towards palmar/plantar (position 1); projecting in a straight line distally parallel with the third metacarpal/metatarsal condyle (position 2); projecting in a straight line towards dorsal with the proximal eminence of P1 located underneath it (position 3); projecting distally with the tip of the plica located between the distal condyle and P1 (position 4) (Fig. 4).

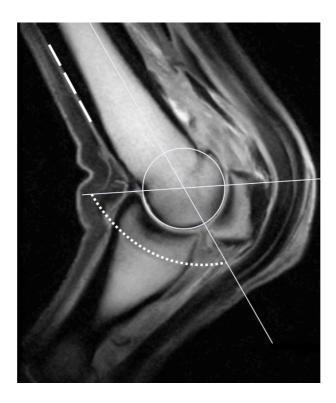


Figure 3. Illustration of the method used to measure the place of contact between the proximal phalanx and the condyle with the limb fixed in a hyperextended position. The long dashed line is representing the line parallel to the dorsal third metacarpal/metatarsal cortex. The short dotted line is representing the angle that was measured and defined as the angle of the place of contact between the proximal phalanx and the condyle.

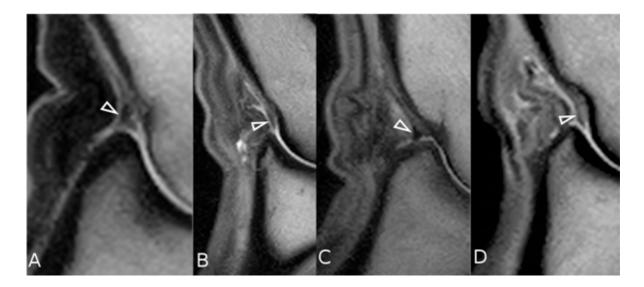


Figure 4. Illustration of the different observed positions of the dorsoproximal synovial plica with the limb fixed in a hyperextended position. A) The plica is shortened with the tip of the plica bended towards palmar/plantar (position 1), B) The plica is projecting distally in a straight line (position 2), C) The plica is projecting dorsally with the proximal eminence located underneath it (position 3), D) The plica is projecting distally with the tip interposed in between the proximal aspect of the proximal phalanx and the condyle (position 4).

Statistical analysis

The unit of analysis was the measurement on a joint within a horse. All continuous outcome variables were checked for a normal distribution. To determine significant differences between front or hind limb, left or right limb, medial or lateral side of the joint and the different positions of the plica for the different outcome variables (dorsal and palmar length of the plica, thickness of the plica, angle of contact between P1 and condyle) a linear mixed model (PROC MIXED) was built (maximum likelihood method), with horse included as a random effect to account for clustering of limbs within a horse. The same model was used to determine significant differences between front or hind limb, left or right limb and the different positions of the plica for the outcome variable dorsal angle during hyperextension. In all models all predictor variables were first tested univariably. All predictors with P < 0.2 in the univariable model were withheld for the multivariable model, which was built stepwise backward, gradually excluding non-significant variables. For the final models, pairwise comparisons for categorical predictors were made, with Scheffé adjustment for multiple comparisons. All biologically relevant two-way interactions of significant fixed effects were tested. Significance was set at P < 0.05. Descriptive and statistical analysis was performed in SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Five Warmblood horses (2 geldings, 2 stallions and 1 mare) were used in this study with a mean and median age of 2.7 years and 3.0 years respectively, ranging from 1.5 to 4 years, The mean \pm s.d. angle of the normal and hyperextended position as measured on MRI was $145.6 \pm 6.7^{\circ}$ and $124.4 \pm 6.1^{\circ}$ respectively. The mean \pm s.d. dorsal length, palmar length and thickness of the plica and the mean \pm s.d. place of contact between P1 and the third metacarpal/metatarsal condyle, subdivided between limb and lateral/medial are summarized in table 1. No abnormal signal intensities were recorded at the place of the contact between P1 and the third metacarpal/metatarsal condyle on the different sequences.

	Left Front		Right Front		Left Hind		Right hind	
	Lateral	Medial	Lateral	Medial	Lateral	Medial	Lateral	Medial
Dorsal length pad	1.29 ± 0.24	1.54 ± 0.16	1.39 ± 0.23	1.43 ± 0.19	1.63 ± 0.24	1.67 ± 0.36	1.61 ± 0.16	1.82 ± 0.16
Palmar/plantar length pad	0.35 ± 0.22	0.38 ± 0.32	0.39 ± 0.18	0.38 ± 0.25	0.44 ± 0.19	0.44 ± 0.21	0.36 ± 0.15	0.38 ± 0.20
Thickness pad	0.14 ± 0.02	0.18 ± 0.06	0.18 ± 0.04	0.14 ± 0.05	0.26 ± 0.07	0.26 ± 0.14	0.25 ± 0.07	0.24 ± 0.07
Place of contact	104.48 ± 6.25	108.98 ± 4.95	100.99 ± 7.52	104.27 ± 9.08	107.50 ± 5.86	112.02 ± 4.54	109.64 ± 6.11	111.96 ± 4.87

Table 1. Overview of the mean \pm s.d. dorsal length (in cm), palmar length (in cm) and thickness (in cm) of the pad measured with the limb in a normal standing position, the mean \pm s.d. place of contact between P1 and the metacarpal/metatarsal condyle (in °) measured with the limb fixed in a hyperextended position, subdivided between limb and lateral/medial.

The dorsal length of the plica differed between a front and hind limb and between the medial and lateral aspect of the joint. No association was detected between the dorsal length of the plica and a left/right limb or the position of the plica during hyperextension. The plica was on average 0.26 cm longer in a hind limb (P < 0.01) and 0.14 cm longer at the medial aspect of the joint (P = 0.03). The palmar length of the plica differed not significantly between the different limbs or the position of the plica. The thickness of the plica differed between a front or hind limb and the different positions of the plica. However, the exact interaction between thickness of the plica and front or hind limb or the position of the plica during hyperextension could not be tested due to the low sample size. The angle of place of contact between P1 and the condyle was different between a front and hind limb, between the lateral and the medial aspect of the joint and between different positions of the plica. The angle of place of contact was 3.48° larger (meaning that the dorsoproximal eminence of P1 was located more proximal at the level of the distal third metacarpal condyle) in a hind limb (P = 0.02) and 3.01° larger at the medial aspect of the joint (P = 0.02). With the plica in position 2, the angle of place of contact was 7.12° smaller compared to when the plica was in position 1 (P < 0.001); and the angle of place of contact was 8.75° smaller compared to when the plica was in position 3 (P < 0.001). The dorsal angle during hyperextension was not different between the front or hind limbs or between a left or right limb. However, a difference of dorsal angle during hyperextension was detected between different positions of the plica. The dorsal angle was 9.95° larger (or less extended) when the plica was in position 2 compared to position 1 (P < 0.001). With the plica in position 4 the dorsal angle was 9.31° larger compared to position 1 (P = 0.04).

In 18 limbs, the position of the plica during hyperextension was symmetrical between lateral en medial. The plica was located in position 1 in 7 of these 18 limbs; in 7 other limbs

the plica was located in position 2; in 3 limbs the plica was in position 3 and finally the plica was located in position 4 in one limb. In the 2 remaining limbs, the medial part of the plica was in position 1 whereas the lateral part was either in position 2 or 4 (Fig. 4).

