Quantitative Assessment of the Equine Hoof Using Digital Radiography and Magnetic Resonance Imaging

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Dedicated to my parents

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Abbreviations:

DR – digital radiography

MR – magnetic resonance

MRI – magnetic resonance imaging

CT – computed tomography

BB – ball bearing

1 Introduction

The equine hoof is an anatomically complex structure *generally consisting of the horn capsule, which contains the distal phalanx, the distal aspect of the middle phalanx, the navicular bone, the hoof cartilage, the bursa podotrochlearis, the extensor and deep digital flexor tendon, as well as other soft tissue structures associated with the distal interphalangeal joint and podotrochlear apparatus.*

The intimate connection between the distal phalanx and the horn capsule is characterized by interdigitating lamellar attachments of the epithelium to the underlying dermis suspending the distal phalanx in the hoof capsule. This complex anatomy supports the entire body system. The lamellar attachments consist of the interdigitating lamellae epidermales and lamellae dermales, the stratum lamellatum. In between the stratum lamellatum and the distal phalanx is the stratum reticulare, which aids in attachment of the distal phalanx to the lamellae epidermales and provides the vascular supply to the lamellae. (BUDRAS and KOENIG 2002, POLLITT 1995, ROBERTS et al. 1980). At the distal extent of the dermis parietis (junction of the wall and the sole), the lamellae continue into the papillae dermales, and the epidermis overlying the papillae forms the terminal horn tubules (POLLITT 1995).

In equine laminitis, a sequela of multiple disorders, this complex lamellar apparatus undergoes injury commonly leading to separation of the lamellae dermales and epidermales (EADES et al. 2002, FALEIROS et al. 2009, MOORE et al. 2004). Disruption of lamellae leads to several recognized patterns of displacement of the distal phalanx within the hoof capsule; 1) rotational displacement 2) symmetrical distal displacement, a pattern often called sinking; and 3) uniaxial/asymmetrical distal displacement in the sagittal plane so that the thickness of the displaced side of the hoof increases and the distance between displaced side of the distal phalanx and the ground surface of the sole decreases (CRIPPS and EUSTACE 1999, BELKNAP and PARKS 2011).

Radiographic measurements assessing the position of the distal phalanx relative to the hoof capsule include the following: 1) distance between the dorsal surface of the distal phalanx and the surface of the dorsal hoof wall (LINFORD et al. 1993); 2) vertical distance from the proximal margin of the hoof capsule or coronet to the extensor process of the distal phalanx ("founder distance") (CRIPPS and EUSTACE 1999); and 3) distance from the dorsodistal aspect of the distal phalanx to the ground surface of the sole, namely the sole depth (BELKNAP and PARKS 2008). The first two measurements are well documented in normal and laminitic horses. Sole depth is less well documented in healthy horses (LINFORD et al. 1993, KUMMER et al. 2006).

Using analogue radiographs, two distinct soft tissue opaque layers are observed in the dorsal hoof wall (LINFORD et al. 1993); DR, with better contrast resolution than analogue radiographs, enhances our ability to delineate and assess these two layers. The outer more radiopaque layer may be the keratinized hoof wall, comprised of the strata externum and medium, and the more radiolucent layer may represent the strata lamellatum and reticulare (LINFORD et al. 1993); the composition of the two layers has not been investigated. Radiographic assessment of distinct soft-tissue layers in the sole area has not been documented in the sense comparing the layers seen on radiographs with other imaging modalities or histopathology. Given that the stratum lamellare and stratum reticulare, in contrast to the strata externum and medium, are likely not affected by trimming or abnormal hoof growth, alteration of these layers could be a more reliable diagnostic measurement in evaluation of laminitis than total hoof wall or sole thickness. Early changes in the thickness of these layers due to initial events such as oedema and the onset of lamellar separation may result in earlier disease diagnosis.

Magnetic Resonance Imaging (MRI) has revealed soft-tissue changes of the dorsal hoof wall in acute and chronic phases of laminitis (ARBLE et al 2009, MURRAY et al. 2003). While the relationship of the epidermis and dermis dorsal to the distal phalanx was examined in both MRI laminitis studies (ARBLE et al 2009, MURRAY et al. 2003), the sole region was not evaluated.

Lower expense and higher availability makes DR the more commonly used modality to evaluate laminitis despite the superior soft-tissue resolution of MRI. Knowledge gained from MRI can be applied to the soft tissue layers detectable on DR. Our objectives were to establish normal measurements of the epidermal and dermal elements of the wall and sole using DR and MRI and to evaluate inter- and intra-observer correlation. We aimed to use MRI to document the tissue components present in the two soft tissue layers seen on DR in the dorsal hoof wall and sole. The results of this study may enable the clinician to obtain more sensitive and in depth information of equine digital changes.

2 Literature

2.1 Anatomy

Equine clinicians often use the term "foot" to describe the hoof of a horse (DYSON and MURRAY 2011, FUERST and LISCHER 2012, PARKS 2011, STASHAK 2002). However, from an anatomical point of view the entire distal limb including the carpus/tarsus, metacarpus/-tarsus and phalanges with encasing soft tissues and horn capsule forms the foot (BUDRAS and KOENIG 2002, NICKEL et al. 2001). The hoof (ungula) per definitionem is the elastic horncapsule. A more clinically used defintion of "hoof" also includes the structures encased inside the horncapsule like the distal phalanx and distal part of the middle phalanx, navicular bone, hoofcartilage, bursa podotrochlearis, insertion of the extensor and deep digital flexor tendon and other structures closely associated with the distal interphalangeal joint and the podotrochlear apparatus (BUDRAS and KOENIG 2012). The hoof capsule encases the latter structures and represents a modification of the skin (Integumentum commune). Resembling the basic set-up of the skin, the hoof capsule consists of three layers:

the **subcutis** *(tela subcutanea ungulae)*

the **dermis** *(corium ungulae) with stratum reticulare and stratum papillare*

the **epidermis** *(epidermis ungulae) with stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum*

The hoof capsule can be differentiated in:

hoof wall *(Paries corneus, Lamina)*

hoof sole *(Facies solearis)*

The hoof can further be differentiated in the following segments:

coronary band *(limbus ungulae)*

coronet *(corona ungulae)*

hoof wall *(paries ungulae)*

hoof sole *(solea ungulae)*

hoof frog *(cuneus ungulae)*

hoof cushion *(torus ungulae)*

(BUDRAS and KOENIG 2002, HABERMEHL 1996, NAV 2012)

The ***coronary band*** *is only a few millimeters wide and starts distal to the hair covered skin. The subcutis in this area is modified to a cushion like structure (pulvinus limbi) that continues into the palmar/plantar hoof cushion. The dermis of the coronary band forms slim papillae and the epidermis tubulae (BUDRAS and KOENIG 2002, POLLITT 2011).*

Distal to the coronary band is the ***coronet*** *with a thick subcutis that forms the pulvinus coronae. The papillae of the dermis and the tubulae of the epidermis in the area of the coronary are stronger than the papillae and tubulae of the coronary band. The horn of the coronary epidermis is organized in three layers which are continuously pushed distally parallel to the facies parietalis of the distal phalanx (BUDRAS and KOENIG 2002, HABERMEHL 1996, POLLITT 1995).*

The horn layers of the coronet cover the horn of the ***hoof wall*** *segment (paries ungulae). This layer only becomes visible at the sole as the white line (zona alba) (BUDRAS and KOENIG 2002). A subcutis is lacking in this segment and the stratum reticulare of the dermis is directly connected to the distal phalanx without an intermediate periost (HABERMEHL 1996). The stratum papillare of the dermis forms primary and secondary lamellae (lamellae dermales) that interdigitate with the primary and secondary lamellae of the epidermis (lamellae epidermales) (BUDRAS and KOENIG 2002, HABERMEHL 1996, POLLITT 1995).*

The ***hoof sole*** *also does not show a subcutis and the dermis is direcly connected to the distal phalanx. At the distal extent of the parietal dermis (junction of the wall and the sole), the lamellae continue into the terminal dermal papillae and epidermal sockets which are also interdigitating and supported by the deep dermis of the sole (POLLITT 1995).*

*The **frog** has important shock-absorbing abilities enabled by his V-shaped structure and thick subcutis that is modified to the cushion of the frog (pars cunealis pulvini digitalis) (BUDRAS and KOENIG 2002). The dermis of the frog is organized in spiral formed villi that are shorter than the papillae of the sole dermis and the epidermis of the frog is organized in matching spiral shaped papillae that form soft horn. The frog continues into the **hoof cushion**, which shows a similar build-up (BUDRAS and KOENIG 2002, HABERMEHL 1996).*

The keratinized hoof capsule with its internal lamellar structure is connected to the distal phalanx over laminar attachments of the dermis. (Figure 4) These laminar attachments suspend the distal phalanx within the hoof capsule. Therefore the connecting laminae are also called the suspensory apparatus of the hoof which supports the entire body weight of the horse (POLLIT 1995; ROBERTS et al. 1980). The suspensory apparatus consists of the interdigitating epidermal and dermal lamellae. The layer between the dermal lamellae and the distal phalanx is also called the sublamellar or deep dermis (POLLIT 1995; ROBERTS et al. 1980). This layer aids in attachment of the distal phalanx to the laminar epidermis, and provides the vascular supply to the lamellae (BUDRAS and KOENIG 2002, POLLIT 1995; ROBERTS et al. 1980).

2.2 Diseases of the equine hoof

Due to the horse's role as a riding and draft animal, lameness plays a major role in equine medicine. The hoof – especially in the forelimb – is one of the most common locations for lameness in the equine patient. A multitude of disorders can affect the equine hoof and cause lameness. It is difficult if not impossible to give a complete list of these disorders and comprehensive information on hoof diseases are found in textbooks on equine lameness and surgery (FUERST and LISCHER 2012, PARKS 2011, STASHAK 2002). The following overview refers to the aforementioned texts.

Disorders of the hoof capsule

Thrush

White line disease

Hollow or loose wall

Hoof wall cracks (toe cracks, quarter cracks, heel cracks)

Keratoma

Canker

Scalping injuries

Disorders of the soft tissues of the hoof

Laminitis

Aseptic pododermatitis (bruising of the laminae)

Septic pododermatitis (hoof abscess)

Puncture wounds ("street nail")

Disorders of the collateral cartilages (mineralization/sidebones, necrosis/quittor)

Primary lesions of the deep digital flexor tendon within the hoof capsule

Disorders of the podotrochlear apparatus

Desmopathy of the distal sesamoidean impar ligament

Desmopathy of the collateral ligaments of the navicular bone

Desmopathy of the collateral ligaments of the distal interphalangeal joint

Injury of the distal digital anular ligament

Navicular bursitis

Disorders of the osseous structures of the hoof

Osteitis of the third phalanx (pedal osteitis)

Fractures of the distal phalanx

Subchondral bone cyst of the distal phalanx

Fractures of the distal sesamoid bone (navicular bone)

Navicular disease

Arthritis of the distal interphalangeal joint.

*Detailed description of pathology, diagnosis and therapy of the aforementioned conditions are found in the literature (FUERST and LISCHER 2012, PARKS 2011, STASHAK 2002). In the following only **laminitis** will be described in more detail as this entity is the focus of the current study.*

2.3 Laminitis

*Laminitis is a devastating disease of the equine foot and remains a major cause of morbidity and death in horses (BELKNAP et al. 2012, MARR 2012, WYLIE et al. 2013). Equine laminitis has been associated with multiple disorders affecting the gastrointestinal, respiratory, reproductive, endocrine or musculoskeletal system (HOOD 1999, POLLITT 2011, STASHAK 2002, KIENZLE et al. 2013). The **pathogenesis of laminitis** is not completely understood (KATZ et al. 2012, De GRAAF-ROELFSEMA 2013, WYLIE 2013). Multiple factors can lead to alterations in the hemodynamics of the hoof and inflammation-induced damage to the lamellae (EADES et al., 2002; MOORE et al., 2004, PATAN-ZUGAJ et al. 2014). This damage may disrupt the intimate connections between the primary and secondary lamellae of the epidermis and dermis of the hoof (KARIKOSKI et al. 2014; WANG et al. 2014). This lamellar separation often results in the painful structural collapse of the foot and the eventual demise of the animal.*

***Microscopic evaluation** of the sagittal hoof wall lamellar sections from horses euthanized 48 hours after experimentally induced laminitis by carbohydrate overload showed disintegration of the basement membrane and failure of its attachment to basal epidermal cells (POLLITT 1996). In a study evaluating early histologic changes in insulin-induced laminitis, death of the secondary epidermal lamellae and epidermal basal cells was shown at six hours (DE LAAT et al. 2013). Histopathology of chronically affected digits reveals hyperplasia of the epidermal* *laminae with wedge formation, which forces the epidermal and dermal laminae apart (COLLINS et al. 2010, ROBERTS 1980). (Fig. 5)*

The disruption of the lamellae leads to several recognized patterns of displacement of the distal phalanx:

1) rotation of the distal phalanx about the distal interphalangeal joint subsequent to dorsal lamellar injury and following traction of the deep digital flexor tendon so that the dorsal surface of the distal phalanx separates from the dorsal hoof wall forming a distally diverging angle, a pattern that is usually called ***rotation*** *(Figure 5);*

2) symmetrical distal displacement in which the distal phalanx retains its normal alignment with the phalanges, which creates an increase in the distance from the coronary band to the proximal margin of the extensor process, an increase in dorsal hoof thickness, and decreased distance from the solear margin of the distal phalanx to the sole surface, a pattern that is usually called ***sinking****; and*

3) asymmetrical distal displacement in the sagittal plane so that the thickness of the displaced side of the hoof increases and the distance between displaced side of the distal phalanx and the adjacent sole surface decreases a pattern that is usually called ***unilateral sinking*** *(BELKNAP and PARKS 2011, CRIPPS and EUSTACE 1999).*

In horses with distal displacement of the distal phalanx, excessive pressure on the sole due to the anatomic displacement of the distal phalanx may not only lead to flattening of the keratinised sole. The unstable distal phalanx may also lead to pain and compromise of the solear tissues from compression of the solear dermis containing vasculature and innervation. The resulting solear compromise may lead to prolapse of the distal phalanx through the sole (POLLITT and COLLINS 2011).

Predisposing factors *for, or causes of equine laminitis include endotoxemia and sepsis, metabolic disorders, toxins, corticosteroids and mechanical overload (HEYMERING 2010, STASHAK 2002, PATAN-ZUGAJ et al. 2014). Common causes for endotoxemia and sepsis in* *the horse are gastrointestinal diseases like colitis and enteritis, pleuropneumonia and retained placenta/metritis (HOOD 1999, POLLITT 2011, STASHAK 2002, WHITE et al. 2009, BELKNAP et al. 2012, WYLIE et al 2013). Equine metabolic syndrome and pituitary pars intermedia dysfunction are the most common metabolic disorders associated with equine laminitis (POLLITT 2011, MENZIES-GOW 2012, JOHNSON et al. 2012). Mechanical overload was historically associated with extreme exercise on hard surface (road founder) but today it is rather a secondary problem commonly seen in horses with severe lameness (support limb laminitis) (HEYMERING 2010, VIRGIN et al. 2011, ORSINI 2012). Carbohydrates in high loads and fructanes - that are produced by grasses under certain climatic conditions - can alternate hindgut fermentation and lactic acid production inducing endotoxemia and leading to grain overload laminitis or grass founder (LONGLAND and CAIRNS 1998, SPROUSE et al. 1987, NOURIAN et al. 2007). Laminitis can be induced experimentally by application of black walnut extract (GARNER et al. 1975) and is associated with systemic and intraarticular administration of large doses of corticosteroids (HEYMERING 2010, POLLITT 2011, STASHAK 2002).*

***Acute laminitis** presents clinically with an acute-onset of lameness usually in both front limbs, with or without the hind limbs, and rarely unilateral or only in the hind limbs (DYSON 2011). The degree of lameness varies and may be severe enough to cause reluctance of the horse to move, a typical stance with the hind limbs placed far underneath the body (saw-horse stance) and extended periods of lying down (DYSON 2011, STASHAK 2002). The hoof may feel warm, pulsation of the digital arteries is usually increased, pressure or percussion applied to the hoof especially in the toe area leads to a pain reaction of the horse and careful palpation of the coronary band may reveal an unusual depression indicating sinking of the distal phalanx (DYSON 2011, STASHAK 2002).*

Evaluating the anamnesis for predisposing factors and causes helps to differentiate the diagnosis (DYSON 2011).

Chronic laminitis *is defined as the continuation of the acute phase and begins with the first sign of movement of the distal phalanx within the hoof capsule (POLLIT and COLLINS, 2011, STASHAK 2002). In horses with chronic laminitis lameness is highly variable, hoof abscesses are often encountered and the hoof capsule may deform and in severe cases separation at the coronary band (coronary band rupture) and even complete hoof wall ablation (exungulation) can occur (BELKNAP and PARKS 2011, CRIPPS and EUSTACE 1999, POLLIT and COLLINS 2011, STASHAK 2002).*

Radiographic examination *initially and during the course of the disease is critical to establish and adjust treatment protocol and prognosis (BELKNAP and PARKS 2011, CRIPPS and EUSTACE 1999, POLLIT and COLLINS 2011, STASHAK 2002). Diagnostic imaging in equine laminitis will be addressed in more detail below.*

Therapy for laminitis *can be divided into medical therapy, hoof care and orthopaedic farriery, surgical therapy and supportive management (VAN EPS 2012, BAKER 2012). At the same time addressing the primary cause or predisposing factors plays a major role in laminitis therapy (BELKNAP and PARKS 2011, STASHAK 2002).* ***Medical treatment options*** *consist of nonsteroidal anti-inflammatory drugs (phenylbutazone, flunixin meglumine, ketoprofen) vasodilator therapy (acepromazin, isoxsuprine, pentoxifylline, nitroglycerin) and anticoagulant therapy (aspirin, heparin) (BELKNAP and PARKS 2011, POLLITT 2011, STASHAK 2002). Cryotherapy was shown to have disease-modulating effects in the very early phase of laminitis (VAN EPS 2012).*

Principles of orthopaedic farriery *in laminitis cases are decreasing pressure on the sole and cushion the hoof, support of the frog, reducing the tension of the deep digital flexor tendon by facilitating break over and heel elevation, and maintenance of the hoof (BELKNAP and PARKS 2011, CRIPPS and EUSTACE 1999, POLLIT and COLLINS, 2011, STASHAK 2002). A multitude of options on how to address these goals are described in the literature.*

Described ***surgical treatments*** *for equine laminitis are distal check ligament desmotomy,* *deep digital flexor tenotomy, hoof wall resection and coronary grooving (BELKNAP and PARKS 2011, STASHAK 2002).*

There is currently no medication available that is able to arrest the triggering of laminitis and laminitis lesions are generally considered irreversible (POLLITT 2011, VAN EPS 2010).

There does not seem to be much consent about the ideal treatment of equine laminitis but a lot of debate and research on the topic is still going on. Given this situation the above list of treatment options is just a short and incomplete overview about what is described in the literature and some might disagree with some of the listed options. Individual case details, course of disease and clinicians preference will influence the treatment plan.