Discussion

The overall goal of this study was to describe the exact location of the dorsoproximal synovial plica during hyperextension of the MCP/MTP joint to get a better insight in its function. Previously, it has been stated that the position and structure of the plica suggests that it acts as a cushioning surface or contact interface between P1 and the third metacarpal bone where these are in contact during full extension of the joint (White 1990). This study demonstrated that different positions of the synovial plica are possible during hyperextension of the joint. A close contact with the plica and the dorsoproximal aspect of the distal third metacarpal/metatarsal condyle was detected, at least with position 1 and 4, suggesting a close relation between the plica and the dorsoproximal aspect of the sagittal ridge during hyperextension.

In this study, low-field MRI was performed to describe the exact location of the pad during hyperextension of the joint. It is generally accepted that MRI is the best imaging modality to image soft tissue structures (Kraft and Gavin 2001). Although smaller structures are better identified using high-field MRI (Smith *et al.* 2011), the overall quality of the images obtained on our low-field MRI system was considered to be sufficient for the purpose of this study.

In this study we used T1W, T2W and PDW SE sequences in all limbs in normal and hyperextended position. A T1W SE sequence gives the best anatomical detail and is considered to be the standard accepted sequence for baseline information of the musculoskeletal system (Kleiter *et al.* 1999; Smith *et al.* 2011). Additional T2W and PDW SE sequences were obtained for evaluation of synovial structures, as the hyperintense synovial fluid creates a better contrast between the synovial plica and the surrounding synovial fluid (Mair *et al.* 2005). With the limb hyperextended an additional STIR sequence was obtained to identify the medullary fluid by eliminating the medullary fat signal of the bones (Mair *et al.* 2005).

Scans in the dorsal and sagittal planes are generally performed for evaluation of the articular surface of joints (Kleiter *et al.* 1999), but for this study only sagittal plane scans were obtained to evaluate the proximal synovial plica in the MCP/MTP joint (Dyson and Murray 2007).

No abnormal low or high signal intensity was detected on the images at the level of the dorsoproximal aspect of P1 or the dorsoproximal third metacarpal/metatarsal condyle at the level where both are in contact during hyperextension. Magnetic resonance imaging has a high sensitivity for the detection of abnormalities at the level of subchondral bone such as bone sclerosis or bone bruise (Smith *et al.* 2012) which means that most likely no abnormalities were present at the level of the bone at the place of contact during hyperextension. It is possible that post-mortem changes and freeze-thaw effect could have an effect on the MR images. It has been reported that there is no subjective difference between images of the same digits obtained before death and after postmortem freezing and thawing (Murray *et al.* 2006). However, it has been demonstrated that freezing-thawing processes can induce changes of the signal-to-noise ratio in images and may alter the reliability of signal intensity in ex vivo MRI examinations (Bolen *et al.* 2011).

To facilitate the extension of the MCP/MTP joint, the suspensory ligament and accessory ligament of the deep digital flexor tendon were sectioned at the proximal aspect of the metacarpus and the suspensory ligament, accessory ligament of the deep digital flexor tendon, the deep digital flexor tendon and superficial digital flexor tendon were sectioned at the proximal aspect of the metatarsus. Without sectioning of the tendons, up to 12 kN is needed to obtain the same amount of hyperextension as is detected at the level of the MCP/MTP joint during jumping (Brama *et al.* 2001). It was not possible to obtain this amount of pressure with a non-magnetic loading frame that could be used in a MRI device. Therefore, sectioning of the flexor tendons was performed to obtain hyperextension with the amount of pressure that could be obtained using a wooden frame. This procedure was considered to be acceptable for the purpose of the study as it was shown in a preliminary study that it had no influence on the position of the bones relative to each other during hyperextension.

In human medicine, plicae are described at the level of the wrist, knee, elbow and hip joint (Kim *et al.* 2006; Katz *et al.* 2010; Al-Hadithy *et al.* 2011; Ishii *et al.* 2012). In normal conditions, these synovial plicae slip to and fro between the articular surfaces without significant mechanical impingement (Bencardino *et al.* 2011). However, in each of these

joints, symptomatic plicae are described with concurring histological abnormalities (Kim et al. 2006; Katz et al. 2010; Al-Hadithy et al. 2011; Ishii et al. 2012). Synovial plica proliferation is also described in the horse, mainly located at the level of the MCP joint with concurring histological abnormalities at the level of the plica (Van Veenendaal and Moffatt 1980; Steyn et al. 1989; Kannegieter 1990; White 1990; Dabareiner et al. 1996). The dorsal length of the medial part of the synovial plica was significantly longer compared to the lateral part and overall significantly longer in the hind limb. However, the repeatability of these measurements was not assessed in this study. Most of the cases of synovial plica proliferation are described at the level of the front limbs without a predilection for medial or lateral (Van Veenendaal and Moffatt 1980; Steyn et al. 1989; Kannegieter 1990; White 1990; Dabareiner et al. 1996). A correlation of this condition with possible differences in length of the plica described in this study seems therefore unlikely. Possibly, the position of the plica during hyperextension is a predisposing factor for the development of proliferation of the synovial plica. It is possible that the plica located in position 4 can be bruised during hyperextension. A correlation between the histogical appearance of the plica and its position during hyperextension would help to confirm this.

The angle of contact between the proximal aspect of P1 and the condyle was significantly larger at the level of the medial dorsal eminence of P1 compared to the lateral dorsal eminence. Fragments at the dorsal aspect of P1 are predominantly detected at the level of the medial dorsal eminence of P1 (Kawcak and McIlwraith 1994; Declercq *et al.* 2009). These fragments probably result from compression of the dorsoproximal portion of P1 against the distal third metacarpal/metatarsal bone during hyperextension of the joint (Yovich and McIlwraith 1986). This is in agreement with the larger angle of contact at the level of the medial dorsal eminence of P1, most likely resulting in higher stress at that level.

In this study a significantly larger angle of contact between P1 and the condyle was detected at the level of the hind limb. A possible explanation could be the sectioning of all flexor tendons and ligaments at the level of the hind limb as higher degree of hyperextension was obtained in the hind limb. However, no significant difference was detected between the degree of hyperextension in a front or hind limb. Meaning that the higher position of the dorsal eminences of P1 was not caused by a higher amount of hyperextension of the MCP/MTP joint. Therefore it is unlikely that sectioning of the tendons is the cause of the larger angle of contact between P1 and the condyle in the hind limb. This difference in angle of contact between P1 and the condyle suggests that the dorsal eminences are possible bigger

in a hind limb, compared to the front limb. This could mean that a higher incidence of dorsoproximal P1 fragments can be present at the level of the hind limb. However, no predilection for dorsoproximal fragments of P1 at the level of the hind limb is described (Declercq *et al.* 2009).

Osteochondral fragmentation at the level of the synovial plica is a condition described in the MCP/MTP joint (Declercq *et al.* 2008). The origin of these synovial fragments still remains unclear. A possible hypothesis is that this osteochondral fragment is a pre-existing cartilaginous structure that develops a fibrous reactive pattern due to a mechanical stimulus such as the repetitive contact with P1 during full extension of the joint (Declercq *et al.* 2008). This close contact between the plica and the dorsoproximal aspect of P1 has been clearly demonstrated in this study.