***The prognosis** is often determined by the severity of the initial insult to the laminae regardless of treatment (BELKNAP and PARKS 2011, POLLITT 2011, VAN EPS 2010). In mild cases without severe lamellar pathology the prognosis can be good depending on the primary cause, early recognition and optimal management. In cases with substantial damage to the lamellae the prognosis ranges from guarded to infaust depending mainly on the initial insult but also on the primary disease, patient related factors and management (BELKNAP and PARKS 2011, COLLINS 2011, CRIPPS and EUSTACE 1999, POLLIT and COLLINS, 2011, STASHAK 2002).*

2.4 Diagnostic imaging in equine laminitis

*Due to the encasing keratinized hoof capsule direct evaluation of the lamellar apparatus using standard soft tissue examination (e.g., palpation and ultrasound imaging) cannot be performed for detection of early signs of laminar injury. Thus, the clinician is limited primarily to radiographs to assess digital changes occurring in laminitis. The **primary radiographic changes** that can be detected in equine laminitis are caused by*

a) separation between distal phalanx and hoof wall and

b) thickening of the hoof wall due to inflammation and separation of the laminae

(BUTLER et al. 2008).

A faint radiolucent line between the distal phalanx and the sole or hoof wall, representing serum collections between epidermal and dermal lamellae may develop in the further course of disease. This line may later become more radiolucent and wide, representing necrotic laminar tissue and gas containment in the hoof wall (BELKNAP and PARKS 2011, BUTLER et al. 2008, STASHAK 2002). In more chronic cases radiographic changes may involve the toe of the distal phalanx and include increased radiolucency of the sole margin and new bone formation on the dorsal aspect of the distal phalanx (BUTLER et al. 2008).

Based on the aforementioned changes observed with displacement of the distal phalanx ***, radiographic measurements*** *assessing the position of the distal phalanx in relation to the hoof capsule include*

1) distance between the dorsal surface of the distal phalanx and the surface of the dorsal hoof wall (LINFORD et al. 1993),

2) the vertical distance from the proximal margin of the hoof capsule or coronet to the extensor process of the distal phalanx ("founder distance") (CRIPPS and EUSTACE 1999) and

3) the distance from the dorsodistal aspect of the distal phalanx to the sole surface, to assess distal displacement of the distal phalanx (sole depth) (BELKNAP and PARKS 2008).

The first two of these measurements are well documented both in normal and laminitic horses. Sole depth has been documented to a lesser degree in healthy horses (LINFORD et al. 1993, BELKNAP and PARKS 2008). A lack of sole hoof data may exist for several reasons, including tradition, as well as high variability due to trimming and irregular wear. Therefore the thickness of the sole is likely to be more variable than the relationship between the dorsal hoof and distal phalanx. However, our clinical experience is that the depth of sole is important in assisting with determining the prognosis. Additionally, interpretation of finite *element analysis results that investigated hoof function in response to weight bearing suggests that the sole is important in redirecting the forces of weight bearing to the lamellae (ARBLE et al. 2009). Therefore, the integrity of the sole is probably important both in normal weight bearing and protecting the underlying soft tissues from direct trauma and compression by the distal phalanx. Consequently, despite any potential variability, the thickness of the sole and its layers is valuable information.*

Two distinct soft tissue opaque layers are observed in the dorsal hoof wall on analogue radiographs (LINFORD et al. 1993). DR has greatly enhanced our ability to delineate and assess these two layers due to higher contrast resolution. The suggestion has been made that the outer more opaque layer is the keratinized hoof wall (epidermis) and the more lucent layer is the underlying lamellae and deep dermis (LINFORD et al. 1993). However, this suggestion has not been documented yet. Two distinct soft tissue opaque layers can also be observed in the sole area however, to our knowledge, radiographic assessment of distinct soft tissue layers in the sole area not been described. According to the aforementioned hypothesis for the hoof wall, the outer layer in the sole most likely represents the epidermis and the inner layer represents the terminal papillae and deep dermis. Because the lamellar/papillar and inner dermal layers, in contrast to the outer epidermis, are not affected by trimming or abnormal hoof growth, alteration of these layers could be a more reliable diagnostic measurement in laminitis evaluation than total hoof wall or sole thickness. Early changes in the thickness of these layers due to initial events such as oedema and the onset of laminar separation may be detectable resulting in earlier disease diagnosis.

***Venography** of the digit can be used to evaluate the functional integrity of the venous blood flow in the hoof and can be performed in the standing, sedated horse by injecting a positive radiographic contrast agent into the lateral or medial palmar vein after applying a tourniquet (BUTLER et al. 2008, D'ARPE et al. 2010, BALDWIN 2010). In chronic laminitis cases, displacement of the distal phalanx and changes in the laminae cause compression of* *the vessels leading to alterations of the vasculature detectable on digital venograms (BELKNAP and PARKS 2011, BUTLER et al. 2008, STASHAK 2002).*

The normal anatomy of the sole has been described using ultrasonography (OLIVIER-CARSTENS 2004). The use of ***ultrasonography*** *is very limited in the evaluation of the equine laminitis patient, Doppler ultrasonography has been shown to be a helpful complementary tool to detect digital blood flow changes in horses with laminitis or at risk of laminitis (AGUIRRE et al. 2013).*

Dynamic contrast-enhanced ***computed tomography (CT)*** *has been conducted in a study to evaluate equine laminar blood flow and vascular permeability in normal horses (KRUGER et al. 2008). The technique could be useful in evaluating the blood supply in diseased horses, due to detailed depiction of the vasculature of the distal limb.*

Magnetic Resonance Imaging (MRI) *to evaluate laminitic equine patients has been reported recently. Detailed soft tissue changes of the dorsal hoof wall in both the acute and chronic phase of the disease, as specified above, have been described (ARBLE et al. 2009, MURRAY at al, 2003, WYLIE 2013). The different layers of the hoof wall were identified on MRI, but were not correlated to the two soft tissue layers seen on radiographs. While the relationship of the dorsal hoof wall to the distal phalanx was examined in both MRI laminitis studies (MURRAY at al, 2003, KLEITER et al. 1999), the sole region was not evaluated. The experience of MRI findings in clinical cases with acute laminitis is limited (DYSON and MURRAY 2011).*

Because of lower expense and higher availability, DR remains the more commonly used modality to evaluate laminitis despite the superior soft tissue resolution of MRI. However, the knowledge gained from MRI can be used to improve differentiation of soft tissue layers detectable on DR.

3 Material and Methods

The practical part of the study was carried out at the Ohio State University. Statistics were performed at the Ohio State University and the Ludwig-Maximilians University. Fifty cadaver front feet were obtained from 25 horses subjected to euthanasia for non-lameness reasons. Multiple breeds were represented, including Quarter Horses (7), Thoroughbreds (4), Standardbreds (3), American Paint Horses (2), American Paso Finos (2), mixed breed horses (2), American Saddlebred (1), Arabian (1), Pinto (1), Rocky Mountain horse (1) and Tennessee Walking horse (1). Horses ranged in age from 3 to 37 years (mean=15 years). The distal extremities were removed proximal to the metacarpophalangeal joint, thoroughly cleaned, and shoes and nails were removed. To prevent desiccation, the feet were sealed into plastic bags, immediately cooled and frozen the same day at -20°C (KLEITER et al. 1999).

Lateromedial and horizontal dorsopalmar radiographs of each frozen distal extremity were made using a digital radiography system (Eklin Medical Systems, Santa Clara, CA). A thin layer of barium (Barium sulfate E-Z-Paste [60% w/w], E-Z-EM Canada Inc., Lake Success, NY) was applied onto the surface of the sole avoiding the frog and hoof wall *(Figure 1 and 6)*. A 5 mm, round metal marker (ball bearing) was placed on the hoof capsule at the level of the coronet in the mid-sagittal plane for lateromedial radiographs and mid-dorsal plane for dorsopalmar images to assist with magnification correction. For radiography, the distal extremities were positioned upright. Any metal detected radiographically was removed to prevent MRI susceptibility artifacts (URRACA del JUNCO et al. 2011). *Barium was thoroughly removed after radiographic examination.*

The distal limbs were thawed for 18-24 h before MR imaging. The hoof and sole surfaces were covered with fatty material (Lundy’s Refined Lard, Premium Standard Farm/Lundy Packing, Clinton, NC), *(Fig. 2 and 3)*, placed horizontally in a knee coil (Philips

SENSE Knee-8 Coil, Achieva, 8 channel, receive-only) (*Figure 7*) and imaged using a 3 Tesla magnet (Philips Achieva 3T, Cleveland, OH). After a localizer spin echo sequence, proton density weighted turbo spin echo and 3D gradient echo (GRE) T2* images were acquired in transverse, sagittal and dorsal planes. The sequence parameters are summarized in Table 1. The transverse plane was oriented perpendicular to the dorsal hoof wall. The sagittal and dorsal planes were oriented perpendicular to the weight bearing surface of the hoof.

All DR and MR images were stored in DICOM format and evaluated using a DICOM viewer (eFilm Merge Healthcare, Milwaukee, WI).

Following MR imaging, the distal extremities were sectioned in a sagittal plane using a band saw. (*Figure 8 a*) Tissue specimens were collected from the distal half of the dorsal hoof wall and lateral, medial and mid-sagittal aspects of the sole. The samples were fixed in 10% neutral-buffered formalin for at least 48 hours, trimmed for paraffin embedding and tissue sectioning. Slides were stained with hematoxylin and eosin and contained the stratum lamellatum and stratum reticulare. Histopathologic evaluation was performed by a board-certified veterinary pathologist to confirm absence of lamellar disease. Only feet without lamellar disease were included in the study.

The DR and MR images were randomized, measured and reviewed by two board-certified veterinary radiologists, one board-certified equine surgeon and one radiology resident. One observer reviewed and measured all of the images three times. *The anatomic specimens were reviewed by a board-certified pathologist and measured by a radiology resident. DR images were also reviewed by an anatomist and measurements of the distal interphalangeal joint – as described below – were obtained by the same individual and a board-certified radiologist.*

Dorsal hoof wall measurements were made using lateromedial radiographs *(Figure 1 a))*. The DR images were recalibrated to account for magnification using a 5 mm diameter ball bearing in each image. The proximal dorsal hoof wall thickness was measured ~5mm distal to the extensor process and perpendicular to the dorsal surface of the hoof wall and the distal dorsal hoof wall thickness was measured ~6mm proximal to the tip of the distal phalanx (LINFORD et al. 1993). Three measurements were made: the full thickness of the wall (proximally R1+2; distally R3+4); the inner less opaque layer (proximally R2; distally R4); and the outer more opaque layer (proximally R1; distally R3). On the dorsopalmar radiographs, the same three measurements of distolateral (R5, 6) and distomedial (R7, 8) hoof thickness were from the most distal and lateral/medial aspect of the distal phalanx perpendicular to the outer margin of the lateral/medial hoof wall *(Figure 1 b)).*

The sagittal sole thickness was measured on the lateromedial radiograph perpendicular to the ground surface from the dorsodistal tip of the distal phalanx to the distal margin of the barium-painted sole (*Figure 1 a)*). The overall sole thickness (R9+10) was measured, plus the outer more radiopaque layer (R10) and the inner less radiopaque layer (R9). On the dorsopalmar image, the lateral and medial sole thickness (R11+12 and R13+14) was measured from the most distal and lateral/medial aspect of the distal phalanx to the surface of the barium-painted sole (*Figure 1 b)*).

The palmar cortex length (PCL) was measured from the tip of the distal phalanx to the palmar articular margin of the distal phalanx on the lateromedial radiograph. The dorsodistal hoof wall thickness to palmar cortex length ratio (R3+4 PCR) was calculated. The coronary band to extensor process distance was measured from the proximal aspect of the coronary band to the proximal aspect of the extensor process on the lateromedial radiograph. The location of the coronary band was obtained by window/levelling the DR images to determine the junction of the hairline and the hoof wall.

The joint space of the distal interphalangeal joint was measured medially and laterally in two different locations each: (1) from the junction of the condyle and the distal articular surface of the middle phalanx to the articular surface of the distal phalanx following a perpendicular line; (2) from the most distal aspect of the distal articular surface of the *lateral/medial condyle of the middle phalanx to the articular surface of the distal phalanx following a perpendicular line (Figure 9). If for the first measurement of the distal interphalangeal joint space a perpendicular line from the junction of the condyle and the distal articular surface of the middle phalanx would not meet the articular surface of the distal phalanx, a horizontal line from the most distal and abaxial aspect of the articular surface of the distal phalanx was drawn and the intersection of the two lines was used as a reference point.*

For MRI measurements, selected slices were used (Figure 2). A mid-sagittal slice, used for dorsal hoof wall and sagittal sole thickness measurements (M15 to M18; M23+M24), was selected by displaying a reference line on a dorsal plane image and visually locating the mid-line of the foot. A dorsal slice, used for lateral and medial hoof wall and sole thickness measurements (M19 to M25), was selected at the palmar edge of the articular surface of the distal phalanx.

Similar measurements made using DR were made using MR images – *except the distal interphalangeal joint measurements (Figure 3)*. Additionally, the tela subcutanea soleae and the papillae dermales including the dermis were measured.

For measurements on anatomic specimens a mid-sagittal slice was used for dorsal hoof wall and sagittal sole thickness measurements (R1 to R4 und R9+10) and a dorsal slice was used for additional measurements of the sole (R11 to R14) (Figure 8). The mid-sagittal slice was selected by visually locating the midline of the foot and the dorsal slice was selected by sectioning the foot from palmarly in 5 mm slices in a dorsal plane and selecting the slice at the palmar edge of the articular surface of the distal phalanx (Figure 8). Similar measurements performed on DR images were performed on anatomic specimens with exception of the lateral and medial hoof wall measurements and the distal interphalangeal joint space measurements. The additional measurements of the deeper layer of the dermis and *the lamellar/papilliform layer of the dermis performed on MR images were also not performed on anatomic specimen.*

The thickness measurements were compared to the outer more radiopaque layer seen on DR images with the strata externum and medium noted on MR images and the inner, less radiopaque layer seen on DR with the strata lamellatum and reticulare seen on MR images.

Descriptive statistics were performed for each measurement. Using the first set of measurements from all four observers, interobserver correlation was calculated using a pairwise Pearson's method. The three measurement sets from one observer were then used to calculate an intraobserver correlation using the same method. Comparisons of the difference between the MR and DR measures with the mean on the two measures were made using Bland-Altman plots. Comparisons of the lateral and medial aspects of the hoof wall *and lateral and medial aspects of the distal interphalangeal joint* were made using paired Student's t-test and $P<0.008$ was considered significant to adjust for multiple comparisons. Bland-Altman plots were used to measure the agreement between the DR and MR measurements (BLAND and ALTMAN 2007). All statistical analyses were done using R statistical software (http://www.r-project.org/).

Additionally, to analyze the agreement between measurements made on DR, MR and anatomic specimens, a Pasing & Bablok Regression as well as Bland-Altman plots were performed. To further evaluate inter- and intraobserver variability the coefficient of variation was derived from the one-way analysis of variance. These statistical analyses were performed using another statistical software (http://www.medcalc.org/).

4 Results

The DR and MRI data are listed in Table 2 and 3 *and a schematic illustration of the DR measurements is found in the Supplementary item 1. Values for measurements made on anatomic specimens are found in Table 2. The values of the measurements of the distal interphalangeal joint space are listed in Table 4.*

Based on MR images, the less radiopaque soft tissue layer surrounding the distal phalanx on DR images consists of both the stratum lamellatum and the stratum reticulare (dorsal hoof wall/sole).

Using Bland-Altman plots to compare the difference between the MR and DR measures with the mean of the two measures, the following 3 types of agreement were noted and are presented in Table 2: 1) – No difference with a narrow confidence interval centered around zero; 2) – within the confidence interval but the MR values were less than the DR values; and 3) –means are not significantly different than zero but variation increases as thickness increases. The average difference between the lateral and medial hoof wall (R5+6/M19+20 and R7+8/M21+22), and the lateral and medial sole thickness (R12+12/M25+26 and R13+14/M23+24) are listed in Table 3.

There was good overall inter- and intra-observer correlation for all values between DR (>0.98/0.98) and MRI (>0.99/0.99) measurements. *The minimal coefficient of variation for intra-observer variability was 1.3% and the maximum value was 17.4%. The minimal coefficient of variation for inter-observer variability was 1.7% and the maximum value was 19.6%.*

The proximal and distal dorsal hoof wall thickness measured on DR and MR images were different (P<0.0001). The mean proximal dorsal hoof wall thickness was 18.3mm (SD=1.6mm) on DR (R1+2) and 16.9mm (SD=1.3mm) on MR (M15+16). The associated *dermal measurements were 7.6mm (SD=01.0mm) on DR (R2) and 6.7mm (SD=0.9mm) on MR (M16).*

The distal dorsal hoof wall thickness was 17.9mm (SE=0.1mm) on DR (R3+4) and 16.8mm (SE=0.1mm) on MR (M17+18).

The dermal (Strata lamellatum et reticulare) measurements for DR (R4) and MR (M18) were 7.2mm (SE=0.1mm) and 6.7mm (SE=0.1mm), respectively. The proximal and distal dorsal hoof wall thickness measured on DR and MR images were different (P<0.0001). The proximal and distal dorsal hoof wall measurements made from the anatomic specimen were different when compared to the DR measurements (P<0.0001), but not when compared to the MR measurements (P>0.0001). (Graph 1-3) The mean anatomic proximal dorsal hoof wall measurement was 16.1mm (SD=1.6mm) and the associated dermal (Strata lamellatum et reticulare) measurement was 6.0mm (SD=1.3mm). The mean anatomic distal dorsal hoof wall measurement was 16.2mm (SD=1.6mm) and the dermal measurement was 6.0mm (SD=1.4mm).

The dorsal hoof wall thickness to palmar cortex length ratio was 26.8% (SD=2.6%) on DR (R3+4 – PCR) and 28.8% (SD=0.04%) on MR (M17+18 – PCR). The associated dermal dorsal hoof wall thickness to palmar cortex length ratio was 10.7% (SD=1.8%) on DR (R4 – PCR) and 11.6% (SD=0.04%) on MR (M18 – PCR). The anatomic dorsal hoof wall to palmar cortex length ratio was 26% (SD=0.033%) and was not different when compared to DR (P<0.0001), but was different when compared to MR (P=0.0235). Mean lateral/medial hoof wall measurements were 19.3mm (SD=2.7mm)/19.3mm (SD=2.3mm) on DR (R5+6/R7+8) and 13.5mm (SD=1.9mm)/13.5mm (SD=1.7mm) on MR (M19+20/M21+22). The lateral/medial dermal measurements were 9.1mm (SE=0.1mm)/9.3mm (SE=0.1mm) on DR (R6/R8) and 5.5mm (SE=0.1mm)/ 5.8mm (SE=0.1mm) on MR (M20/M22).