The position of the proximal synovial plica in the MCP/MTP joint was in most limbs symmetrical between the medial and the lateral part. Only in 2 limbs of 2 different horses a difference in position of the lateral and medial part was detected. No reason for the difference in lateral-medial position of the plica in these two limbs was detected. It is possibly that this medial-lateral asymmetry was coincidental, as the repeatability of the position of the plica during hyperextension was not assessed in this study

The angle of contact between P1 and the condyle was significantly smaller when the plica was in position 2 compared to when the plica was in position 1 or 3. In addition, with the plica in position 2 the dorsal angle during hyperextension was significantly larger compared to when the plica was in position 1; meaning that there was less extension of the MCP/MTP joint in the group with the plica in position 2. It is possible that the distal projection of the plica (position 2) represents an intermediate position that will change to one of the other three positions when the joint is in full hyperextension (significantly larger angle of contact P1/condyle for position 1 and 3). To consolidate this hypothesis, further research on limbs fixed sequentially in different angles of extension should be performed.

Interesting in this study was that the dorsal angle during hyperextension was 9.31° larger or the joint was less extended when the plica was in position 4 than when the plica was in position 1. The dorsal angle during hyperextension was not significantly different when the plica was in position 2 and 4. Changing the angle of the extension on the same limb could also reveal if this position of the plica (position 4) is an in intermediate position between the

plica projecting distally (position 2) and the position with the tip of the plica bending palmar/plantar (position 1) or projecting dorsally (position 3).

The mean \pm s.d. thickness of the proximal synovial plica was 2.07 ± 0.13 mm in this study. This is in agreement with the number available in the literature, ranging from 2 to 4 mm (White 1990; Denoix 1996).

This study demonstrated that during hyperextension of the joint, the proximal synovial plica can have different positions. Either the tip of the plica is projecting distally, dorsally, is interposed in between P1 and the condyle or the tip of the plica is bending towards palmar/plantar. During hyperextension, a close relation is present between the proximal synovial plica, the proximal aspect of P1 and the distal third metacarpal/metatarsal condyle. The plica therefore most likely acts as a contact interface between P1 and the cannon bone. However, considering the different positions of the plica during hyperextension the plica does not seem not to function as a cushioning interface between P1 and the cannon bone during hyperextension of the joint.

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Chapter 7 General Discussion

A veterinary pre-purchase examination is an important professional service offered by the veterinarian designed to identify any abnormalities or potential problems that can make the horse unsuitable for its intended use (Bladon and Main 2003; Van Hoogmoed *et al.* 2003). During this pre-purchase examination, survey radiographs of the distal limb are often taken in order to identify these abnormalities or potential problems (Van Hoogmoed *et al.* 2003). The predictive value of findings on radiography is controversial but nevertheless, radiography has become an integral part of a pre-purchase examination (Van Hoogmoed *et al.* 2003) and the presence or absence of radiological abnormalities has become an important factor in the horse business (Stock *et al.* 2005).

The MCP/MTP joint is a high motion joint with high loading and is therefore frequently injured (Pool and Meagher 1990). Variations in appearance of the dorsoproximal aspect of the sagittal ridge have been observed during radiographic examinations of the MCP/MTP joint in different breeds (Kane *et al.* 2003b; Wittwer *et al.* 2006; Verwilghen *et al.* 2009). However, there is a discrepancy in detected variations between these breeds.

When these variations are identified in a sound horse, the veterinarian will be asked to evaluate how these will influence the future performance of the horse (Van Hoogmoed *et al.* 2003). The scientific information on these detected variations of the dorsoproximal aspect of the sagittal ridge is limited. The presence of these variations at the level of the sagittal ridge was not associated with loss of performance at young age in Thoroughbreds (Kane *et al.* 2003a). However, studies performed on Thoroughbred horses cannot be just extrapolated to Warmbloods. These latter are not being trained until they reach the age of 3 years and will not be challenged at high speeds as is done in Thoroughbreds at the age of 2 and 3 years. Warmbloods will only start reaching their toplevel the earliest at 8 years old (Hinchcliff and Hamlin 2004; O'Sullivan and Lumsden 2004).

In addition, the difference in type of career between these breeds makes a difference in evaluating the potential influence of these variations. It is easier in Thoroughbreds, running at high speed, to evaluate limiting performance or subtle lameness. In contrast to a Warmblood horse, of which the type of training frequently differs from horse to horse, the objective determination of the cause of not "performing up to the expected level" is very difficult. A thorough study of the variations at the dorsoproximal aspect of the sagittal ridge is therefore needed to help in the decision making during a pre-purchase examination.

To evaluate the relevance of these variations, ideally, a longitudinal study would be needed. However, this is difficult to perform in Warmblood horses. First, a long follow up period is necessary, because of the long career of a Warmblood horse (up to 15 years). Secondly, because of the different uses of a Warmblood horse (jumping, dressage, eventing), a large population is needed. Horses competing in different sports are predisposed for different injuries (Murray *et al.* 2006). Last, Warmblood horses are frequently sold and therefore lost for follow up.

In the present study we started by defining the exact prevalence of the variations at the dorsoproximal aspect of the sagittal ridge of the MCP/MTP joint in Warmblood horses. After screening radiographs of 308 stallions, we detected a total of 5 variations. The most commonly observed variation was a "smooth" appearance of the ridge (51.5%), followed by an "irregular" outlining (19.3%). The remaining variations detected were a "cam" like lesion (8.9%), "lucency" (8.1%) and "indentation" (12.2%). Based on our study, a discrepancy was found in the number of detected variations at the dorsoproximal aspect of the sagittal ridge, and their frequency of occurrence, compared with an other population of Warmbloods (Verwilghen *et al.* 2009) and compared with Thoroughbreds (Kane *et al.* 2003b). In a population of Warmbloods, only a mild surface irregularity of the proximal border of the sagittal ridge was described in 6.8% of the joints and a well defined osteochondral defect at that level in 1% (Verwilghen *et al.* 2009). In Thoroughbreds, only a notch in approximately 24% of the joints, lucency (1%) or a fragment/loose body (0.5%) was described (Kane *et al.* 2003b).

This difference in number and frequency could be expected as different frequencies are reported between different populations of Thoroughbreds for other abnormalities at the level of the MCP/MTP joint: for example the reported frequency of osteochondrosis, ranges from 7.5% to 61% (Howard and Rantanen 1992; Kane *et al.* 2003b; Furniss *et al.* 2011). This difference in number and frequency is most likely explained by the fact that the prevalence of common radiographic findings in the limbs of young horses are influenced by genetics and probably are caused by different genes (Stock and Distl 2006).