The majority of the sole thickness measured on DR and MR were different (P<0.0001) with exception of the medial epidermal sole measurement, which did not differ (P=0.37). There *was a mixed agreement between the anatomic measurements and the DR/MR measurements with the majority of the DR measurements being different (P<0.0001). The anatomic and MR values of the mid sagittal sole thickness and the dermal lateral/medial sole thickness were similar (P>0.0001) (Graph 1-3).*

The mean mid-sagittal sole thickness was 13.7mm (SD=2.4mm) on DR (R9+10), 12.3mm (SD=2.3mm) on MR (M23+24) and 10.7mm (SD=3.3mm) on anatomic specimens. The associated dermal measurement was 5.6mm (SD=1.0mm) on DR (R9), 4.6mm (SD=0.8mm) on MR (M24) and 10.7mm (SD=3.7mm) on anatomic specimens. The lateral/medial sole thickness measurements were 20.7mm (SD=4.4mm)/18.9mm (SD=4.2mm) on DR (R11+12/R13+14), 18.7mm (SD=4.7mm)/17.4mm (SD=4.4mm) on MR (M25+26/M23+24) and 15.0mm (SD=5.6mm)/13.0mm (SD=4.3mm) for anatomic measurements. The mid-sagittal dermal sole thickness was 5.6mm (SD=1.0mm) on DR (R9), 4.6 mm (SD=0.8mm) on MR (M24) and 4.3mm (SD=1.3mm) for anatomic specimens. The lateral/medial dermal sole measurements were 7.5mm (SD=1.7mm)/7.4mm (SD=4.1mm) on DR (R12/R14), 6.2mm (SD=01.3mm)/6.1mm (SD=1.1mm) on MR (M24/26) and 5.3mm (SD=3.7mm)/4.7mm (SD=2.5mm) for anatomic measurements.

The sole thickness to palmar cortex length ratio was 20.2% (SD=5.3%) on DR (R9+10 – PCR), 21.1% (SD=0.04%) on MR (M23+24 – PCR) and 26% (SD=3.3%) on anatomic specimens. The associated dermal sole thickness to palmar cortex length ratio was 9.1% (SD=7.4%) on DR (R9 – PCR), 8.0% (SD=0.04%) on MR (M24 – PCR) and 6.8% (SD=2.0%) on anatomic specimens.

Measurements before and after barium application onto the surface of the sole varied (P <0.0001), with a mean value of 2 mm. The mid-sagittal sole thickness had the least significant difference with a mean of 1.7 mm (SD=0.1mm). The lateral and medial sole thickness values were more different, with a mean of 2.3mm/2.3mm respectively.

There was good overall inter- and intra-observer correlation between DR (>0.98/0.98) and

MR (>0.99/0.99) measurements.

MRI measurements of the deep/sublamellar dermis and interdigitating epidermal and dermal layer had fair to moderate correlation to the lucent soft tissue opaque band surrounding the distal phalanx on DR images. This was lowest (0.32) for the medial lamellar/deep dermal sole thickness. There was good overall intra- and interobserver correlation between the DR measurements of the distal interphalangeal joint with a better correlation of the intraobserver variability. The minimal coefficient of variation for intra-observer variability was 3.0% and the maximum value was 6.3%. The minimal coefficient of variation for inter-observer variability was 10.6% and the maximum value was 15.9%.

The measurements of the lateral and medial distal interphalangeal joint (R15 to R17) are listed in Table 4 and the average difference between the lateral and medial aspect of the distal interphalangeal joint measurements are listed in Table 5.

5 Publication

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Quantitative assessment of the equine hoof using digital radiography and magnetic resonance imaging

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Summary

Reasons for performing study: Evaluation of laminitis cases relies on radiographic measurements of the equine foot. Reference values have not been established for all layers of the foot.
Objectives: To establish normal hoof wall and sole measurements using digital radiography (DR) and magnetic resonance imaging (MRI) and to document tissue components present in the dorsal hoof wall and solar layers seen on DR.
Study design: Prospective observational case–control study.
Methods: Digital radiography and MRI were performed on 50 cadaver front feet from 25 horses subjected to euthanasia for nonlameness-related reasons. Four observers measured hoof wall (dorsal, lateral and medial) and sole thickness (sagittal, lateral and medial) using DR and magnetic resonance images. One observer repeated the measurements 3 times. Inter- and intraobserver correlation was assessed.
Results: Digital radiography and MRI measurements for the normal hoof wall and sole were established. Inter- and intraobserver pairwise Pearson's correlation for DR ($r>0.98$) and MRI measurements ($r>0.99$) was excellent. Based on MRI, the less radiopaque layer on DR is comprised of the stratum lamellatum and stratum reticulare.
Conclusions: Normal DR and MRI measurements for the hoof wall and sole were established. On DR images, the less radiopaque layer of the foot observed corresponds to the critical tissues injured in laminitis, the strata lamellatum and reticulare. These reference measurements may be used by the clinician to detect soft-tissue changes in the laminitic equine foot and provide a foundation for future research determining changes in these measurements in horses with laminitis.

Keywords: horse; diagnostic imaging; magnetic resonance imaging; laminitis

Introduction

The equine foot is an anatomically complex structure, in which lamellar attachments of the epithelium to the underlying dermis suspend the distal phalanx in the hoof capsule and thereby support the entire musculoskeletal system. The lamellar attachments consist of the interdigitating lamellae epidermales and lamellae dermales, the stratum lamellatum [1]. In between the stratum lamellatum and the distal phalanx is the stratum reticulare, which aids in attachment of the distal phalanx to the lamellae epidermales and provides the vascular supply to the lamellae [1–4]. At the distal extent of the dermis parietis (junction of the wall and the sole), the lamellae continue into the papillae dermales, and the epidermis overlying the papillae forms the terminal horn tubules [1,3].

In equine laminitis, a sequela of multiple disorders, this complex lamellar apparatus undergoes injury commonly leading to separation of the lamellae dermales and epidermales [5–7]. Disruption of lamellae leads to several recognised patterns of displacement of the distal phalanx within the hoof capsule, as follows: 1) rotational displacement; 2) symmetrical distal displacement, a pattern often called sinking; and 3) uniaxial/asymmetrical distal displacement in the sagittal plane so that the thickness of the displaced side of the hoof increases and the distance between the displaced side of the distal phalanx and the ground surface of the sole decreases [8,9].

Radiographic measurements assessing the position of the distal phalanx relative to the hoof capsule include the following: 1) distance between the dorsal surface of the distal phalanx and the surface of the dorsal hoof wall [10]; 2) vertical distance from the proximal margin of the hoof capsule or coronet to the extensor process of the distal phalanx ('founder distance') [11]; and 3) distance from the dorsodistal aspect of the distal phalanx to the ground surface of the sole [12]. The first 2 measurements are well documented in normal and laminitic horses. Sole depth is less well documented in healthy horses [10,13].

Using analog radiographs, 2 distinct soft tissue opaque layers are observed in the dorsal hoof wall [10]; digital radiography (DR), with better resolution than analog radiographs, enhances our ability to delineate and assess these 2 layers. The outer, more radiopaque layer may be the keratinised hoof wall, comprised of the strata externum and medium, and the more radiolucent layer may represent the strata lamellatum and reticulare [10]; the composition of the 2 layers has not been investigated. Radiographic assessment of distinct soft-tissue layers in the sole area has not been documented in the sense of comparing the layers seen on radiographs with other imaging modalities or histopathology. Given that the stratum lamellare and stratum reticulare, in contrast to the strata externum and medium, are likely not to be affected by trimming or abnormal hoof growth, alteration of these layers could be a more reliable diagnostic measurement in evaluation of laminitis than total hoof wall or sole thickness. Early changes in the thickness of these layers due to initial events such as oedema and the onset of lamellar separation may result in earlier disease diagnosis.

Magnetic resonance imaging (MRI) has revealed soft-tissue changes of the dorsal hoof wall in acute and chronic phases of laminitis [14,15]. While the relationship of the epidermis and dermis dorsal to the distal phalanx was examined in both MRI studies of laminitis, the sole region was not evaluated.

Lower expense and higher availability makes DR the more commonly used modality to evaluate laminitis despite the superior soft-tissue resolution of MRI. Knowledge gained from MRI can be applied to the soft-tissue layers detectable on DR. Our objectives were to establish normal measurements of the epidermal and dermal elements of the wall and sole using DR and MRI and to evaluate inter- and intraobserver correlation. We aimed to use MRI to document the tissue components present in the 2 soft-tissue layers seen on DR in the dorsal hoof wall and sole. The results of this study may enable the clinician to obtain more sensitive and in-depth information about equine digital changes.

Materials and methods

Fifty cadaver front feet were obtained from 25 horses subjected to euthanasia for nonlameness-related reasons. Multiple breeds were represented, including Quarter Horses (7), Thoroughbreds (4), Standardbreds (3), American Paint Horses (2), American Paso Finos (2), mixed breed horses (2), American Saddlebred (1), Arabian (1), Pinto (1), Rocky Mountain horse (1) and Tennessee Walking horse (1). Horses ranged in age from 3 to 37 years (mean 15 years). The distal extremities were removed proximal to the metacarpophalangeal joint, thoroughly cleaned, and shoes and nails removed. To prevent desiccation, the feet were sealed into plastic bags, immediately cooled and frozen on the same day at -20°C [16].

Lateromedial and horizontal dorsopalmar radiographs of each frozen foot were made using a digital radiography system[a]. A thin layer of barium (Barium sulfate E-Z-Paste [60% w/w])[b] was applied to the surface of the sole, avoiding the frog and hoof wall. A 5 mm, round metal marker (ball bearing) was placed on the hoof wall at the level of the coronet in the mid-sagittal plane for lateromedial radiographs and the mid-dorsal plane for dorsopalmar images to assist with magnification correction. For radiography, the feet were positioned upright. Any metal detected radiographically was removed to prevent MRI susceptibility artefacts [17].

The distal limbs were thawed for 18–24 h before magnetic resonance (MR) imaging. The hoof and sole surfaces were covered with fatty material (Lundy's Refined Lard)[c], placed horizontally in a knee coil (Philips SENSE Knee-8 Coil, Achieva, 8 channel, receive-only)[d] and imaged using a 3 T magnet (Philips Achieva 3T)[d]. After a localiser spin echo sequence, proton density-weighted turbo spin echo and 3-dimensional gradient echo T2* images were acquired in transverse, sagittal and dorsal planes. The sequence parameters are summarised in Table 1. The transverse plane was oriented perpendicular to the dorsal hoof wall. The sagittal and dorsal planes were oriented perpendicular to the weightbearing surface of the hoof.

All DR and MR images were stored in DICOM format and evaluated using a DICOM viewer[e].

Following MR imaging, the feet were sectioned in a sagittal plane using a band saw. Tissue specimens were collected from the distal half of the dorsal hoof wall and lateral, medial and mid-sagittal aspects of the sole. The samples were fixed in 10% neutral-buffered formalin for at least 48 h, then trimmed for paraffin embedding and tissue sectioning. Slides were stained with haematoxylin and eosin and contained the stratum lamellatum and stratum reticulare. Histopathological evaluation was performed by a board-certified veterinary pathologist to confirm the absence of lamellar disease. Only feet without lamellar disease were included in the study.

The DR and MR images were randomised, measured and reviewed by 2 board-certified veterinary radiologists, one board-certified equine surgeon and one radiology resident. One observer reviewed and measured all of the images 3 times. Dorsal hoof wall measurements were made using lateromedial radiographs (Fig 1a). The DR images were recalibrated to account for magnification using a 5 mm diameter ball bearing in each image. The proximal dorsal hoof wall thickness was measured ~5 mm distal to the extensor process and perpendicular to the dorsal surface of

TABLE 1: Magnetic resonance imaging sequence parameters

	FOV (mm)	Voxel (mm)	TR (ms)	TE (ms)	Thickness/ gap (mm)	Flip angle (°)
PD-w TSE	100 × 180	0.5 × 0.5	6187	30	2/0	–
T2* 3DGRE	100 × 180	0.4 × 0.4	16	4.7	2/0	25

Abbreviations: FOV = field of view; PD-w TSE = proton density-weighted turbo spin echo; TE = time to echo; TR = time to repetition; and 3DGRE = three-dimensional gradient recall echo.

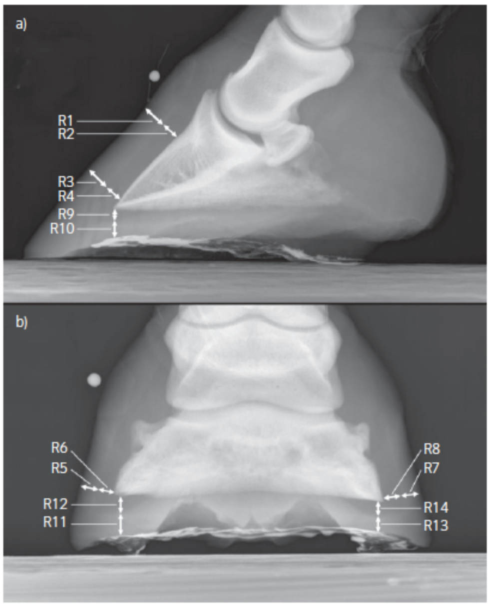

Fig 1: Lateromedial (a) and horizontal dorsopalmar radiographs (b) of a front hoof with barium on the sole showing the measurement sites. See Table 2 for key to the labels.

the hoof, and the distal dorsal hoof wall thickness was measured ~6 mm proximal to the tip of the distal phalanx [10]. Three measurements were made: the full thickness of the wall; the thickness of the inner, less radiopaque layer; and the thickness of the outer, more radiopaque layer. On the dorsopalmar radiographs, the same 3 measurements of distolateral and distomedial hoof thickness were made from the most distal and lateral/medial aspect of the distal phalanx perpendicular to the outer margin of the lateral/medial hoof wall (Fig 1b).

The sagittal sole thickness was measured on the lateromedial radiograph perpendicular to the ground surface from the dorsodistal tip of the distal phalanx to the distal margin of the barium-painted sole (see Fig 1a). The overall sole thickness was measured, plus the thicknesses of the outer, more radiopaque layer and the inner, less radiopaque layer. On the dorsopalmar image, the lateral and medial sole thickness was measured from the most distal and lateral/medial aspect of the distal phalanx to the surface of the barium-painted sole (see Fig 1b).

The palmar cortex length was measured from the tip of the distal phalanx to the palmar articular margin of the distal phalanx on the lateromedial radiograph. The dorsodistal hoof wall thickness to palmar cortex length ratio was calculated. The coronary band to extensor process distance was measured from the proximal aspect of the coronary band to the proximal aspect of the extensor process on the lateromedial radiograph. The location of the coronary band was obtained by window/levelling the DR images to determine the junction of the hairline and the hoof wall.

For MRI measurements, selected slices were used (Fig 2). A mid-sagittal slice, used for dorsal hoof wall and sagittal sole thickness measurements, was selected by displaying a reference line on a dorsal plane image and visually locating the mid-line of the foot. A dorsal slice, used for lateral and medial hoof wall and sole thickness measurements, was selected at the palmar edge of the articular surface of the distal phalanx.

Similar measurements made on DR images were made using MR images (Fig 3). Additionally, the tela subcutanea soleae and the papillae dermales including the dermis were measured.

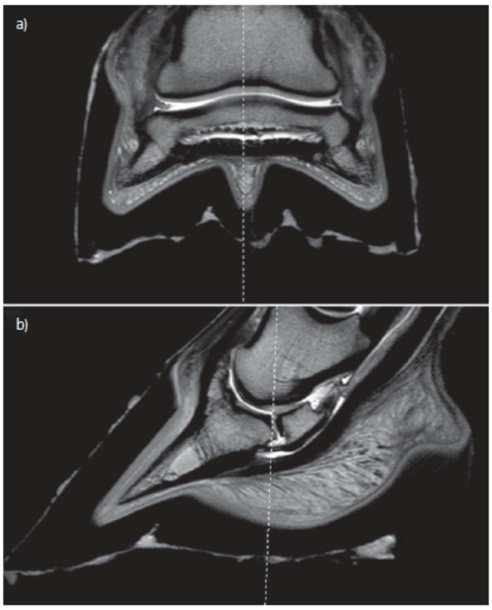

Fig 2: Proton density-weighted turbo spin echo magnetic resonance dorsal (a) and mid-sagittal images (b) of a front foot with lard on the hoof wall, showing the location of the slices used to make the measurements. The dotted line on the dorsal image (a) shows the location of the mid-sagittal slice, while the dotted line on the mid-sagittal image (b) shows the location of the dorsal slice.

The thicknesses were compared of the outer, more radiopaque layer seen on DR images with the strata externum and medium noted on MR images and the inner, less radiopaque soft-tissue layer seen on DR with the strata lamellatum and reticulare seen on MR images.

Descriptive statistics were performed for each measurement. Using the first set of measurements from all 4 observers, interobserver correlation was calculated using a pairwise Pearson's method. The 3 measurement sets from one observer were then used to calculate an intraobserver correlation using the same method. Comparisons of the difference between the MR and DR measures with the mean of the 2 measures were made using Bland-Altman plots. Comparisons of the lateral and medial aspects of the hoof wall were made using paired Student's *t* test, and $P<0.008$ was considered significant to adjust for multiple comparisons. All statistical analyses were performed using R statistical software (http://www.r-project.org/).

Results

The DR and MRI data are listed in Table 2 (Supplementary item 1). Based on MR images, the less radiopaque soft-tissue layer surrounding the distal phalanx on DR images consists of both the stratum lamellatum and the stratum reticulare (dorsal hoof wall)/sole. Using Bland-Altman plots to compare the difference between the MR and DR measures with the mean of the 2 measures, the following 3 types of agreement were noted and are presented in Table 2: 1) no difference, with a narrow confidence interval centred around zero; 2) within the confidence interval, but the MR values are less than the DR values; and 3) means are not significantly different from zero, but variation increases as thickness increases. The average differences between the lateral and medial hoof wall thickness and between the lateral and medial sole thickness are listed in Table 3.

There was good overall inter- and intraobserver correlation for all values between DR ($r>0.98/0.99$) and MRI measurements ($r>0.99/0.99$).

Discussion

Assessment of hoof wall and sole thickness using radiographs is critical in the clinical assessment of laminitis, especially regarding displacement of the distal phalanx. We attempted to assess the majority of measurements currently being used in radiographic assessment of laminitis cases, including dorsal hoof wall, lateral and medial hoof wall and sole thicknesses. Evaluation of the lateromedial radiographs for dorsal hoof wall thickness and sole thickness at the toe is critical in symmetrical distal and rotational displacement of the distal phalanx, whereas evaluation of the dorsopalmar radiographs for lateral and medial hoof wall and sole thicknesses is critical for the detection of uniaxial/asymmetric distal displacement of the distal phalanx [8,9].

Comparing hoof wall measurements in our cadaveric study with those obtained in live horses, the dorsal hoof wall thicknesses we obtained using DR are slightly larger than those in one study [10] and similar to other studies [11,13]. All of these studies used clinically normal (sound) horses as their study population, whereas we included histologically normal feet. Our study did not include any Warmbloods, whereas a previous study used Warmbloods exclusively [13]. To account for magnification and breed variation when assessing dorsal hoof wall thickness, a ratio of dorsal hoof wall thickness to palmar cortex length is used [10]. Our average value for this ratio is slightly greater than reported for analog radiographs [10] but similar to studies using DR [13,14].