The origin of these variations in appearance of the dorsoproximal aspect of the sagittal ridge is unknown. Irregularities and depressions of the subchondral bone at the mid and distal aspect of the dorsal condylar sagittal ridge are associated with osteochondrosis dissecans in horses (Yovich *et al.* 1985; Nixon 1990). Typical histological changes associated with

dyschondroplasia are clear alterations in staining of the mineralized matrix of the cartilage and adjacent subchondral bone or retained cartilage cores surrounded by clusters of chondrocytes (Henson *et al.* 1997; Jeffcott and Henson 1998). Histology demonstrated in all of the samples of the present study a clear demarcated cortex, covered towards distally with normal appearing hyaline cartilage and towards proximally with longitudinal aligned collagen fibres. Based on these results, the origin of these variations is not osteochondrotic. However, since only 25 samples were examined in this study and the quality of the staining was sometimes suboptimal, more subtle lesions indicating a possible osteochondrotic origin of these variations, could have been missed.

No information is available if these variations are already detected at birth (congenital) or if they only develop at later age (joint adaptation to workload). Based on histology a reactive process is excluded, however a slow ongoing process remains possible. Evolution of radiographic changes in young horses up to the age of 11 months has been demonstrated (Dik et al. 1999) and cartilage and its supporting subchondral bone undergo substantial changes at young age. Both undergo major changes in composition under the age of 5 months and a slower rate of evolution is detected at a later age. These changes result in differences in composition of the cartilage and subchondral bone between the dorsoproximal aspect of P1 and the more centrally located regions (Brama et al. 2002a; Brommer et al. 2005). The maturation of cartilage is also influenced by the type of exercise (Brama et al. 2002b). However, maturation of cartilage and subchondral bone has up to now only been demonstrated at the proximal articular surface of P1. A similar evolution is possible at the level of the proximal aspect of the dorsal condylar sagittal ridge and the condyles but has until now not been examined. During hyperextension of the MCP/MTP joint the dorsoproximal aspect of P1 comes into contact with the apposing surface of the distal metacarpal condyle (Brama et al. 2001). This abutment of P1 against the condyle can result in an adaptation at young age of the dorsoproximal aspect of the distal metacarpal/metatarsal condyles to sustained forces in a same way as described at the level of the proximal articular surface of P1. The period of maturation of cartilage and subchondral bone (Brama et al. 2002a; Brommer et al. 2005) corresponds with the period of radiographic evolution of osteochondral abnormalities (Dik et al. 1999).

Based on histology the "cam" group and the "indentation" group in our study showed a close resemblance with lesions associated with femoroacetabular impingement in the human hip joint (Pitt *et al.* 1982). Due to femoral head or acetabular abnormalities, femoroacetabular

impingement occurs as a result of abnormal contact between the proximal femur and acetabular rim (Keogh and Batt 2008). Based on histology, the osseous excrescence or reactive area at the level of the femoral head-neck junction (Hong *et al.* 2010; Bredella *et al.* 2013) and associated pit herniation are lesions in the human hip joint that show resemblance with our "cam" and "indentation" group (Pitt *et al.* 1982). Avulsion of the aceteabular labrum and cartilage damage are associated lesions with femoroacetabular impingement in the human hip joint (Hong *et al.* 2010).

The origin of these lesions at the level of the human hip joint is unclear, but could be congenital or developmental where abnormalities are formed during adolescence related to an abnormal femoral head conformation (Bredella *et al.* 2013). To further elucidate the possible origin of the different appearances of the dorsoproximal aspect of the sagittal ridge in horses, a follow up study performed on young Warmblood horses from birth till the age of 1 or 2 years should be performed.

Based on previous research, initial cartilage degeneration in the MCP/MTP joint starts at the dorsal articular margin of P1 (Brommer et al. 2004a) and early degenerative changes are frequently encountered at the dorsal aspect of the distal third metacarpal/metatarsal condyle (Brama et al. 2000). Cartilage degeneration can therefore be initiated by the variations detected at the dorsoproximal aspect of the sagittal ridge in the equine MCP/MTP joint. The assessment of articular cartilage abnormalities, however, is difficult. Arthroscopy is one way to evaluate the articular cartilage surface, although points of discussion remain. First, the entire cartilage surface of the equine MCP/MTP joint cannot be inspected and evaluated (Vanderperren et al. 2009). Osteoarthritis is known to be heterogeneous with severely and at seemingly unaffected sites within the joint (Palmer et al. 1995). Therefore, an evaluation of the complete surface of the articular cartilage should provide a more complete and correct assessment of the status of the articular cartilage. Secondly, the visual evaluation of the status of the articular cartilage is not accurate. Arthroscopy underestimates the severity of the lesions with mild cartilage damage and overestimates the extent of damage with more established lesions (Brommer et al. 2004b). In addition, the inter-observer reliability of arthroscopic grading of cartilage lesions is poor (Spahn et al. 2011). Other techniques available for a more objective evaluation of the articular cartilage quality, like a high frequency (40 Mhz) ultrasound biomicroscope (Spriet et al. 2005) or near-infraredspectroscopy device (Spahn et al. 2007) are available and are able to detect early changes in articular cartilage. However, none of these are available for equine arthroscopy. An indentation instrument was evaluated for the more objective assessment of cartilage quality in equines but was found not to be sensitive enough to detected early changes in cartilage properties (Brommer *et al.* 2006).

A second technique available for the assessment of articular cartilage is MRI. The evaluation of the quality of the articular cartilage of the MCP/MTP joint depends on the system used (low- or high-field) and is also highly susceptible to partial volume averaging even when using a 1.5 T MRI- unit (Werpy 2007). Only the most severe lesions are evident while less severe lesions are frequently not detected using a low-field standing magnet (Murray et al. 2009; Sherlock et al. 2009; Olive 2010). Audigié et al. 2007 mentioned that the cartilage is even too thin at the level of the MCP/MTP joint for evaluation with a low-field MRI (Audigie et al. 2007). In human medicine it is recommended to acquire images using magnets with a field strength of 1.5 T or greater for morphologic and compositional assessment of cartilage resolution of articular cartilage (Roemer et al. 2011). Specialized MRI sequences are available to detect loss of proteoglycans within the cartilage matrix, which is considered to be one of the first pathophysiologic events in osteoarthritis. Delayed gadolinium-enhanced MRI of cartilage is able to detect early changes in the proteoglycan content of articular cartilage (Taylor et al. 2009) and is described for use in equine MRI studies (Pease 2012; Saveraid and Judy 2012). However, both with high- or low-field MRI, there is a high likelihood of false positive results for detection of cartilage lesions compared with histopathology (Smith et al. 2012). As specimen obtained from the slaughterhouse were used in this thesis, delayed gadolinium-enhanced MRI of cartilage was not possible because this requires the use of IV injection of gadolinium.