Sole thickness is an important measurement used by clinicians for assessment of the relationship of the distal phalanx and sole in laminitis cases [12]. Our sagittal sole thickness using DR was greater than in a study where no radiographic marker was used on the sole surface [10] but similar

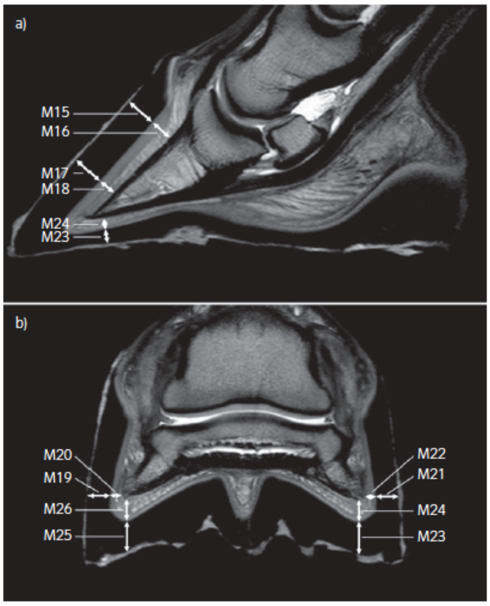

Fig 3: Proton density-weighted turbo spin echo magnetic resonance mid-sagittal (a) and dorsal images (b) of a front hoof with lard on the hoof wall, showing the same measurement sites that were made using radiographs. See Table 2 for the key to the labels. The dermis parietis was further divided into the stratum lamellatum and stratum reticulare (not shown).

TABLE 2: Measurements of 50 cadaver feet in images obtained with digital radiography (DR) and magnetic resonance imaging (MRI)

	DR (mm)		MRI (mm)		Figure legend	
	Mean	s.d.	Mean	s.d.	Fig. 1	Fig. 2
Proximal dorsal hoof wall thickness[a]	18.3	1.6	16.9	1.3		
Proximal dorsal epidermal thickness[a]	10.7	1.3	10.2	1.1	R1	M15
Proximal dorsal dermal thickness[a]	7.6	1.0	6.7	0.9	R2	M16
Proximal dorsal lamellar thickness	–	–	3.7	0.7		
Proximal dorsal deep dermal thickness	–	–	3.0	0.7		
Distal dorsal hoof wall thickness[a]	17.9	1.8	16.8	1.6		
Distal dorsal epidermal hoof wall thickness[a]	10.7	1.2	10.1	1.0	R3	M17
Distal dorsal dermal thickness[a]	7.3	1.3	6.8	1.3	R4	M18
Distal dorsal lamellar thickness	–	–	3.9	1.0		
Distal dorsal deep dermal thickness	–	–	2.8	0.7		
Lateral hoof wall thickness[b]	19.3	2.7	13.5	1.9		
Lateral epidermal thickness[a]	10.1	1.4	7.9	1.1	R5	M19
Lateral dermal thickness[b]	9.1	1.9	5.5	1.4	R6	M20
Lateral lamellar thickness	–	–	2.7	0.8		
Lateral deep dermal thickness	–	–	2.8	1.1		
Medial hoof wall thickness[b]	19.3	2.3	13.5	1.7		
Medial epidermal thickness[a]	9.9	1.5	7.7	1.0	R7	M21
Medial dermal thickness[b]	9.3	1.8	5.8	1.6	R8	M22
Medial lamellar thickness	–	–	2.8	0.9		
Medial deep dermal thickness	–	–	3.0	1.1		
Sagittal sole thickness[a]	13.7	2.4	12.3	2.3		
Sagittal epidermal sole thickness[a]	8.1	2.1	7.7	2.1	R9	M23
Sagittal dermal sole thickness[a]	5.6	1.0	4.6	0.8	R10	M24
Sagittal papillar sole thickness	–	–	1.4	0.6		
Sagittal deep dermal sole thickness	–	–	3.3	0.8		
Lateral sole thickness[c]	20.7	4.4	18.7	4.7		
Lateral epidermal sole thickness[c]	13.2	4.3	12.5	4.6	R11	M25
Lateral dermal sole thickness[a]	7.5	1.7	6.2	1.3	R12	M26
Lateral papillar sole thickness	–	–	1.8	0.6		
Lateral deep dermal sole thickness	–	–	4.4	1.2		
Medial sole thickness[c]	18.9	4.2	17.4	4.4		
Medial epidermal sole thickness[c]	11.5	3.9	11.3	4.3	R13	M27
Medial dermal sole thickness[a]	7.4	1.4	6.1	1.1	R14	M28
Medial papillar sole thickness	–	–	1.9	0.8		
Medial deep dermal sole thickness	–	–	4.2	1.0		
Founder distance	6.9	2.4	–	–		
Ratios	DR		MR			
Ratio of dorsal hoof wall to palmar cortex	26.8%	2.6	28.8%	0.04		
Ratio of dorsal dermis to palmar cortex	10.7%	1.8	11.6%	0.04		
Ratio of sagittal sole to palmar cortex	20.2%	5.3	21.1%	0.04		
Ratio of sagittal sole dermis to palmar cortex	9.1%	7.4	8.0%	0.04		

Bland-Altman: [a]no difference, with a narrow confidence interval centred around zero; [b]narrow confidence interval, but the MR values are less than the DR values; and [c]means are not significantly different from zero, but variation increases as thickness increases. Anatomical terms in the left column are more descriptive and correlate to the *Nomina Anatomica Veterinaria* [1] terms used in the manuscript as follows: hoof wall thickness = strata externum, medium, lamellatum et reticulare; epidermal thickness = strata externum et medium; dermal thickness = strata lamellatum et reticulare; lamellar thickness = stratum lamellare; deep dermal thickness = stratum reticulare.

to a study where the mid-sagittal aspect of the sole was marked with barium [13]. Thus, although differences in study population may account for some of the variability between studies, the similarity between our study and the latter study probably indicates that a more accurate measurement can be obtained with barium on the sole.

As both lateral and medial hoof wall thicknesses and lateral and medial sole thicknesses have been used on the dorsopalmar view to assess for uniaxial/asymmetric distal displacement of the distal phalanx, both were assessed in the present study. Although there were minor differences in the individual dermal and epidermal thicknesses of the lateral and medial hoof wall on the dorsopalmar view, thicknesses of the entire lateral and medial hoof walls were close to identical in the digits assessed in this study. The same was not true for lateral and medial sole measurements. The lateral aspect of the sole was significantly thicker than the medial aspect owing to different thicknesses of the strata externum and medium; this disparity was noted on DR and MRI measurements. Interestingly, the thickness of the strata lamellatum and reticulare of the sole was not significantly different when comparing lateral with medial measurements using either modality. This may be due to a disparity in trimming of the lateral and medial sides. As this was a cadaveric study, force on the sole due to weightbearing was not present and should not be the cause of the lateral vs. medial differences. This suggests that differences between

TABLE 3: Comparison of lateral and medial aspects of the hoof wall and sole measurements from 50 cadaver feet in images obtained with digital radiography and magnetic resonance imaging

	Measurements	Average difference (mm)	P value*
Digital radiography	Lateral vs. medial hoof wall epidermal	0.2	0.05
	Lateral vs. medial hoof wall dermal	-0.2	0.03
	Solar lateral vs. solar medial	1.9	0
	Solar lateral epidermal vs. solar medial epidermal	1.8	0
	Solar lateral dermal vs. solar medial dermal	0.1	0.5
Magnetic resonance imaging	Lateral vs. medial hoof wall epidermal	0.2	0.02
	Lateral vs. medial hoof wall dermal	-0.3	0
	Solar lateral vs. solar medial	1.2	0
	Solar lateral epidermal vs. solar medial epidermal	1.1	0
	Solar lateral dermal vs. solar medial dermal	0	0.7

*P<0.008 considered significant to adjust for multiple comparisions.

lateral and medial sole thicknesses (including dermal and epidermal components) should be used with caution when assessing the dorsopalmar DR images for uniaxial/asymmetric displacement, and changes in measurement of the dermis soleae may be combined with comparison of measurements of the entire lateral and medial hoof wall thickness and evaluation of the symmetry of the distal interphalangeal joint in making this determination.

The ability to obtain consistent measurements depends on accurate distinction of anatomical landmarks on high-quality images. Three techniques used in this study can give clinicians more distinct visualisation of external landmarks when using DR and MRI to obtain measurements; these are as follows: 1) adjustment of brightness and contrast instead of a radiographic marker to establish the dorsal aspect of the hoof wall on our DR images [18,19]; 2) placement of barium on the sole to delineate the ground surface of the sole from the distal hoof wall on our DR images; and 3) coating of the hoof wall surface with lard for our MRI images because the strata externum and medium of the hoof wall is normally difficult to detect on MRI due to low water content [14]. In margin more visible [11,13]. We were able to see the dorsal hoof wall surface well by adjusting image brightness/contrast. The intra- and interobserver variability in measurements overall was very low for DR and MRI measurements and slightly better for the MRI measurements, suggesting that both imaging modalities are valuable for objective quantitative assessment of the equine foot. The materials for delineating the hoof wall are readily available and easy to apply. If one is applying barium to the sole margin, this should be done after imaging any radiographic view in which the barium would be superimposed on important foot anatomy.

In an attempt to determine the anatomical composition of the 2 layers detectable in the dorsal hoof wall, we hoped to make a comparison of DR with MR images. However, direct comparison of absolute values should not be made because of inherent differences in the modalities. While a single MR slice is essentially a 2-dimensional representation of a 2-dimensional structure, a DR image is a 2-dimensional representation of a 3-dimensional structure (i.e. summation of multiple MR 'slices'). Thus, different amounts of hoof wall are represented by each modality. We used Bland-Altman plots to view the agreement between the measurements made using the 2 modalities. Measurements made of the dorsal hoof wall had good agreement, but the lateral and medial hoof wall and the lateral and medial sole thicknesses were not as good, with the MR thicknesses being smaller than those measured using DR. Given that the lateral and medial hoof wall and sole thicknesses on DR are measured on dorsal plane images, the curvature of the foot (narrower dorsally and wider palmarly) is likely to play a role in the differences. Digital radiographic images are a summation of the thicknesses and MRI are a single 'slice' of the foot. Albeit not proven, we believe that the outer, more radiopaque layer of the hoof wall on DR corresponded to the stratum externum and medium on MRI, whereas the inner, more radiolucent layer on DR corresponded to the stratum lamellatum and stratum reticulare on MRI. Sectioning a cadaveric foot in the same plane as the MRI and comparing measurements is a logical next step.

The more radiolucent layer of the dorsal hoof wall (lateromedial view) and lateral and medial hoof walls (dorsopalmar view) may provide a more sensitive and accurate assessment of the symmetric and asymmetric distal displacement of the distal phalanx. The same is true for the layers of the sole, where lateral and medial thicknesses of the radiolucent layer were similar, but the thicknesses of the strata externum and medium were noticeably different between lateral and medial sides. Given that the keratinised sole thickness is even more likely to be affected by routine trimming than the dorsal hoof wall, assessment of the inner, more radiolucent layer may be more useful for assessing changes in disease states, particularly for horses with laminitis.

As the use of equine digital MRI becomes more common, reference values for normal hoof wall structures that are important in the assessment of laminitis are valuable. Our study provides extensive data regarding various parts of the hoof wall. Our dorsal wall thicknesses from MRI images are similar to a measurement made on the front feet of normal horses included in an MRI study of chronic laminitis [15]. The MRI data are a possible reference for clinical and research MRI studies of equine feet.
thickness of the hoof wall or sole is not known. This is a concern for the DR measurements, but should not be a factor in high-field MRI studies that are performed in recumbent, anaesthetised horses. Interestingly, our dorsal hoof wall thickness measurements were similar to measurements made on standing horses [10], indicating that weightbearing may not affect these measurements greatly.

In conclusion, we documented the following features: 1) normal hoof wall and sole measurements using DR and MRI; 2) the inner, more radiolucent layer of the dorsal hoof wall observed on DR is likely to correspond to the stratum lamellatum and stratum reticulare; 3) there is good correlation between observers in determining the thickness of this radiolucent layer; and 4) there is lower variability in thicknesses of this layer between lateral and medial measurements on the dorsopalmar view compared with the strata externum and medium, which may indicate that quantification of the thickness of this layer may be more reliable for assessment of early lamellar injury/damage than assessment of the entire hoof wall. For assessment of uniaxial distal displacement, comparison of the lateral/medial hoof wall thicknesses and sole dermis measurements may be more accurate than measurement of the entire sole thicknesses. Future studies are needed to perform the same quantifications presented in the present study on feet from clinical laminitis cases in order to quantify further the differences in measurements of the different layers in affected animals.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Our Institution's Animal Care and Use Committee approved the study. Owners gave informed consent for inclusion of their horses in the study.

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Authorship

I. N. M. Grundmann contributed to study design, data collection, data analysis and manuscript preparation. W. T. Drost, L. J. Zekas and J. K. Belknap contributed to study design, image analysis, data analysis and manuscript preparation. R. B. Garabed contributed to statistical data analysis. S. E. Weisbrode contributed to histopathology interpretation. A. H. Parks and J. Maierl contributed to data analysis. M. V. Knopp contributed to MRI data collection. All authors reviewed the manuscript.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary item 1: Schematic of a lateromedial (A) and dorsopalmar radiograph (B) of a front foot showing the thickness of various hoof and solar layers (in mm ± SD).

6 Discussion

Assessment of hoof wall and sole thickness using radiographs is critical in the clinical evaluation of laminitis, especially regarding displacement of the distal phalanx. We attempted to assess the majority of measurements currently being used in radiographic assessment of laminitis cases, including dorsal hoof wall, lateral and medial hoof wall, and sole thickness. *Additionally we attempted to establish new measurements involving the distal interphalangeal joint, which to our knowledge has not been described for possible evaluation of laminitis.*

Evaluation of the lateromedial radiographs for dorsal hoof wall and sole thickness at the toe is critical in symmetrical distal and rotational displacement of the distal phalanx, whereas evaluation of the dorsopalmar radiographs for lateral and medial hoof wall and sole thickness is critical for the detection of uniaxial/asymmetric distal displacement of the distal phalanx (CRIPPS and EUSTACE 1999, BELKNAP and PARKS 2011).

To establish and evaluate another method for assessment of uniaxial/asymmetric displacement of the distal phalanx (unilateral sinking), the distal interphalangeal joint space was measured in different locations.

Comparing hoof wall measurements in our cadaveric study to those obtained in live horses, the dorsal hoof wall thickness measurements we obtained using DR are slightly larger than those in one study (LINFORD et al. 1993) and similar to others studies (CRIPPS and EUSTACE 1999, KUMMER et al. 2006). All of these studies used clinically normal (sound) horses as their study population, whereas we included histologically normal feet. Our study did not include any Warmbloods, whereas a previous study used Warmbloods exclusively (KUMMER et al. 2006). To account for magnification and breed variation when assessing dorsal hoof wall thickness, a ratio of the dorsal hoof wall thickness to palmar cortex length is used (LINFORD et al. 1993). Our average value for this ratio is slightly greater than reported for analogue radiographs (LINFORD et al. 1993) but similar to studies using DR (KUMMER et al. 2006, ARBLE et al. 2009). *The reason for this discrepancy may be the fact that the dorsal surface of the dorsal hoof wall may be more difficult to depict on analogue radiographs, even when using a bright light to help delineating the dorsal margin. Displaying the dorsal margin of the dorsal hoof wall may be even more difficult on analogue images, if not impossible, when the images are overexposed. On DR images one is able to adjust the image brightness/contrast to more clearly display margins, even in mildly to moderately overexposed images.*

Sole thickness is an important measurement used by clinicians for assessment of the relationship of the distal phalanx and sole in laminitis cases (BELKNAP and PARKS 2008). Our sagittal sole thickness using DR was greater than a study where no radiographic marker was used on the sole surface (LINFORD et al. 1993) but similar to a study where the mid-sagittal aspect of the sole was marked with barium (KUMMER et al. 2006). Thus, although differences in study population may account for some of the variability between studies, the similarity between our study and the latter study probably indicates that a more accurate measurement can be obtained with barium on the sole.

As both lateral and medial hoof wall thickness and lateral and medial sole thickness have been used on the dorsopalmar view to assess for uniaxial/asymmetric distal displacement of the distal phalanx (KUMMER et al. 2006), both were assessed in the current study as well. Although there were minor differences in the individual dermal and epidermal thickness of the lateral and medial hoof wall on the dorsopalmar view, thickness of the entire lateral and medial hoof walls were close to identical in the digits assessed in this study. The same was not true for lateral and medial sole measurements. The lateral aspect of the sole was significantly thicker than the medial aspect owing to different thickness of the strata externum and medium; this disparity was noted on DR and MRI measurements. Interestingly, the thickness of the strata lamellatum and reticulare of the sole was not significantly different when comparing lateral with medial measurements using either modality. This may be due to a disparity in trimming of the lateral and medial sides. As this was a cadaveric study, force on the sole due to weight bearing was not present and should not be the cause of the lateral vs. medial differences. This suggests that differences between lateral and medial sole thickness (including dermal and epidermal components) should be used with caution when assessing the dorsopalmar DR images for uniaxial/asymmetric displacement, and changes in measurement of the dermis soleae may be combined with comparison of measurements of the entire lateral and medial hoof wall thickness and evaluation of the symmetry of the distal interphalangeal joint in making this determination.

The ability to obtain consistent measurements depends on accurate distinction of anatomic landmarks on high-quality radiographs. Three techniques used in this study can give clinicians more distinct visualization of external landmarks when using DR and MRI to obtain measurements; these are as follows:

1) adjustment of brightness and contrast instead of a radiographic marker to establish the dorsal aspect of the hoof wall on our DR images (SCHMIDT et al. 2008, van der STELT 2008);

2) placement of barium on the sole to delineate the ground surface of the sole from the distal hoof wall on our DR images; and

3) coating of the outer hoof wall with lard for our MR images because the strata externum and medium of the hoof wall is normally difficult to detect on MRI due to low water content (ARBLE et al. 2009).

In other studies, material was attached to the dorsal hoof wall to make its margin more visible (CRIPPS and EUSTACE 1999, KUMMER et al. 2006). We were able to see the dorsal hoof wall well by adjusting image brightness/contrast. *However, as mentioned above in the sole region barium application very well helped to delineate the margin of the sole and improved accuracy of measurements. The sole of the equine hoof does not show a plane* *surface but a concavity and undulations in the area of the frog. Additionally the horn of the sole is usually not as smooth in all areas as in the region of the hoof wall. Both can lead to superimposition on radiographs what makes exact calliper placement for measurements more difficult. Barium application to certain areas of the sole is a cost-efficient, safe and technically easy way to increase accuracy of evaluation and measurements in the sole region.*

We established values for distal interphalangeal joint measurements and showed good inter- and intraobserver agreement for these measurements. Assessment of the distal interphalangeal joint space has been described for the evaluation of degenerative joint disease and hoof imbalance but not for laminitis in the horse (BUTLER et al. 2008, DYSON 2003), However, to our knowledge reference values for distal interphalangeal joint space measurements have not been reported. While it is logical that loss of articular cartilage – a common feature in the course of osteoarthritis – would lead to partial or complete narrowing of the joint space and mediolateral imbalance of the hoof would lead to widening of the joint space on one side of the joint, we propose that in case of unilateral sinking the joint space should also become uneven. In cases where the lamellar injury is more severe on one side, leading to unilateral separation of the lamellae and sinking of the distal phalanx, one would expect the distal interphalangeal joint space to be wider on the same side due to two reasons (Figure 10):

(1) there is less attachment of the distal phalanx with the encasing hoof capsule which leads to less resistance to proximodistal pressure and thus less cartilage compression;

(2) the horse would most likely try to bear more weight on the less severely affected side which would lead to less cartilage compression on the other – more affected – side.