For this ex vivo evaluation of articular cartilage, it was decided to work with the objective measurement of the "Cartilage Degeneration Index" developed by Brommer *et al.* 2003 for the evaluation of the proximal articular cartilage surface of P1 (Brommer *et al.* 2003). This technique uses Indian ink staining of the cartilage surface and is based on the fact that Indian ink particles have a high affinity for articular cartilage with surface fibrillation and depletion of proteoglycans, whereas normal intact articular cartilage with a high proteoglycan-rich matrix will prevent the particles from entering (Meachim 1972; Chang *et al.* 1997). It has the advantage over the histopathological scoring systems for articular cartilage degradation (Mankin *et al.* 1971; McIlwraith *et al.* 2010) that it is able to evaluate the entire surface of the articular surface rather than only small samples obtained for histology. It has already been used in experimental studies in different species (Roberts *et al.*

1986; Chang et al. 1997; Cantley et al. 1999) to subjectively evaluate the quality of articular cartilage. The technique developed by Brommer et al. 2003 is different because they use an objective evaluation by analysing the grey levels of digitally imaged cartilage surfaces of P1. This resulted in a calculated CDI that allows the objective evaluation of the degree of cartilage degeneration (Brommer et al. 2003). The technique develop by Brommer et al. 2003 is however not directly applicable for our study. This technique was only validated using 2D photographs for the proximal articular surface of P1, considered to be a relatively "flat surface" and doesn't include area calculations on curved surfaces like the distal third metacarpal/metatarsal condyles. Therefore a modified technique that created a complete 3D model of the articular surfaces of the distal metacarpal/metatarsal condyle and P1 and allowed area calculations on curved surfaces was used (Declercq et al. 2011). Once the 3D models were generated, the cartilage regions of interest were selected using a paintbrush metaphor, and this surface area was measured based on the 3D geometry. The process of selecting the regions of interest had a good intra-observer repeatability (Declercq et al. 2011). The CDI, defined as the percentage of darkening, was calculated as the ratio between stained and unstained grey values of the selected cartilage surface. This procedure was slightly different compared to Brommer et al. 2003, who first averaged the grey values of each image, then computed the difference between the average values. However, the results of the measurements were in the same range in the 2 studies (Brommer et al. 2003; Declercq et al. 2011).

In the present research project, this technique of CDI calculation was applied on MCP/MTP joints of Warmblood horses collected in the slaughterhouse. The major drawback of this study design was the lack of clinical data of the horses. Important data as the breed and age of the horse could be retrieved, but other clinical information such as the presence of MCP/MTP joint related lameness was not available. Although care was taken not to include joints presenting variations or abnormalities other than those present at the dorsoproximal aspect of the sagittal ridge (Hauspie *et al.* 2010), it cannot be entirely excluded that some of these joints might have been included. However, a large number of horses were available for this study (86 horses), possibly compensating for this weakness.

This study revealed no significant association between the different variations at the dorsoproximal aspect of the sagittal ridge and a higher CDI or increased cartilage damage neither at the level of the complete MCP/MTP joint, the distal third metacarpal/metatarsal condyle or the proximal articular surface of P1. This suggests that the presence of these

detected variation at the dorsoproximal aspect of the sagittal ridge does not have an influence on the joint and are therefore anatomic variations.

Although the advantage of the CDI-technique is the evaluation of the complete articular surface, its major limitation is that only the articular surface is evaluated. First, degenerative joint disease is a multifactorial problem, not only involving the articular surface, but also involving the subchondral bone (Kawcak *et al.* 2001) or joint capsule (Palmer and Bertone 1994), which were not evaluated with the current study design. Secondly, the technique of staining of articular cartilage with Indian ink is based on the increased uptake of these ink particles in the presence of surface fibrillation and proteoglycan depletion (Chang *et al.* 1997), but a recent study suggests that the picture of proteoglycan loss early in osteoarthritis may not be complete and additional factors may also contribute to the process in early osteoarthritis (Taylor *et al.* 2009).

Previous research demonstrated that initial cartilage degeneration starts at the dorsal articular margin of P1 (Brommer *et al.* 2004a) and early degenerative changes are frequently encountered at the dorsal aspect of the distal third metacarpal/metatarsal condyle (Brama *et al.* 2000). It is possible that the variations in appearance of the dorsoproximal aspect of the sagittal ridge will have a more localized effect at the dorsal aspect of the joint, since during hyperextension, the dorsoproximal aspect of P1 comes into contact with the dorsal aspect of the distal third metacarpal/metatarsal condyle (Brama *et al.* 2001). Calculation of the CDI specifically for the dorsal and palmar/plantar aspect of the condyle and articular surface of P1 would possibly further elucidate this more localized effect.

In addition to the detected variations at the dorsoproximal aspect of the sagittal ridge, a synovial plica is present at that level. This latter is projecting distally from the dorsoproximal attachment of the joint capsule and tapers to a thin edge. Position and structure suggests that the pad acts as a cushioning surface or contact interface between the dorsal rim of P1 and the distal metacarpal/metatarsal condyle during hyperextension (White 1990; McIlwraith *et al.* 2005), even though its exact position during hyperextension of the MCP/MTP joint and its function have not been determined.

In the last study of this research project, different positions of the plica were detected during hyperextension of the joint, clearly demonstrating a close contact between the dorsoproximal aspect of P1 and the synovial plica. Based on the different positions of the plica during hyperextension, a cushioning function of the plica seems less likely. However, it

is not described if this cushioning function is performed by the distal tip of the plica or by the base of the plica. Based on histology of the bone specimen, longitudinal collagen fibres were detected just proximal to the articular cartilage. This could suggest some kind of cushioning function at that level. However, in our study, due to the removal of the soft tissues when sampling the specimen for histology, the exact relation of these longitudinal collagen fibres relative to the synovial plica could not be established.

Synovial plica proliferation is a described condition in horses (Van Veenendaal and Moffatt 1980; Steyn *et al.* 1989; Dabareiner *et al.* 1996), and thought to be caused by chronic trauma to the plica. Histopathology could determine if one of the different positions of the plica can predispose for this proliferation by determining its content and structure.

A future MRI study in which limbs are hyperextended multiple times immediately after collection could demonstrate the repeatability of the position of the synovial plica during hyperextension as well as the place of contact of the dorsoproximal aspect of P1 relative to the variations at the dorsoproximal aspect of the sagittal ridge. In addition, a subsequent storage at -20 °C would allow the sampling of the dorsoproximal aspect of P1 with the surrounding soft tissues still in place. This would provide information about the location of the plica relative to the variations at the dorsoproximal aspect of the sagittal ridge.

The main conclusion of the present research work is that variations are present at the dorsoproximal aspect of the sagittal ridge of the MCP/MTP joint ("smooth", "irregular", "cam", "lucency" and "indentation"). These variations do not demonstrate clear abnormalities on histology nor are they associated with abnormalities at the level of the articular cartilage. During hyperextension of the equine MCP/MTP joint, these variations are most likely in close relation with the dorsoproximal aspect of P1 and the synovial plica.

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Summary

A veterinary pre-purchase examination is an important service offered to the owner of a horse. Survey radiographs often reveal abnormalities or variations. The predictive value of these findings is somewhat controversial however has become an important factor in the horse business. Assessing the future clinical relevance of detected changes can be challenging especially when clinical signs are absent. Variations in appearance of the dorsoproximal aspect of the sagittal ridge are described in Thoroughbreds, without any influence on the performance of the horse. The scientific information on detected variations at the dorsoproximal aspect of the dorsal condylar sagittal ridge is limited in Warmblood horses. The present PhD research focus was to describe the prevalence of variations at the dorsoproximal aspect of the dorsal condylar sagittal ridge in Warmblood horses, to evaluate their histological basis, assess their influence on the articular joint cartilage and their interaction with the surrounding soft tissues.

In <u>chapter 1</u> an overview is provided of the anatomy of the equine metacarpo-/metatarsophalangeal joint and the diagnostic imaging modalities currently used in an equine pre-purchase examination.

The scientific aims of this work are presented in <u>chapter 2</u>. The *first objective* of this thesis was to describe the prevalence of variation in radiographic appearance of the dorsoproximal aspect of the condylar sagittal ridge in the equine metacarpometatarsophalangeal joint in Warmblood horses.

The evaluation of the histological appearance of these detected variations at the dorsoproximal aspect of the sagittal ridge was the *second objective* of this thesis.

The *third objective* of this thesis was to assess the potential association of these variations in appearance of the dorsoproximal aspect of the sagittal ridge with articular cartilage degeneration.

The *fourth objective* of this thesis was to describe the influence of hyperextension of the metacarpo-/metatarsophalangeal joint on the position of the synovial plica surrounding the proximal aspect of the dorsal condylar sagittal ridge.

In <u>chapter 3</u> radiographs of young Warmblood stallions were scrutinized to describe the prevalence of radiographic variations at the dorsoproximal aspect of the sagittal ridge in the metacarpo-/metatarsophalangeal joint. Radiographs of a total of 308 Warmblood stallions were used, giving us a total of 1232 equine metacarpo-/metatarsophalangeal joints available for this study. The radiographic appearance of the dorsoproximal aspect of the sagittal ridge

was classified into the following categories: "smooth", "irregular", "cam", "indentation" and "lucency". A "smooth" appearance was detected in 51.5% of the joints; the ridge was "irregular" outlined in 19.3%. A "cam" like bony excrescence was present in 8.9%, 12.2% of the ridges had an "indentation" and in 8.1% a "lucency" was detected. An "indentation" at the dorsoproximal aspect of the sagittal ridge was significantly more detected at the level of the hind limbs (P < 0.0001). In conclusion, there is variation in the radiographic appearance of the proximal aspect of the dorsal condylar sagittal ridge in the metacarpo-metatarsophalangeal joint of Warmblood horses. However, their background and possible influence on the function of the joint needs further investigation.

In chapter 4 the histological origin of the variations in radiographic appearance of the dorsoproximal of the sagittal ridge of the metacarpo-/metatarsophalangeal joint was described. A total of 25 joints of 12 young Warmblood horses were selected based on a radiographic examination to ensure that of each detected variation at the dorsoproximal aspect of the sagittal ridge 5 specimen were available for histological examination. The "smooth" variation was used as the reference category because this variation was most detected on radiography. Each group demonstrated a bone cortex, covered with hyaline cartilage distally and a transition to longitudinally aligned collagen fibres covered by loosely organized connective tissue proximally. The "smooth" and "irregular" group showed a smooth bone cortex. The "cam" like category presented an expansion of the cortex. In the "indentation" and "lucency" group, a depression in the cortex was detected. The collagen fibres and connective tissue were located in the depression in the "indentation" category whereas the location varied in the "lucency" and "cam" group. In conclusion, the detected differences in appearance of the dorsoproximal aspect of the sagittal ridge on radiography are a representation of anatomical variations resulting from detectable histological differences. Further research is warranted to determine whether these variations are developmental or congenital and to evaluate their potential influence on the joint and surrounding soft tissues during hyperextension

In <u>chapter 5</u> the objective was to investigate whether an association could be found between the variable morphological appearances of the proximal aspect of the dorsal condylar sagittal ridge and the degree of cartilage degeneration, determined by the "cartilage degeneration index" at the level of the metacarpo-/metatarsophalangeal joint, distal metacarpal/metatarsal condyle or proximal articular surface of the proximal phalanx. The distal limbs of 86 Warmblood horses were used for this study. No significant association was

detected between the "cartilage degeneration index" and the presence of one of the detected variations, age, front or hind limb and left or right limb. In conclusion, the different radiographic features at the proximal aspect of the dorsal condylar sagittal ridge have no influence on the cartilage status of the metacarpo-/metatarsophalangeal joint.

In chapter 6 the location of the dorsoproximal synovial plica was described during hyperextension of the metacarpo-/metatarsophalangeal joint in order to get a better insight in its function. The position and structure of the plica suggests that it acts as a cushioning surface or contact interface between the proximal phalanx and the cannon bone where these are in contact during full extension of the joint. The distal limbs of 5 Warmblood horses were collected and magnetic resonance imaging scans were performed. Each joint was scanned twice: first in a neutral standing position and than in a hyperextended position. During hyperextension several positions of the plica were detected, ranging from (1) shortened with the tip of plica bending or angled towards palmar/plantar; (2) projecting distally parallel to the condyle; (3) projecting dorsally with the proximal margin of the proximal phalanx located underneath it or (4) the tip of the plica interposed between the proximal aspect of the proximal phalanx and the condyle. During hyperextension, a close relation is present between the proximal synovial plica, proximal aspect of the proximal phalanx and the condyle with a variable position of the plica. This suggests that the plica acts as a contact interface between the proximal phalanx and the cannon bone. However, due to the different positions of the plica, the plica consequently seems not to act as a cushioning surface.

The final chapter (chapter 7) presents the general discussion and conclusions. Variations in appearance of the dorsoproximal aspect of the sagittal ridge are present in the metacarpo-/metatarsophalangeal joint of Warmblood horses. These variations are not linked with an increase in articular cartilage damage, nor do they present clear abnormalities on histology. During hyperextension of the joint, a close contact is present between these variations at the level of the sagittal ridge, the dorsoproximal aspect of the proximal phalanx and the synovial plica.

Samenvatting

In de "paardenwereld" wordt een aankooponderzoek regelmatig uitgevoerd om na te gaan of het paard het gewenste prestatieniveau zal aankunnen. Radiografie is hierbij een zeer belangrijk hulpmiddel om afwijkingen ter hoogte van de ledematen te identificeren. Het inschatten van het toekomstig belang van waargenomen afwijkingen is een grote uitdaging indien het paard geen klinische symptomen vertoont op het ogenblik van de keuring. Vormvariaties ter hoogte van het proximaal aspect van de sagittaalkam zijn zichtbaar bij volbloeden, zonder invloed op de sportieve prestaties. Tot op heden zijn deze radiografische vormvariaties nooit eenduidig beschreven bij Warmbloedpaarden, noch hun relatie met kraakbeendegeneratie of interactie met de omliggende weke delen. Het algemene doel van dit doctoraatswerk was om hier een beter inzicht in te krijgen.

Het <u>eerste hoofdstuk</u> geeft een overzicht van de anatomie van het kogelgewricht van het paard en de beeldvormingsmodaliteiten die kunnen gebruikt worden bij een aankoop onderzoek.

De doelstelling van dit doctoraatswerk worden weergegeven in het <u>tweede hoofdstuk</u>. De *eerste doelstelling* was om de prevalentie van vormveranderingen ter hoogte van het dorsoproximaal aspect van de sagittaalkam van het kogelgewricht te beschrijven in een populatie van Warmbloedpaarden. De *tweede doelstelling* bestond erin om het histologisch uitzicht van deze radiografisch zichtbare vormveranderingen te beschrijven.