The phenomenon that horses with laminitis bare more weight on certain parts of the foot is well described in the literature (DYSON 2011, STASHAK 2002). The typical stance *described for horses with laminitis is a "saw-horse" stance with increased weight bearing on the heels to relieve pressure to the toe region. Although not described yet, unilateral increase of weight bearing to relieve the more affected side of the hoof in cases with unaxial sinking would be logical as well. However, there are potential factors objecting the latter theory that there would be consistently a wider joint space on the side of distal phalangeal sinking and a more narrow joint space on the less affected side. First of all a horse with unilateral sinking could develop an axial deviation in the distal extremity causing a narrower joint space on the sinking side. Additionally, the difficulty of placing both front feet on a wooden block in a straight, physiological manner avoiding a wide or narrow stance of the horse's feet, should not be underestimated. Especially diseased horses may be reluctant to certain positions as they avoid placing more weight on the affected limb. Even slight errors in obtaining an orthograde radiograph in a horse distributing its weight evenly on both feet and standing straight on both limbs, could result in artificial changes in joint space width. Obtaining radiographs in a painful and often weight-shifting laminitic patient represents a particular challenge in this regard (REDDEN et al. 2003). Any kind of axial deformity is another potential cause for differences in lateral/medial joint space width and therefore could possibly reverse the potential effects of unilateral sinking on the distal interphalangeal joint space width. Nevertheless, we believe that measuring distal interphalangeal joint space width and comparing lateral and medial joint space width deservers further attention as a potential method to evaluate pathology in the equine hoof. Future research evaluating radiographs of horses with uniaxial/asymmetrical displacement of the distal phalanx and ideally linking unilateral lamellar separation and width of the distal interphalangeal joint space would be needed to prove our theory. Certainly the reference values for joint space width presented in the current study could also be of value in the assessment of osteoarthritis in the distal interphalangeal joint. Comparing our lateral/medial distal interphalangeal joint width measurement showed an average difference of -1.67 mm at the junction of the condyle and the* *distal articular surface and a -0.57 mm at the most distal aspect of the distal articular surface of the middle phalanx. The lateral measurements were on average larger than the medial with both, the axial and abaxial measurement methods. The reason for the disparity is unknown. Interestingly the lateral aspect of the sole is significantly thicker than the medial, which we believe is due to disparities in trimming. A relation between sole thickness and distal interphalangeal joint width is not very likely since one would expect contrary results of sole thickness and distal interphalangeal joints space (smaller joint space on the side with thicker sole). Additionally as this was a cadaveric study weight bearing could not have played a role. However, it would be interesting if the same results and differences can be obtained in alive and weight bearing horses.*

The intra- and interobserver variability of both distal interphalangeal joint space measurements was overall good. However, the more axial measurement at the most distal aspect of the distal articular surface of the middle phalanx seems to be more reliable. For the abaxial measurement the distal reference point was sometimes abaxial to the articular surface of the distal phalanx and a horizontal line had to be drawn as a reference point. It is our impression that this may leave room for inaccuracy. This may also be reflected by the bigger average difference (1.67 mm) between lateral and medial when the more abaxial measurement is used compared to the difference (0.57 mm) when the more axial measurement is used.

The intra- and interobserver variability in measurements overall was very low for DR and MRI measurements and slightly better for the MRI measurements, suggesting that both imaging modalities are valuable for objective quantitative assessment of the equine hoof. The materials for delineating the hoof wall are readily available and are easy to apply. If one is applying barium to the sole margin, this should be done after imaging any radiographic view in which the barium would be superimposed on important foot anatomy.

In an attempt to determine the anatomical composition of the two layers detectable in the dorsal hoof wall, we hoped to make a comparison of DR with MR images. However, direct comparison of absolute values should not be made because of inherent differences in the modalities. While a single MR slice is essentially a 2-dimensional representation of a 2-dimensional structure, a DR image is a 2-dimensional representation of a 3-dimensional structure (i.e. summation of multiple MR 'slices'). Thus different abounds of hoof wall are represented by each modality. We used Bland-Altman plots to view the agreement between the measurements made using the two modalities. Measurements made of the dorsal hoof wall had good agreement, but the lateral and medial hoof wall and the lateral and medial sole thickness measurements were not as good, with the MR thickness measurements being smaller than those measured using DR. Given that the lateral and medial hoof wall and sole thickness on DR are measured on dorsal plane images, the curvature of the foot (narrower dorsally and wider palmarly) is likely to play a role in the differences. Digital radiographic images are a summation of the structures and MRI are a single 'slice' of the foot. Albeit not proven, we believe, that the outer, more radiopaque layer of the hoof wall on DR corresponded to the stratum externum and medium on MRI, whereas the inner more radiolucent layer on DR corresponded to the stratum lamellatum and stratum reticulare on MRI. Sectioning a cadaveric foot in the same plane as the MRI and comparing *additional* measurements is a logical next step. The more radiolucent layer of the dorsal hoof wall (lateromedial view) and lateral and medial hoof walls (dorsopalmar view) may provide a more sensitive and accurate assessment of the symmetric and asymmetric distal displacement of the distal phalanx. The same is true for the distinct layers of the sole, where lateral and medial thickness measurements of the radiolucent layer were similar, but the thickness measurements of the strata externum and medium were noticeably different between lateral and medial sides. Given that the keratinized sole thickness is even more likely to be affected by routine trimming than the dorsal hoof wall, assessment of the inner more radiolucent layer may be more useful for assessing changes in disease states, particularly for horses with laminitis. *Additionally the inner more lucent layer, composed of dermis and lamellae, is the layer primarily affected by laminitis. Radiographic changes in this layer may not only more specifically reflect pathology but perhaps allow for earlier detection of disease. As shown by ARBLE et al. (2009) pathology in the soft tissues of the hoof can be detected in MRI of horses at risk for laminitis even before clinical signs and radiographic changes occur. Future research should focus on the inner lucent layer of the hoof wall and –sole with the intention to detect pathologic radiographic changes in laminitis patients or horses at risk for laminitis as early as possible.*

As the use of equine digital MRI becomes more common, reference values for normal hoof wall structures that are important in the assessment of laminitis are valuable. Our study provides extensive data regarding various parts of the hoof wall. Our dorsal wall thickness measurements from MRI images are similar to a measurement made on the front feet of normal horses included in an MRI study of chronic laminitis (MURRAY et al. 2003). The MRI data are a possible reference for clinical and research MRI studies of equine feet. *The primary difference between DR and MR measurements was better tissue contrast resolution noted on MR images, which may allow for more precise positioning of calipers at the tissue interfaces when making measurements. This was important, because it allowed us to distinguish the tissue components of the two soft tissue layers (outer opaque and inner more lucent) of the hoof wall, which can be delineated on DR.*

The majority of dorsal hoof wall measurements made on anatomic specimens were similar to those made on MR images. However, there was less agreement when compared to those made on DR images. Comparison of sole measurements made on anatomic specimens to those made on MR showed mixed results. When sole measurements made on anatomic specimens were compared to those made on DR images, the majority was significantly different. The reasons for these results are unknown. One could argue that MR images rather *display the exact anatomy due to better tissue contrast resolution, allowing for more exact caliper placement and therefore show more agreement between measurements made on MR and anatomic specimens. However, clearly distinguishing the tissue components of the deep layer of the dermis and the lamellar/papilliform layer of the dermis as performed on MR images, was not possible on gross anatomic specimen. For this reason caliper placement on MR images should be more accurate and MR measurements seem to be a better reference to validate DR measurements than anatomic specimen. However, the accuracy of measurements made on anatomic specimen could have been enhanced by microscopic evaluation, accounting for possible tissue shrinking due to formalin fixation (DOCQUIER et al. 2010).*

The measurements made on DR were consistently about 2 mm greater than those made on MRI. This suggests that hoof wall and sole measurements used to discriminate laminitic from normal horses on DR may apply to MR measurements if one accounts for the 2 mm difference. The thickness difference between DR and MRI is similar to a study evaluating chronic laminitis on radiographs and MRI, where a difference of approximately 2 mm between imaging modalities was reported (MURRAY et al. 2003). Even though DR and MRI correlation was only fair to mild, the actual difference was less than 2 mm and therefore likely not clinically relevant. Additionally the above-mentioned differences between modalities may also play a role in the only fair to mild correlation.

A limitation of our study is that the influence of weight bearing on the thickness of the hoof wall or sole is not known. This is a concern for the DR measurements, but should not be a factor in high-field MRI studies that are performed in recumbent, anesthetized horses. Interestingly, our dorsal hoof wall thickness measurements were similar to measurements made on standing horses (LINFORD et al. 1993), indicating that weight bearing may not greatly affect these measurements (Davies et al. 2007).

In conclusion, we document the following features:

1) normal hoof wall and sole measurements using DR and MRI;

2) the inner, more radiolucent layer of the dorsal hoof wall observed on DR is likely to correspond to the stratum lamellatum and stratum reticulare;

3) there is good correlation between observers in determining the thickness of this radiolucent layer;

4) there is lower variability in thickness of this layer between lateral and medial measurements on the dorsopalmar view compared with the strata externum and medium, which may indicate that quantification of the thickness of this layer may be more reliable for assessment of early lamellar injury/damage than assessment of the entire hoof wall.

For assessment of uniaxial distal displacement, comparison of the lateral/medial hoof wall thickness and sole dermis measurements may be more accurate than measurement of the entire sole thickness. Future studies are needed to perform the same quantifications presented in the present study on feet from clinical laminitis cases in order to quantify further the differences in measurements of the different layers in affected animals.

7 Conclusion

In conclusion, we documented 1) normal hoof wall and sole measurements using DR, MRI *and measurements made on anatomic specimens*; 2) the inner less radiopaque soft-tissue layer of the dorsal hoof wall observed on DR corresponds to the Strata lamellatum et reticulare; 3) good correlation between observers in determining the thickness of this less radiopaque layer; 4) lower variability in thickness of this layer between lateral and medial measurements on the dorsopalmar view compared to the epidermal layer (Strata externum et medium) may indicate that quantification of the thickness of this layer may be more reliable in assessing early lamellar injury/damage than assessment of the entire hoof wall. *Further we established reference values for the width of the distal interphalangeal joint space.* For assessment of uniaxial distal displacement, comparison of the lateral/medial hoof wall thickness and sole dermis measurements may be more accurate than measurement of the entire sole thickness. *Additionally measuring the distal interphalangeal joint space may offer an alternative to evaluate for uniaxial displacement of the distal phalanx.* Future studies are needed to perform the same quantifications presented in the current study on feet from clinical laminitis cases to further quantify differences in the measurements of the different layers in affected animals. *Our measurements not only support established measurements, but also offer new approaches to quantitatively assess the epidermal and dermal anatomy of the equine foot and possible displacement of the distal phalanx.*

8 Summary

The equine foot is an anatomically complex structure in which lamellar attachments of the epidermis to the underlying dermis suspend the distal phalanx in the hoof capsule and therefore support the entire body weight. In equine laminitis, a sequela of multiple disorders, this complex lamellar apparatus undergoes injury commonly leading to separation of the dermal and epidermal lamellae. This disruption of the lamellae leads to several recognized patterns of displacement of the distal phalanx within the hoof capsule. These displacements of the distal phalanx can be detected on radiographs and evaluation of laminitis patients relies on radiographic measurements of the hoof. Reference values are not established for all layers of the foot.

The objective of the study was to establish normal hoof wall and sole measurements using digital radiography (DR) and magnetic resonance imaging (MRI), and to document tissue components present in the dorsal hoof wall and sole layers seen on DR.

Digital radiography and MRI were performed on 50 cadaver front feet from 25 horses euthanized for non-lameness reasons. Four observers measured hoof wall (dorsal, lateral and medial) and sole thickness (sagittal, lateral and medial) using DR and MR images as well as gross anatomic specimens. Inter- and intraobserver correlation was assessed.

Digital radiography and MRI measurements for the normal hoof wall and sole were established. Inter- and intraobserver correlation for DR (>0.98) and MRI (>0.99) measurements was excellent. Based on MRI, the less radiopaque layer on DR is comprised of the Stratum lamellatum and Stratum reticulare.

This less radiopaque layer of the dorsal hoof capsule corresponds to the critical tissues injured in laminitis, the Strata lamellatum et reticulare. Additionally trimming of the hoof does not alter this layer. Therefore measuring this layer may present a more reliable marker for pathologic changes in laminitis cases than measurements of the entire hoof capsule including the outer epidermal layer that is influenced by trimming.

Distal interphalangeal joint space measurements were established and may offer an alternative approach to evaluate for uniaxial displacement of the distal phalanx. Further these reference values could be of value in the assessment of osteoarthritis of the distal interphalangeal joint. Our measurements not only support established measurements, but also offer new approaches to quantitatively assess the anatomy of the equine foot.

Our reference measurements may be used by the clinician to detect soft tissue changes in the laminitic hoof, and provide a foundation for future research determining changes in these measurements in horses with laminitis.

9 Zusammenfassung

Titel: Quantitative Untersuchungen des Pferdehufes mit Hilfe digitaler Radiographie und Magnetresonanztomographie.

Der Pferdehuf ist eine anatomisch komplexe Struktur in der das Hufbein mittels lamellelärer Verbindungen zwischen dem Epithel und der darunterliegenden Dermis in der Hufkaspel aufgehängt ist. Somit trägt diese lamelläre Verbindung das komplette Körpergewicht und wird auch als Hufbeinträger bezeichnet.

Bei der Hufrehe (engl.: laminitis), einer Folgeerkrankung verschiedener Krankheiten des Pferdes, kommt es zur Schädigung dieses komplexen Hufbeinträgers was zur Separation der dermalen und epidermalen Lamellen führt. Diese Separation resultiert in verschiedenen Formen von Dislokation des Hufbeins in der Hufkapsel. Diese Dislokationen können radiologisch detektiert werden und spezielle Messungen auf den Röntgenbildern dienen der Evaluierung von Patienten mit Hufrehe. Während mehrere Messungen und Referenzwerte beschrieben sind, ist dies nicht für alle Schichten des Hufes der Fall.

Das Ziel der vorliegenden Arbeit war es Referenzwerte für unterschiedliche Messungen an der Hufwand und der Sohle auf digitalen Röntgenbildern als auch auf magnetresonanztomographischen Bildern zu etablieren. Weiter sollten die verschiedenen Schichten der Hufwand und –sohle dokumentiert werden, die sich auf digitalen Röntgenbildern unterscheiden lassen.

Digitale Röntgenaufnahmen und magnetresonanztomographische Aufnahmen beider Vorderfüße von 25 Pferden, welche zuvor aus einem anderen Grund als Lahmheit euthanasiert worden sind, wurden angefertigt.

Die Schichten der Hufwand (dorsal, lateral und medial) und Hufsohle (sagittal, lateral und medial) wurden von vier Untersuchern unabhängig sowohl auf den digitalen

Röntgenaufnahmen als auch den magnetresonanztomographischen Aufnahmen und am anatomischen Präparat gemessen. Die Korrelation zwischen den verschiedenen Untersuchern (interobserver correlation) als auch die Korrelation zwischen den verschiedenen Untersuchungen der einzelnen Untersucher (intraobserver correlation) wurde evaluiert. Referenzwerte für Hufwand und –sohlenmessungen auf normalen Röntgenbildern und magnetresonanztomographischen Aufnahmen von normalen Hufen wurden etabliert. Die Korrelation zwischen den Untersuchern und den Untersuchungen der digitalen Röntgenbildern (>0,98) und magnetresonanztomographischen Bildern (>0,99) war hervorragend. Basierend auf den magnetresonanztomographischen Bildern konnte bestätigt werden, dass die auf digitalen Röntgenbildern erkennbare innere, weniger röntgendichte Schicht die tiefe/sublamelläre Dermis und die dermalen und epidermalen Lamellen darstellt. Diese Schicht korrespondiert somit mit den Strukturen, die im Fall der Hufrehe im Zentrum des Krankheitsgeschehens stehen. Außerdem sind diese Schichten, anders als die epidermalen äußeren Schichten des Hufes nicht von hufbearbeitenden Maßnahmen wie Ausschneiden oder Raspeln betroffen. Damit stellen Messungen dieser inneren Schichten wahrscheinlich zuverlässigere Parameter für pathologische Veränderungen bei Hufrehe da als die Messungen der äußeren, epidermalen Schichten bzw. der gesamten Hufwand.

Zusätzlich wurden Referenzwerte für die Weite des Hufgelenkspalts an verschiedenen definierten Lokalisationen des Gelenks etabliert. Diese Werte könnten nicht nur eine Rolle bei der Untersuchung von an Hufrehe erkrankten Pferden, sondern auch im Falle degenerativer Veränderungen des Hufgelenks spielen.

Unsere Referenzwerte können von Tierärzten benutzt werden um Hinweise auf Weichteilveränderungen im Zusammenhang mit Hufrehe zu bekommen und können eine Basis für weitere Forschung auf diesem Gebiet darstellen.