De analyse van de potentiële invloed van deze vormveranderingen op de integriteit van het gewrichtskraakbeen werd de *derde doelstelling*.

De laatste en *vierde doelstelling* was een beter inzicht te verwerven in de interactie van de structuren ter hoogte van het dorsaal aspect van de kogel. Hierbij lag de nadruk op de positie van de plica synovialis tijdens hyperextensie van het kogelgewricht.

In het <u>derde hoofdstuk</u> werd de prevalentie van deze vormvariaties ter hoogte van het proximaal aspect van de sagittaalkam beschreven in een populatie Warmbloedpaarden. Hiervoor werden radiografische opnames van 308 Warmbloedhengsten beoordeeld, wat een totaal van 1323 radiografische projecties van het kogelgewricht opleverde voor deze studie. Het röntgenbeeld van het dorsoproximaal aspect van de sagittaalkam werd ingedeeld in volgende categorieën: vlak en glad afgelijnd (51.5%), onregelmatig afgelijnd (19.3%), knobbelvormige aanwas (8.9%), aanwezigheid van een indeuking (12.2%) of aanwezigheid van een lucente zone (8.1%). Een indeuking kwam significant meer voor ter hoogte van het achterbeen (P < 0.0001). Uit deze studie kon worden besloten dat er radiografisch

vormvariaties zichtbaar zijn ter hoogte van het proximaal aspect van de dorsale sagittaalkam van het kogelgewricht. Hun oorsprong en eventuele invloed op het gewricht vraagt verder onderzoek.

Het doel van het vierde hoofdstuk was een beter inzicht te verkrijgen in het histologisch uitzicht van de radiografisch zichtbare vormveranderingen. Hiervoor werd er van elke radiografische variatie 5 stalen geselecteerd voor verder histologisch onderzoek. Deze werden verzameld bij Warmbloedpaarden. De vlak en glad afgelijnde categorie diende als controle groep omdat deze de meest voorkomende radiografische variatie was. Elke categorie vertoonde een beenderige cortex, distaal afgelijnd met normaal uitziend hyalien kraakbeen, naar proximaal toe was er een overgang naar longitudinaal gealigneerde collageenvezels die bedekt was met losmazig georganiseerd bindweefsel. De vlak en glad afgelijnde en onregelmatig afgelijnde categorie vertoonden geen duidelijke verschillen. Een beenderige aanwas was zichtbaar in de knobbelvormige aanwas categorie. Een depressie van de cortex was zichtbaar in de indeuking en lucente zone categorie. De overgang kraakbeencollageenvezels had een variabele locatie in de knobbelvormige aanwas en lucente zone categorie, terwijl deze zich in de indeuking categorie telkens ter hoogte van de depressie van de cortex bevond. Hieruit kon worden afgeleid dat de radiografische vormvariaties een uiting waren van duidelijke histologische verschillen ter hoogte van de gemineraliseerde weefsels zonder duidelijk pathologisch uitzicht. Verder onderzoek is nodig om de invloed van deze vormverandering op het gewricht en de omgevende weke delen te weten.

De potentiele invloed analyseren van deze vormvariaties van de sagittaalkam op de integriteit van het kraakbeenoppervlak was het doel van <u>hoofdstuk vijf</u>. Deze integriteit werd gemeten door een objectieve "Cartilage Degeneration Index". Been specimens van 86 Warmbloedpaarden werden geanalyseerd in deze studie. De aanwezigheid van een van de vormvariaties ter hoogte van het proximaal aspect van de sagittaalkam was niet significant gecorreleerd met verhoogde kraakbeenschade.

Het doel van <u>hoofdstuk zes</u> was een beter inzicht te verwerven in de interactie van de structuren ter hoogte van het dorsaal aspect van de kogel, met nadruk op de dorsale proximale plica synovialis. Van deze laatste wordt beschreven dat deze structuur tijdens hyperextensie van het gewricht, een contactoppervlak en schokdemperfunctie heeft tussen het dorsoproximaal aspect van het kootbeen en het proximaal aspect van de distale condyl van het pijpbeen. Hiervoor werden een magnetische resonantie onderzoek uitgevoerd op de been

specimens van 5 Warmbloedpaarden gefixeerd in hyperextensie. Hieruit bleek dat, tijdens hyperextensie, het dorsoproximaal aspect, de distale condylen van het pijpbeen en de plica synovialis zich in nauw contact bevonden. De plica zelf bevond zich in verschillende posities: (1) verkort met de tip van de plica gekruld naar palmair/plantair; (2) distaal geprojecteerd evenwijdig met de condyl; (3) dorsaal geprojecteerd met de proximale rand van het kootbeen eronder gelokaliseerd of (4) met de tip van de plica tussen het proximaal aspect van het kootbeen en de distale metacarpale/metatarsale condyl. Deze studie toonde duidelijk aan dat er tijdens hyperextensie een nauw contact is van de plica synovialis met het proximaal aspect van het kootbeen. Dit suggereert dat de plica dient als een contactoppervlak tussen kootbeen en condyl tijdens hyperextensie. Gelet op de verschillende posities van de plica tijdens hyperextensie is een schokdemperfunctie tussen kootbeen en pijpbeencondyl minder waarschijnlijk.

De algemene discussie en conclusies van dit doctoraatswerk zijn vervat in het zevende hoofdstuk. Vormvariaties zijn zichtbaar ter hoogte van het dorsoproximaal aspect van de sagittaalkam van het kogelgewricht in het Warmbloedpaard. Deze vormvariaties zijn niet gelinkt aan verhoogde kraakbeenschade in het gewricht noch hebben ze een duidelijk pathologisch histologisch uitzicht. Tijdens hyperextensie is er wel een nauw contact aanwezig van deze vormvariaties met het kootbeen en de plica synovialis.

Curriculum Vitae

Stijn Hauspie werd geboren op 1 oktober 1983 te Roeselare. Na het beëindigen van het secundair onderwijs aan het Sint-Leocollege te Brugge (wetenschappen-wiskunde), startte hij in 2001 met de studies diergeneeskunde aan de universiteit Gent. Hij behaalde in 2007 het diploma van Dierenarts (optie paard) met onderscheiding.

Onmiddellijk na het afstuderen, startte hij een internship paard, optie Heelkunde, Medische Beeldvorming. Daarna trad hij in dienst bij de vakgroep Medische Beeldvorming van de Huisdieren en Orthopedie van de Kleine Huisdieren.

Geboeid door het wetenschappelijk onderzoek startte hij in 2008 een doctoraatsstudie bij de vakgroep Medische Beeldvorming van de Huisdieren en Orthopedie van de Kleine Huisdieren. Deze studie werd gefinancierd door het Bijzonder Onderzoeksfonds van de universiteit.

Stijn Hauspie is auteur en medeauteur van meerdere wetenschappelijke publicaties en was spreker op meerdere internationale congressen. Hij won ook de 'Hippo Zorg Horse Insurance Award" voor zijn mondelinge presentatie op de voorjaarsdagen 2012.