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11 List of tables

Table 1. Magnetic resonance imaging sequence parameters

Abbreviations: FOV = field of view; PD-w TSE = proton density-weighted turbo spin echo; TE = time to echo; TR = time to repetition; and 3DGRE = three-dimensional gradient recall echo;

	FOV (mm)	Voxel (mm)	TR (ms)	TE (ms)	Thickness/Gap (mm)	Flip angle (degree)
PD –w TSE	100 x 180	0.5 x 0.5	6187	30	2/0	-
T2* 3DGRE	100 x 180	0.4 x 0.4	16	4.7	2/0	25

Table 2. Measurements of 50 cadaver feet in images obtained with digital radiography (DR), magnetic resonance imaging (MRI) *and from anatomic specimens (Anat).*

Bland-Altman (DR vs. MR): [a]no difference, with a narrow confidence interval centered around zero; [b]narrow confidence interval, but the MR values are less than the DR values; and [c]means are not significantly different from zero, but variation increases as thickness increases. Anatomical terms in the left column are more descriptive and correlate to the Nomina Anatomica Veterinaria terms used in the manuscript as follows: hoof wall thickness = strata externum, medium, lamellatum et reticulare; epidermal thickness = strata externum et medium; dermal thickness = strata lamellatum et reticulare; lamellar thickness = stratum lamellare; deep dermal thickness = stratum reticulare

Pasing & Bablock and Bland-Altman (Anat vs DR/MR): [d]narrow confidence interval centered around 1; [e]narrow confidence interval; [f]significant P-value (>0.005)

	DR (mm)		**MRI (mm)**		***Anat (mm)***		**Figure legend**	
	mean	SD	mean	SD	*mean*	*SD*	Fig. 1	Fig. 3
Proximal dorsal hoof wall thickness[a,d]	18.3	1.6	16.9	1.3	*16.0*	*1.6*	R1	M15
Proximal dorsal epidermal thickness[a,d,f]	10.7	1,3	10.2	1.1	*10.0*	*1.4*	R2	M16
proximal dorsal dermal thickness[a,d,e,f]	7.6	1.0	6.7	0.9	*6.0*	*1.3*		
proximal dorsal lamellar thickness	-	-	3.7	0.7				
proximal dorsal deep dermal thickness	-	-	3.0	0.7				
Distal dorsal hoof wall thickness[a,d]	17.9	1.8	16.8	1.6	*16.2*	*1.6*		
Distal dorsal epidermal thickness[a,d,f]	10.7	1.2	10.1	1.0	*10.1*	*1.1*	R3	M17
Distal dorsal dermal thickness[a,d,f]	7.3	1.3	6.8	1.3	*6.0*	*1.4*	R4	M18
Distal dorsal lamellar thickness	-	-	3.9	1.0	-	-		
Distal dorsal deep dermal thickness	-	-	2.8	0.7	-	-		
Lateral hoof wall thickness[b]	19.3	2.7	13.5	1.9	-	-		
Lateral epidermal thickness[a]	10.1	1.4	7.9	1.1	-	-	R5	M19
Lateral dermal thickness[b]	9.1	1.9	5.5	1.4	-	-	R6	M20
Lateral lamellar thickness	-	-	2.7	0.8	-	-		
Lateral deep dermal thickness	-	-	2.8	1.1	-	-		
Medial hoof wall thickness[b]	19.3	2.3	13.5	1.7	-	-		
Medial epidermal thickness[a]	9.9	1.5	7.7	1.0	-	-	R7	M21
Medial dermal thickness[b]	9.3	1.8	5.8	1.6	-	-	R8	M22

	DR (mm)		**MRI (mm)**		***Anat (mm)***		**Figure legend**	
	mean	SD	mean	SD	*mean*	*SD*	Fig. 1	Fig. 3
Medial lamellar thickness	-	-	2.8	0.9	-	-		
Medial deep dermal thickness	-	-	3.0	1.1	-	-		
Sagittal sole thickness[a,e,f]	13.7	2.4	12.3	2.3	*10.7*	*3.7*		
Sagittal epidermal sole thickness[a,d,f]	8.1	2.1	7.7	2.1	*6.3*	*3.3*	R9	M23
Sagittal dermal sole thickness[a,d,f]	5.6	1.0	4.6	0.8	*4.3*	*1.3*	R10	M24
Sagittal papillar sole thickness	-	-	1.4	0.6	-	-		
Sagittal deep dermal sole thickness	-	-	3.3	0.8	-	-		
Lateral sole thickness[c,d]	20.7	4.4	18.7	4.7	*14.9*	*5.6*		
Lateral epidermal sole thickness[d,e,f]	13.2	4.3	12.5	4.6	*9.5*	*3.4*	R11	M25
Lateral dermal sole thickness[a]	7.5	1.7	6.2	1.3	*5.3*	*3.7*	R12	M26
Lateral papillar sole thickness	-	-	1.8	0.6	-	-		
Lateral deep dermal sole thickness	-	-	4.4	1.2	-	-		
Medial sole thickness[c,d]	18.9	4.2	17.4	4.4	*13.0*	*4.3*		
Medial epidermal sole thickness[c,d]	11.5	3.9	11.3	4.3	*8.5*	*3.5*	R13	M27
Medial dermal sole thickness[a,d,f]	7.4	1.4	6.1	1.1	*4.7*	*2.5*	R14	M28
Medial papillar sole thickness	-	-	1.9	0.8	-	-		
Medial deep dermal sole thickness	-	-	4.2	1.0	-	-		
Founder distance	6.9	2.4	-	-	-	-		

Ratios	**DR**	**DR**	**MR**		***Anat***	
	Mean (%)	SD	Mean (%)	SD	*Mean (%)*	*SD*
Ratio of dorsal hoof wall to palmar cortex	26.8	2.6	28.8	0.04	*25.9*	*3.3*
Ratio of dorsal dermis to palmar cortex	10.7	1.8	11.6	0.04	*9.6*	*2.3*
Ratio of sagittal sole to palmar cortex	20.2	5.3	21.1	0.04	*17.3*	*6.6*
Ratio of sagittal sole dermis to palmar cortex	9.1	7.4	8.0	0.04	*6.8*	*2.0*

Table 3. Comparison of lateral and medial aspects of the hoof wall and sole measurements from 50 cadaver feet in images obtained with digital radiography and magnetic resonance imaging

*P<0.008 considered significant to adjust for multiple comparisons.

	Measurements	**Average Difference (mm)**	**P value***
Digital Radiography			
	Lateral vs. Medial hoof wall epidermal	0.2	0.05
	Lateral vs. Medial hoof wall dermal	-0.2	0.03
	Solar lateral vs. Solar medial	1.9	0
	Solar lateral epidermal vs. Solar medial epidermal	1.8	0
	Solar lateral dermal vs. Solar medial dermal	0.1	0.5
Magnetic resonance imaging	Lateral vs. Medial hoof wall epidermal	0.2	0.02
	Lateral vs. Medial hoof wall dermal	-0.3	0
	Solar lateral vs. Solar medial	1.2	0
	Solar lateral epidermal vs. Solar medial epidermal	1.1	0
	Solar lateral dermal vs. Solar medial dermal	0	0.7

Table 4. *Measurements of the width of the distal interphalangeal joint of 50 cadaver feet in images obtained with digital radiography (DR)*

	DR (mm)		***Figure legend***
Distal interphalangeal joint width	*mean*	*SD*	*Fig. 9*
at lateral junction of condyle and distal articular surface	*6.2*	*1.7*	*R15*
at medial junction of condyle and distal articular surface	*6.1*	*1.9*	*R17*
at lateral aspect of condyle at most distal aspect of distal articular surface of middle phalanx	*4.7*	*1.5*	*R16*
at medial aspect of the condyle at most distal aspect of distal articular surface of middle phalanx	*4.4*	*1.3*	*R18*

Table 5. *Comparison of lateral and medial distal interphalangeal joint measurements from 50 cadaver feet in dorsopalmar images obtained with digital radiography*

**P<0.008 considered significant to adjust for multiple comparisons.*

Measurements	***Average Difference (mm)***	***P value****	***DR (lat/med in mm)***	
			mean	*SD*
Lateral vs. Medial junction of condyle and distal articular surface	*-1.67*	*0.12*	*6.1/4.4*	*0.7/0.4*
Lateral vs. Medial aspect of condyle at most distal aspect of distal articular surface of middle phalanx	*-0.57*	*0.18*	*4.6/4.1*	*0.3/0.2*

12 List of figures

Figure 1. Lateromedial (a) and horizontal dorsopalmar radiographs (b) of a front hoof with barium on the sole showing the measurement sites. See Table 2 for key to the labels.

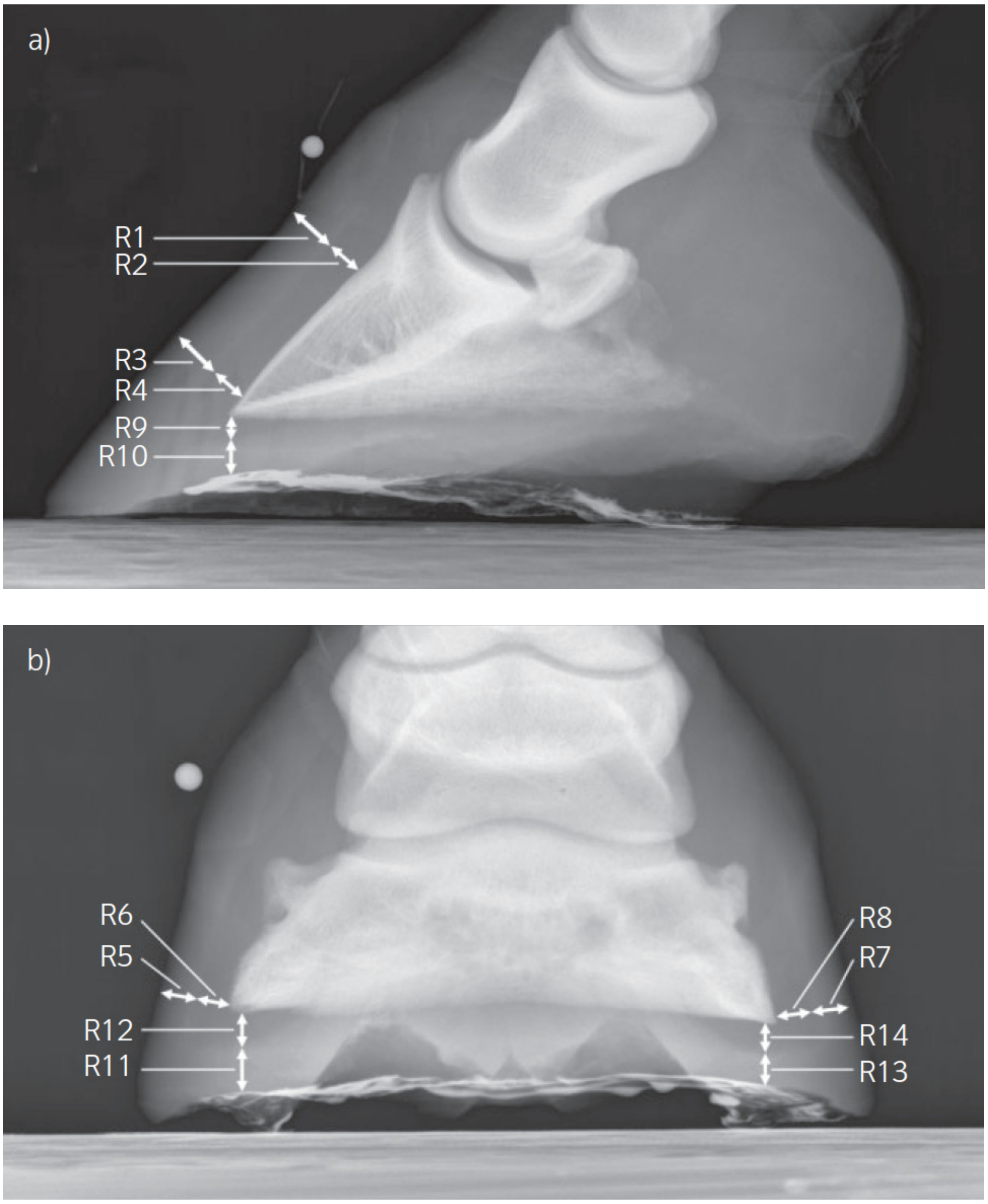


Figure 2. Proton density-weighted turbo spin echo magnetic resonance dorsal (a) and mid-sagittal images (b) of a front foot with lard on the hoof wall, showing the location of the slices used to make the measurements. The dotted line on the dorsal image (a) shows the location of the mid-sagittal slice, while the dotted line on the mid-sagittal image (b) shows the location of the dorsal slice.

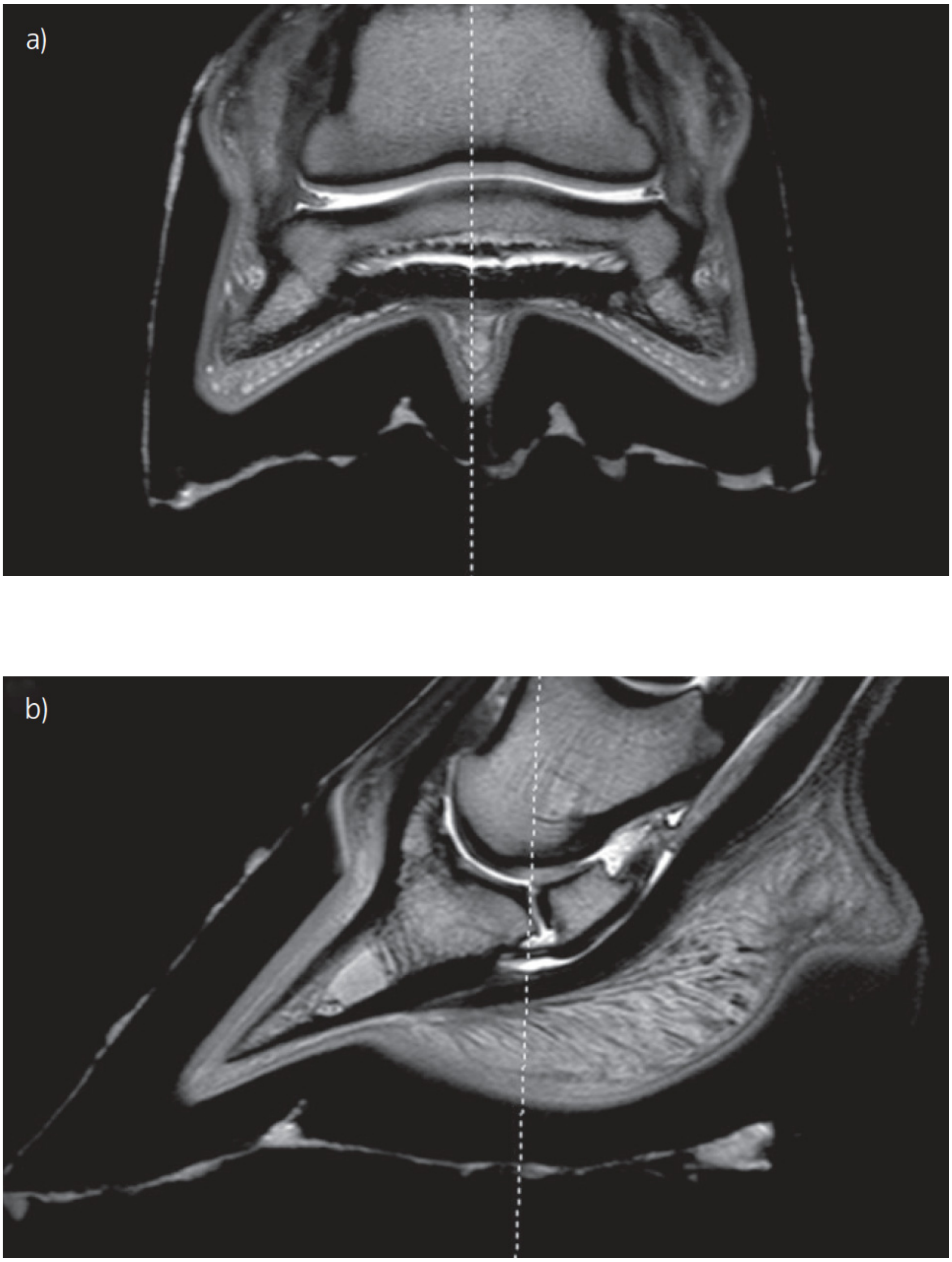
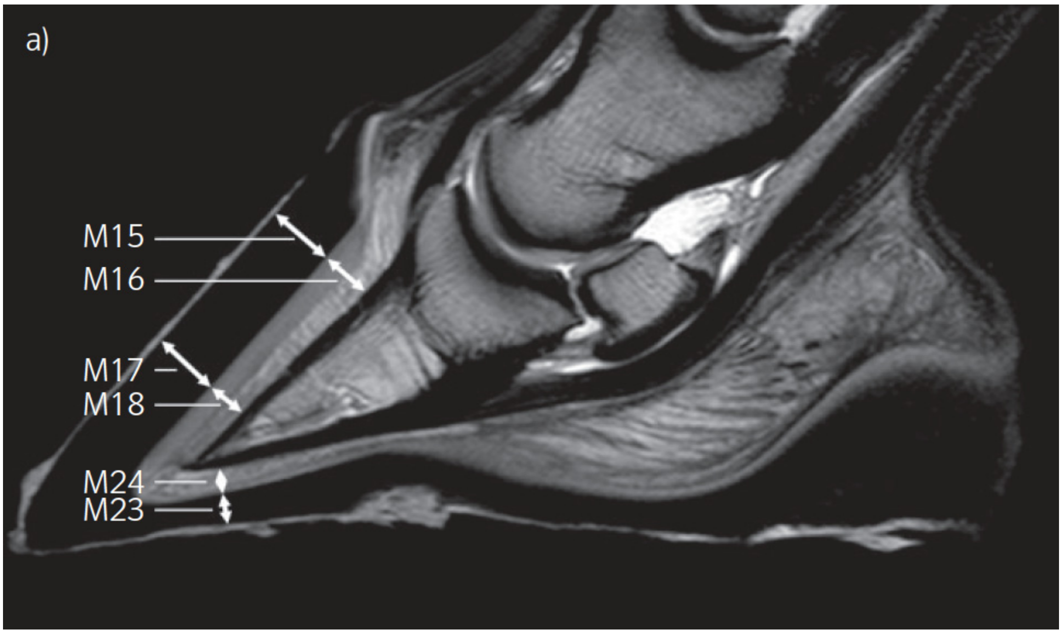


Figure 3. Proton density-weighted turbo spin echo magnetic resonance mid-sagittal (a) and dorsal images (b) of a front hoof with lard on the hoof wall, showing the same measurement sites that were made using radiographs. See Table 2 for the key to the labels. The dermis parietis was further divided into the stratum lamellatum and stratum reticulare (not shown).



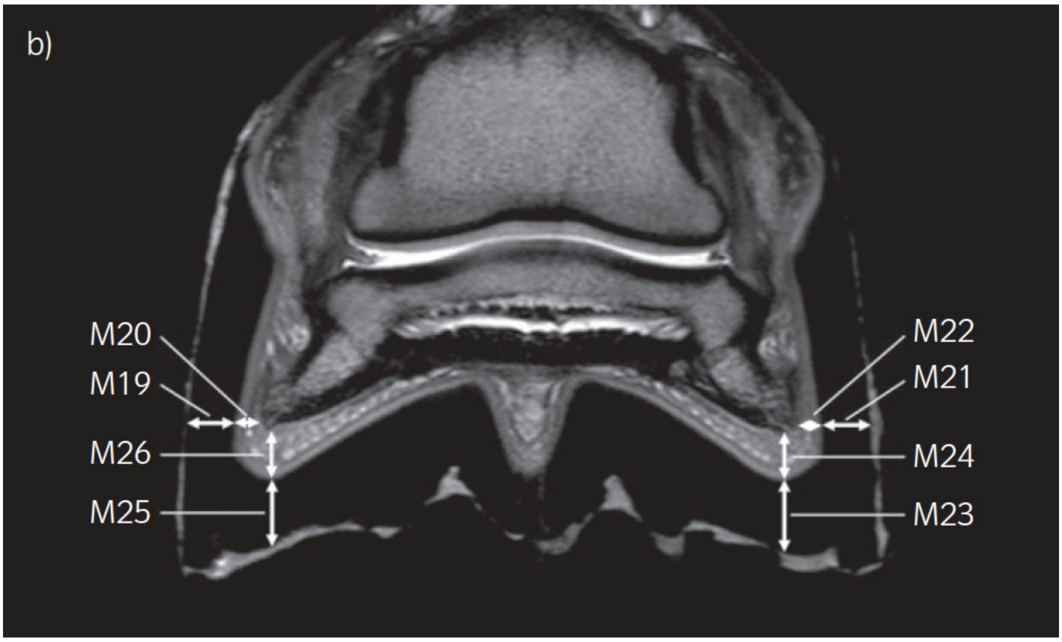


Figure 4. *Illustration of the anatomy of the hoof capsule - proton density-weighted turbo spin echo MR image of a horse's front foot covered in a thin layer of lard. Dorsal is the top of the image.*

hoof wall thickness = strata externum (illustrated only), medium, lamellatum et reticulare; epidermal thickness = strata externum et medium; dermal thickness = strata lamellatum et reticulare; lamellar thickness = stratum lamellatum; deep dermal thickness = stratum reticulare

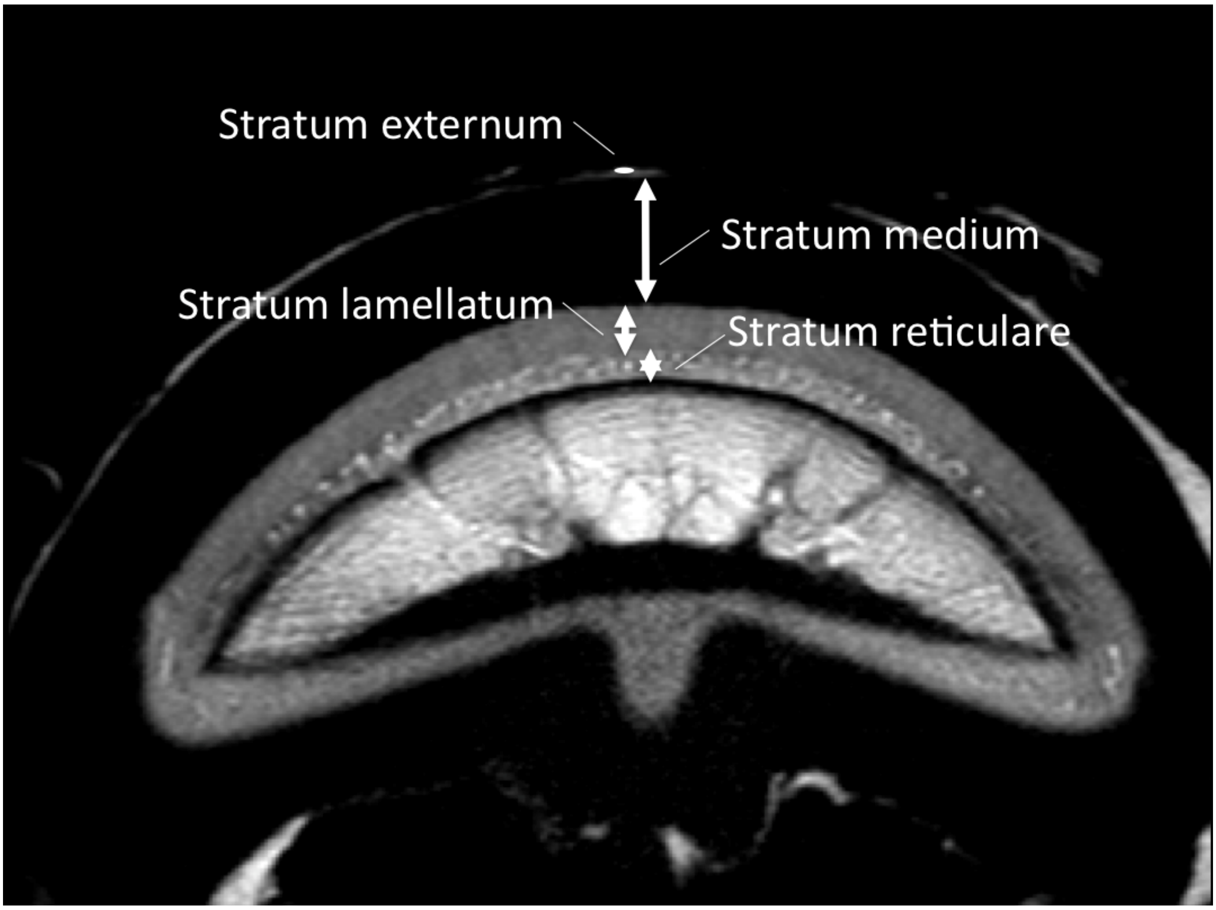


Figure 5. *Mediolateral radiograph of the left front foot of a laminitic horse illustrating rotation and sinking of the distal phalanx: solid curved arrow = rotation of the distal phalanx, solid straight arrow = traction of the deep digital flexor tendon, open arrow = sinking of the distal phalanx, dashed line = malalignement with hyperflexion of the middle and distal phalangeal axis*

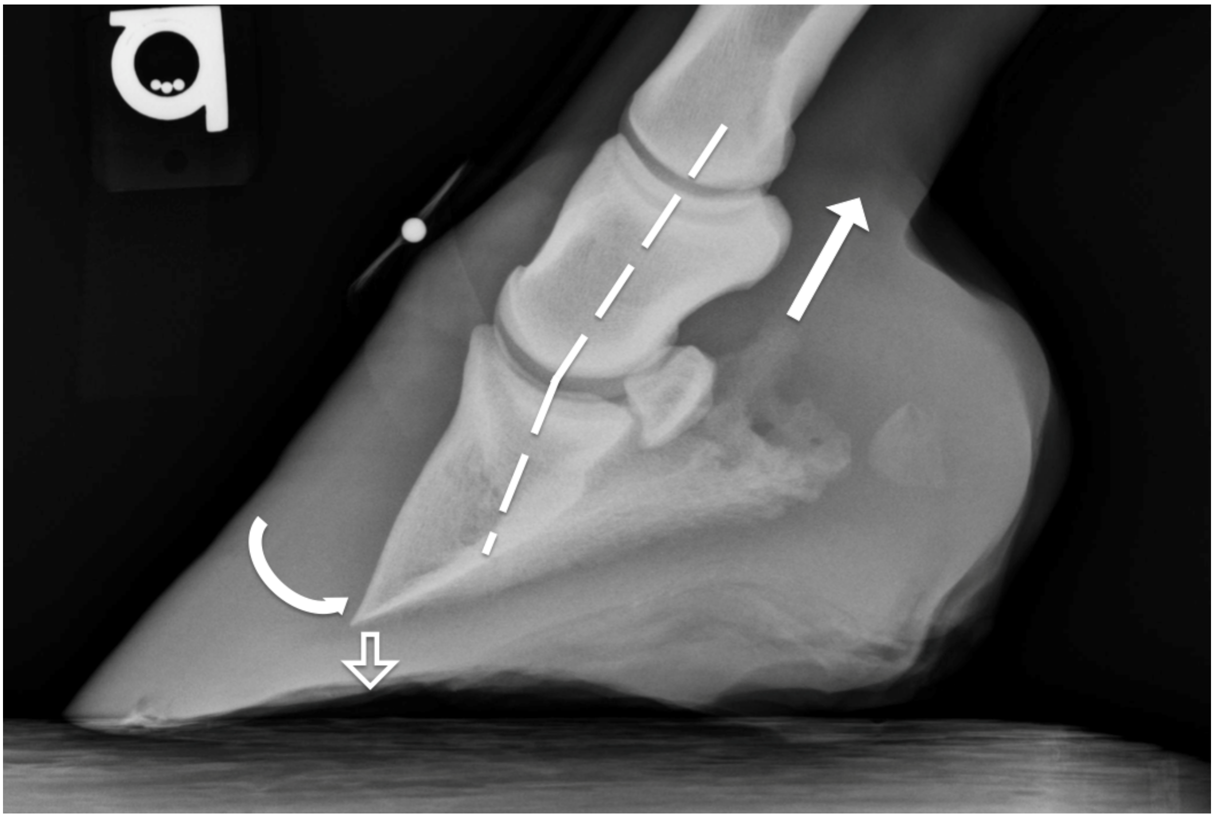


Figure 6. *Illustration of a positive radiographic marker (barium) on the surface of the sole.*

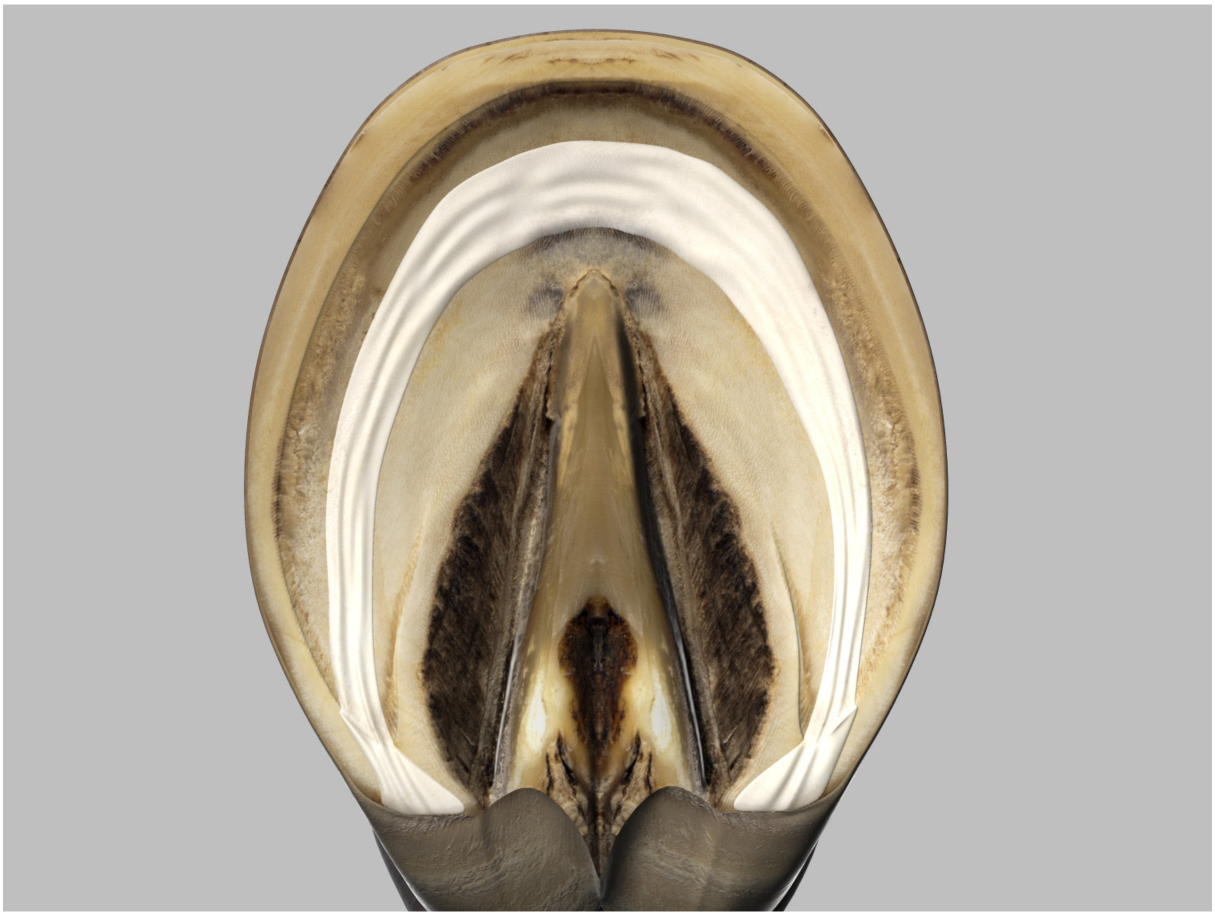
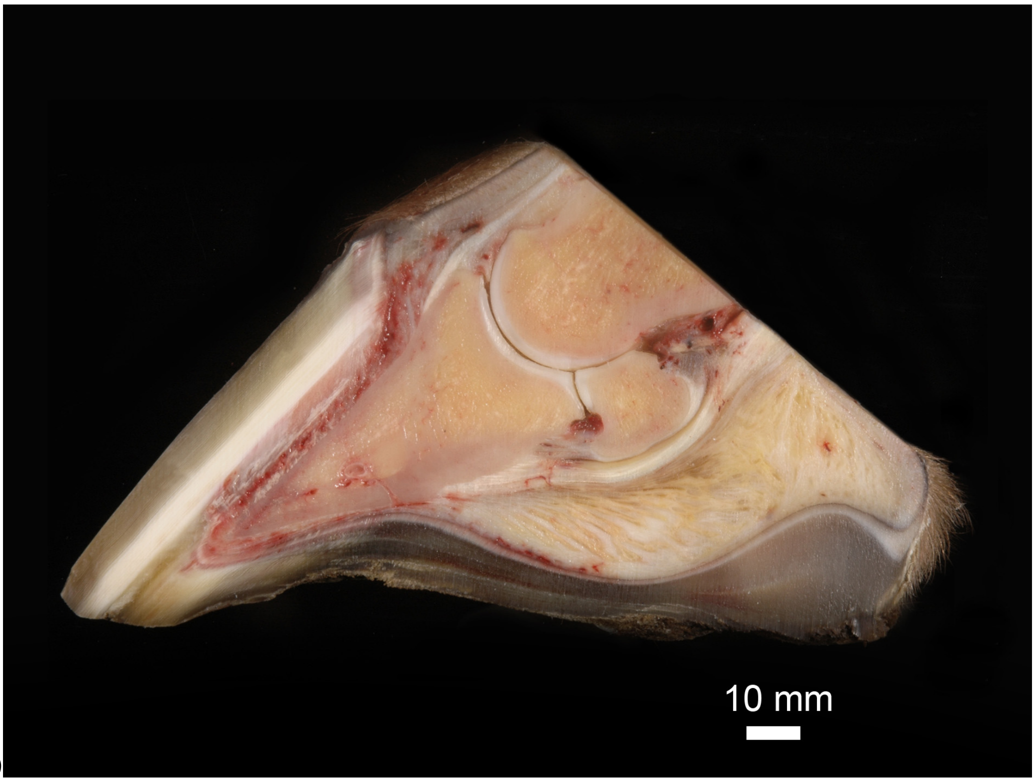
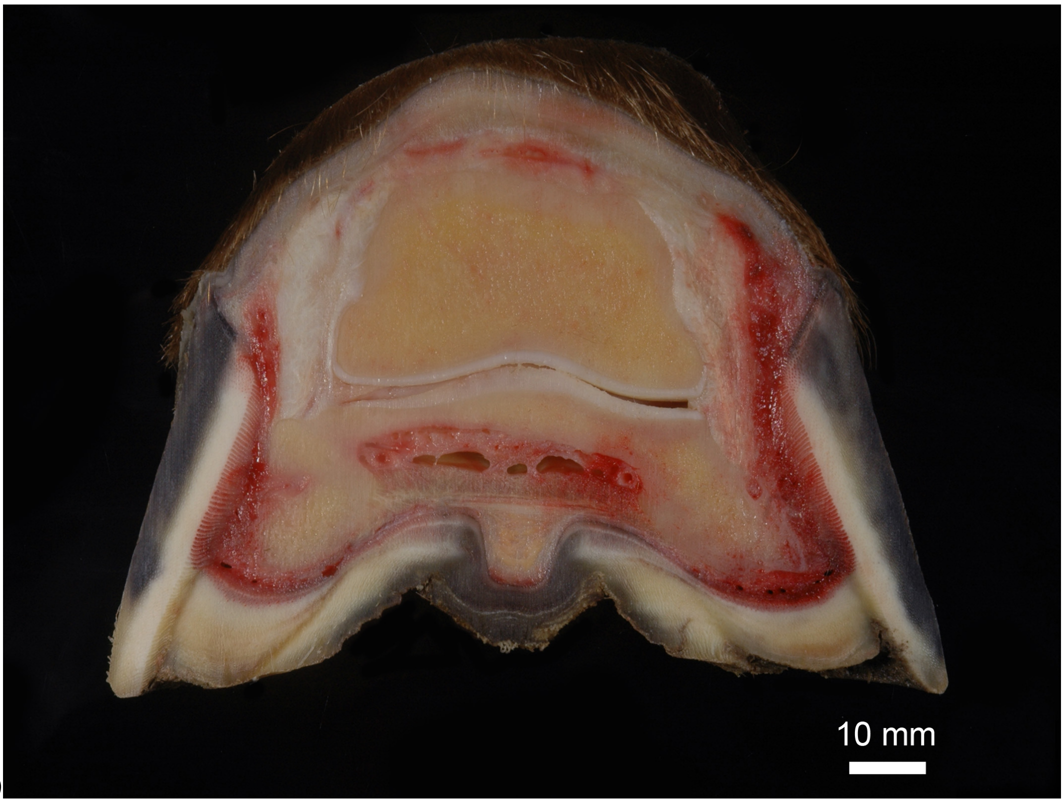


Figure 7. *The Philips SENSE Knee-8 Coil, Achieva, 8 channel, receive-only*

Figure 8. *Post-mortem sagittal (a) and dorsal (b) photographic image of a front foot showing the gross anatomy.*



a)



b)

Figure 9. *Horizontal dorsopalmar radiograph of a front hoof with barium on the sole showing the measurement sites of the distal interphalangeal joint width. R15 = proximodistal width at the deflection point of the lateral epicondyle and the distal articular surface of the middle phalanx to the articular surface of the distal phalanx; R16 = proximodistal width at the most distal aspect of the distal articular surface of the lateral aspect of the middle phalanx to the articular surface of the distal phalanx; R17 = proximodistal width at the deflection point of the medial epicondyle and the distal articular surface of the middle phalanx to the articular surface of the distal phalanx; R18 = proximodistal width at the most distal aspect of the distal articular surface of the medial aspect of the middle phalanx to the articular surface of the distal phalanx;*