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Tot slot mag ik de interns niet vergeten (of "het intern" zoals sommigen plachten te zeggen, een term waar ik zeker niet achter sta). Uit eigen ervaring kan ik zeggen, zonder jullie draait deze kliniek vierkant, dat mag gezegd worden. Elk jaar is er een nieuwe lichting dus te veel om iedereen op te noemen. Maar bij deze: Karen, Babette, Evi, Helga, Evelyn, Wendelien, Krisje, Thomas, Jan, Véronique, jullie waren top, elk op jullie eigen manier... Een kleine aparte vermelding voor de huidige interns, het komt allemaal goed. Een kleine extra dank u voor 2 in het bijzonder: Valérie en Steven. Bedankt bij het helpen verzamelen van de laatste beeldjes.

Barbara, eigenlijk hoor jij ook in het intern rijtje thuis, maar ondertussen ben je toch meer geworden. Op speciaal verzoek krijg je samen met Anneleen hier je eigen alinea (ja mensen, als je het lief vraagt lukt dat dus bij mij). Jullie staan altijd klaar om te luisteren. Anneleen, het is goed om te weten dat jouw koelkast altijd gevuld is.

Naast het werk overdag moet er uiteraard ontspanning zijn.

Wim en Yves, jullie deur van de puinstraat staat altijd open. Gelukkig zetten jullie ook al graag eens een stapje in de wereld. Sill en Delphine, ik wens jullie alvast proficiat. Jullie zullen zeker goede ouders worden. Frank en Tom. Frank, jij kunt me altijd een lekkere trappist aanprijzen (de ene al wat lichter dan de andere). Tom, ik heb respect voor je originaliteit (zowel schrijvend als sprekend), ik had er graag ook een stukje van gehad. Het zou dit schrijven zoveel makkelijker maken. Seppe en Tine, jullie hostel, dat komt goed. Ik voel het! Wim en Evelien, dat verbouwproject, ik heb er alle vertrouwen in. Rob en Annelies, jullie zijn net begonnen aan een volgende etappe in jullie leven, veel geluk samen.

Dan de mensen van de vriendschap. We zijn ondertussen samen een aantal jaren afgestudeerd. Peter en Siets, jullie staan altijd klaar om me te ontvangen met open armen. Iedere keer vragen jullie hoe het met me gaat. Het doet deugd om te merken dat jullie altijd oprecht

luisteren. Bart en Alien, hoeveel avonden heb ik niet bij jullie doorgebracht met top gear als hoofdthema van de avond. Bedankt om er telkens te zijn als het nodig was. Pieter en Alice. Na het lopen kom ik altijd even "buurten". Altijd gezellig: een Campari orange om de lente in te zetten; een kasseisteen uitbreken; een "koffietje" gaan drinken, wat ons uiteindelijk een fles augustijn opleverde. Peter, we hebben al heel wat watertjes doorzwommen. We draaien veel rond de pot, maar toch weten we de essentiële dingen van elkaar. Directe communicatie is niet ons sterkste punt, maar meestal weten we "via via" wel genoeg. Katrien en Joris, jammer genoeg zie ik jullie niet zo vaak meer. Veel succes met de verdere uitbouw van de praktijk. Herlinde en An, jullie zijn 2 zotte trienen, wel van een soort die ik graag heb. Af en toe een spontaan stapje zetten ("nee, we gaan niet te lang blijven"), net op dat moment wanneer ik er het meeste nood aan had. Yvan en Frank, draag zorg voor jullie schatten. Julie, een speciale dank voor jou. Door de jaren heen heb je altijd zitten vloeken op mij, dat ik een gesloten boek ben. Uiteindelijk denk ik toch dat je een van de weinigen bent die echt veel van me weet. Bedankt gewoon om er te zijn en altijd te luisteren. Vincent, monsieur le commissaire, ik ben verdorie toch een beetje jaloers op je paardrijkunsten. Proficiat voor je prestatie. Bedankt voor je af en toe nuchtere observatietalenten.

Jeroen en Lynn. Jeroen, wat startte als af en toe 's middags samen te gaan eten is ondertussen uitgegroeid tot een goeie vriendschap. Samen hebben we veel meegemaakt, gaande van elkaars ochtendlijk humeur oppeppen tot 2 maanden in ons "kot" samen te zitten. Nu gaan we elk afzonderlijk ons verhaal verder uitbouwen. Op naar de toekomst! Lynntje, altijd leuk als jij erbij bent. Draag goed zorg voor "clerqie" (en omgekeerd natuurlijk ook). Het pensenfestijn komt eraan.

Het enige wat ik eigenlijk wil zeggen aan jullie allemaal, bedankt om er altijd te zijn. Zoals een bepaald individu placht te zeggen, "bedankt voor de vriendschap".

Het laatste deel is natuurlijk voor mijn familie bestemd. Dit is een deel van jezelf dat je niet kunt kiezen, toch ben ik blij dat jullie er allemaal zijn, stuk voor stuk. Leen, vroeger hebben we onze meningsverschillen gehad. Tegenwoordig ben ik blij dat we gezellig ergens naartoe kunnen gaan, nu met Peter erbij: naar het strand om vedett te drinken; een kleine verhuis; en ja, binnenkort spring ik wel eens binnen. Housewarming? Annelies, ik ben benieuwd om je "project" in volle glorie te zien, stiekem ben ik wel jaloers op die robot. Louis, ondanks de verspreking van Saint Germain van mijnentwege, ben je er toch met de juiste persoon geëindigd. Zelfs op deze speciale dag, heb je me geholpen. Bedankt! Jullie hebben 3 prachtige schatten van kinderen. Als peter zijnde, is natuurlijk de oudste de tofste, maar samen brengen ze altijd leven in de brouwerij. Dries en Kim, samen met jullie afspreken zorgt altijd tot ontspanning: van de skivakantie (die ene waar men vingers afvroren tot diegene waar ik gepromoveerd was tot gps) tot de gezellige concert

avonden (Dries, je doet het goed op podium) en jazz momenten. Het is zeker gezellig. Lieven en Riitta, You couldn't make it here today. But the mental connection was sure working. Hopefully everything will work out fine for you all in the future. I hope we will see each other soon. It would be nice to visit you more, see how Laura and Lotta grow up into beautiful daughters.

En dan als allerlaatste: de personen zonder wie ik er niet zou zijn vandaag, mijn ouders. Blijkbaar waren ze zo tevreden met mij dat ze spontaan gestopt zijn (uit angst dat het niet meer beter kon zeg ik altijd tegen mezelf). Gelukkig hebben ze op mij gewacht. Bedankt om me zoveel kansen te geven in mijn leven. Ik weet dat ik het soms niet genoeg laat blijken, maar ik ben jullie echt dankbaar moeke en vake. Als ik het lastig heb, weet ik dat ik altijd mag aanschuiven aan tafel zonder teveel vragen. Indien nodig staan jullie klaar om mij te steunen op eender welke manier. Bedankt!! Ik hoop alleen dat ik vanaf nu wat meer zelfstandig ben, maar stiekem geniet ik toch nog van de huiselijke warmte die ik altijd Thuis kan vinden.

En als laatste, voor diegene die het zich nog afvroeg: Ja, ik denk ook aan je.

Stijn



When you're chewing on life's gristle

Don't grumble, give a whistle

And this'll help things turn out for the best...

And...Always look on the bright side of life...

(Monty Python's Life of Brian)