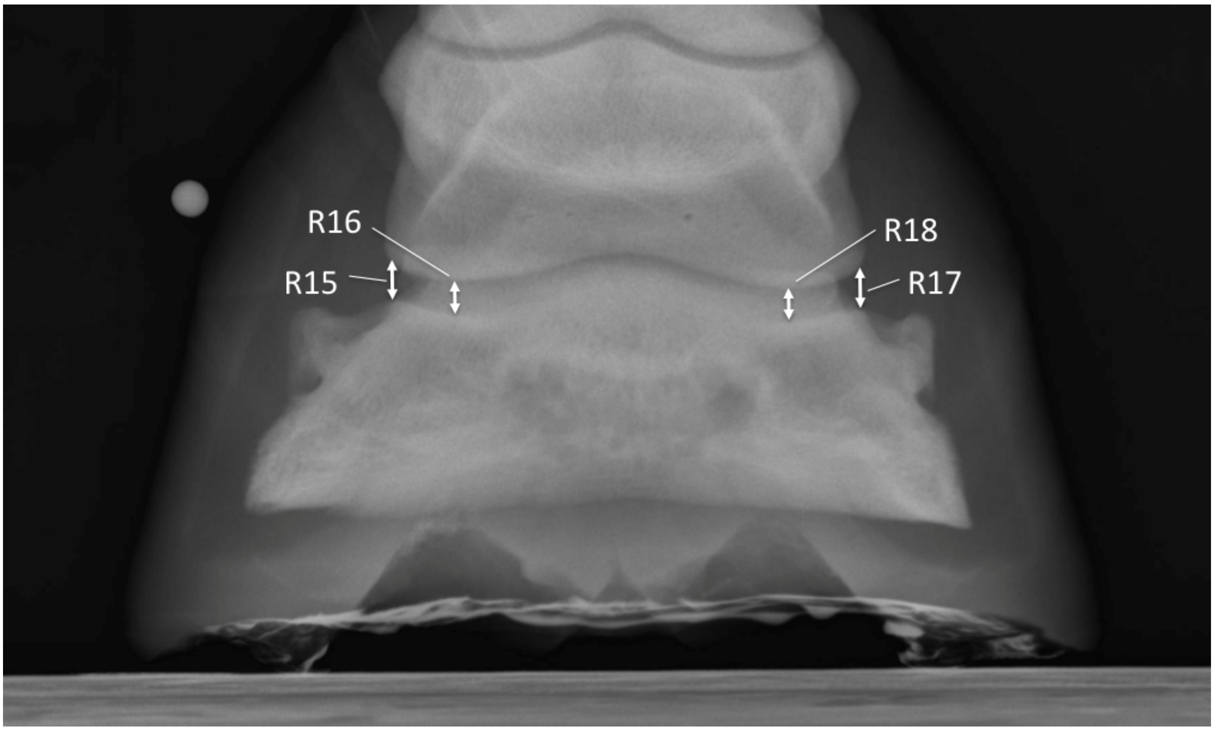
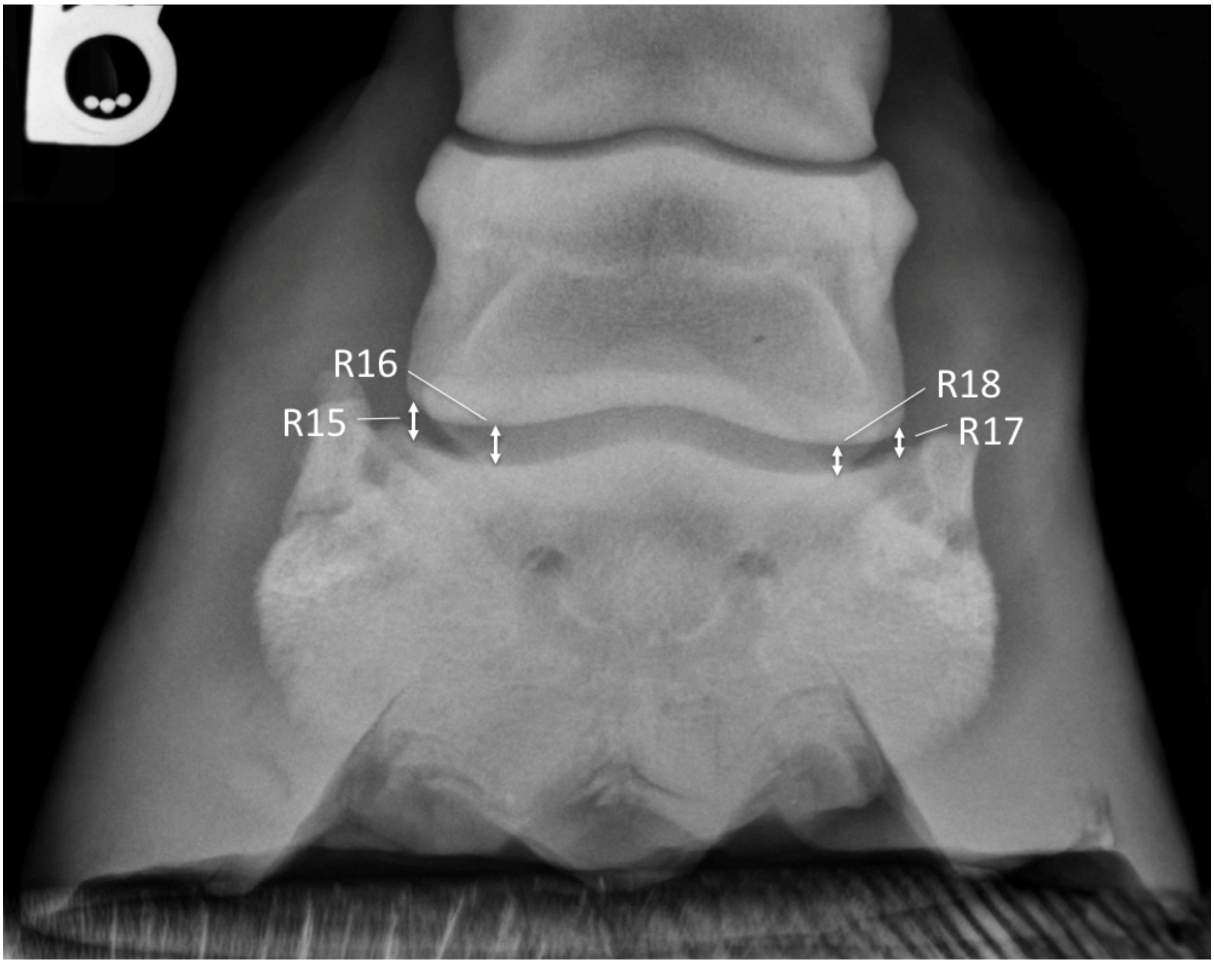
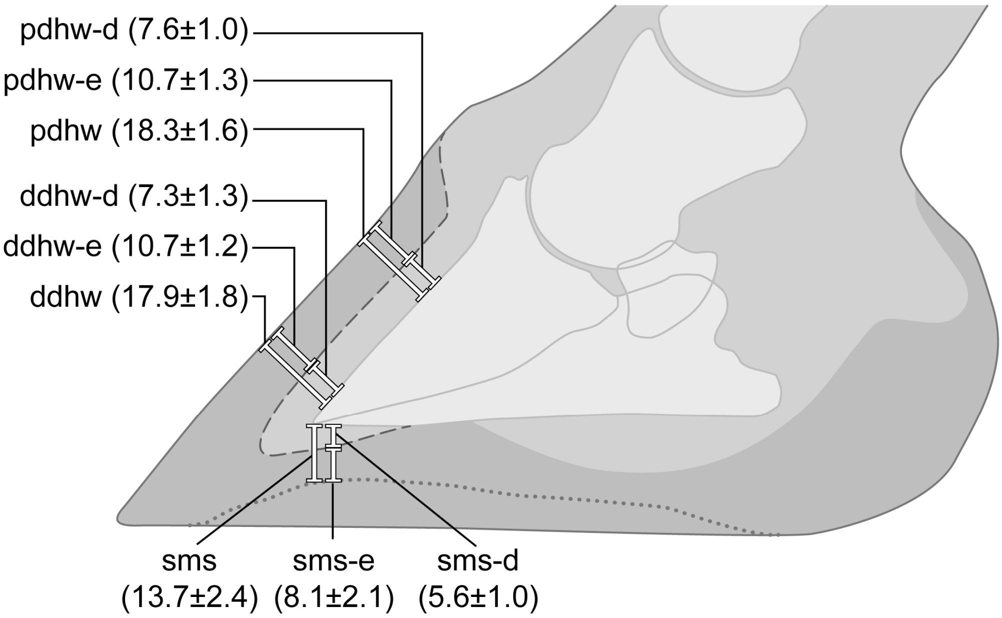
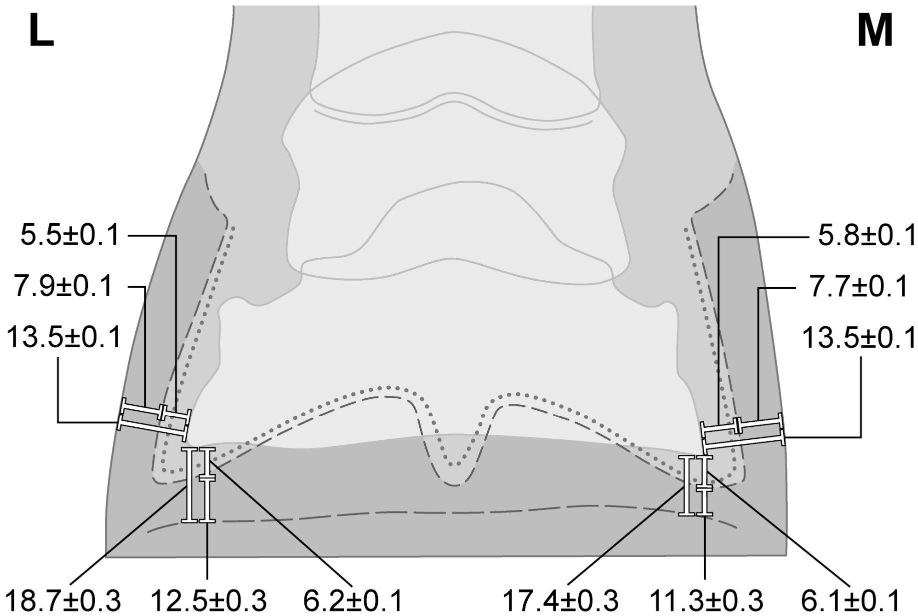


Figure 10. *Horizontal dorsopalmar radiograph of the same left front foot of the laminitic horse in Figure 5 illustrating uniaxial, medial sinking and a wide stance with narrowing of the medial distal interphalangeal joint space with narrower measurements of R17 and R18 in comparison to R15 and 16*



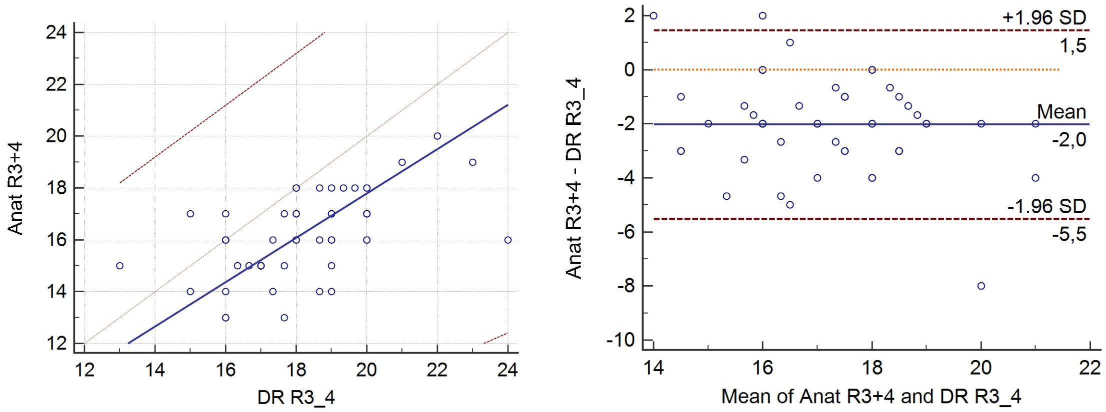
Supplementary item 1. Schematic of a lateromedial (a) and dorsopalmar radiograph (b) of a front foot showing the thickness of various hoof and sole layers (in mm ± SD).



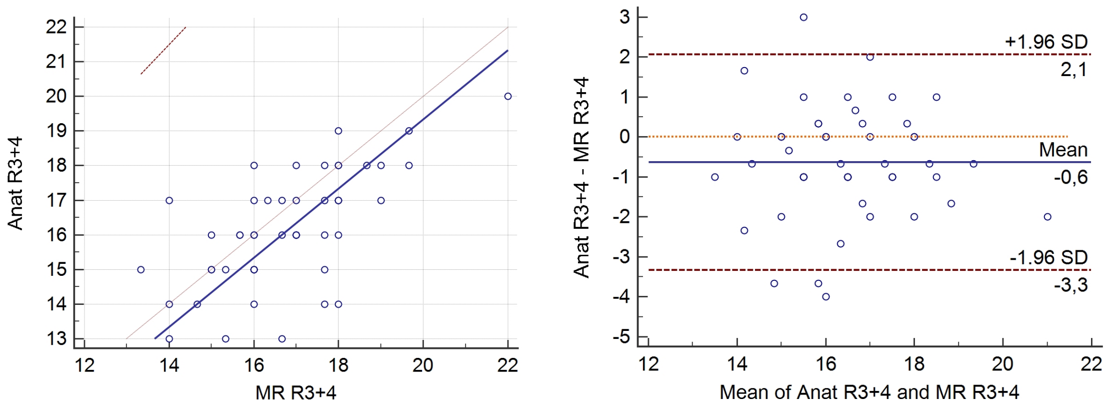


13 *List of graphs*

Graph 1. *Regression graph and Bland-Altman Plot from the distal dorsal hoof wall measurement: a) Anatomic vs. DR measurement; b) Anatomic vs. MR measurement*

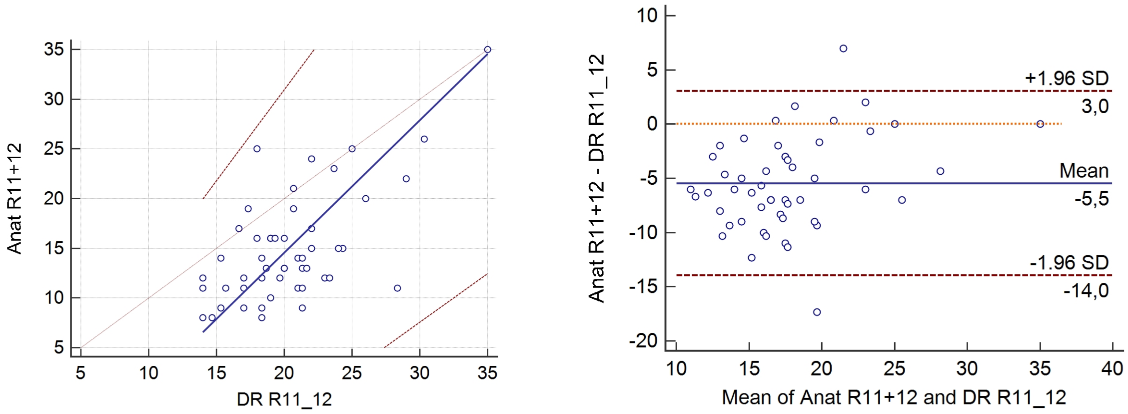


a)

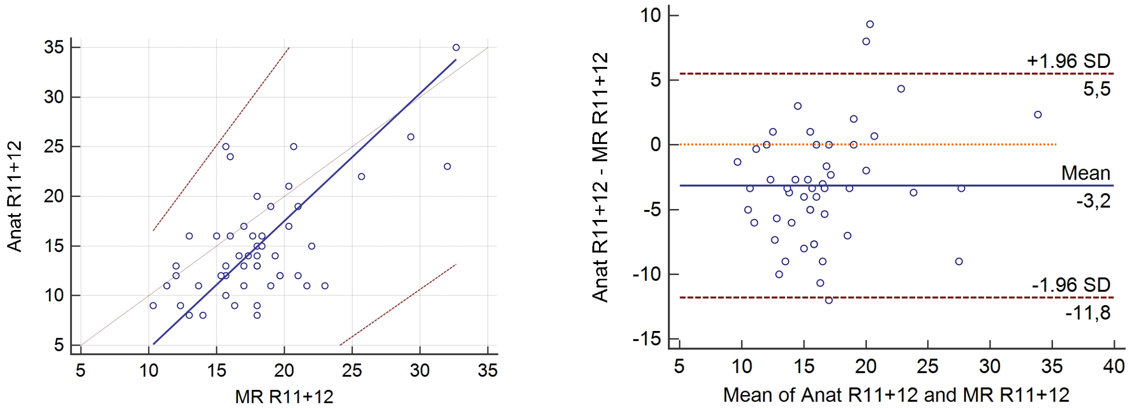


b)

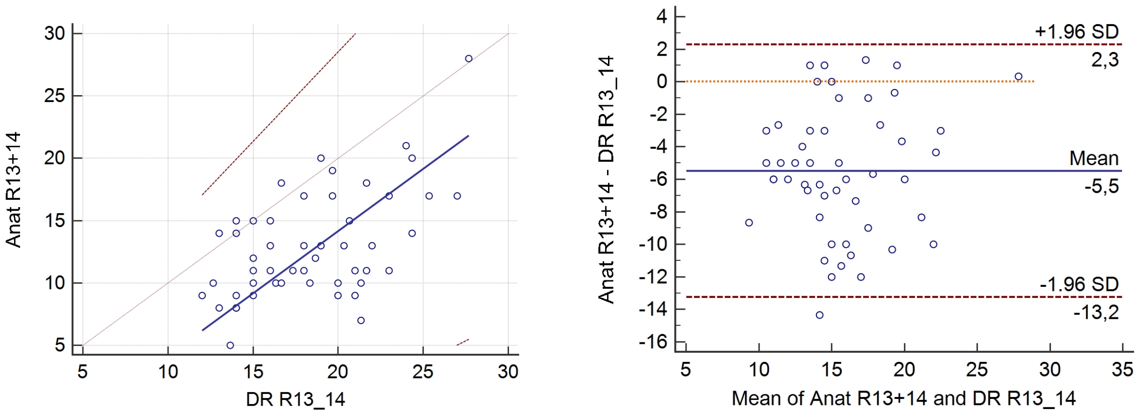
Graph 2. *Regression graph and Bland-Altman Plot from the lateral and medial sole measurements: a) Anatomic vs. DR lateral sole measurement; b) Anatomic vs. MR lateral sole measurement; c) Anatomic vs. DR medial sole measurement; d) Anatomic vs. MR medial sole measurement*



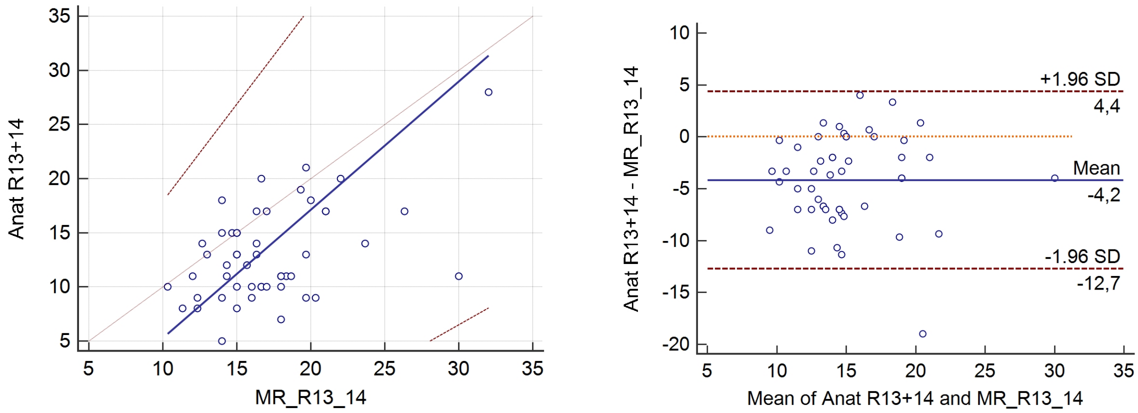
a)



b)

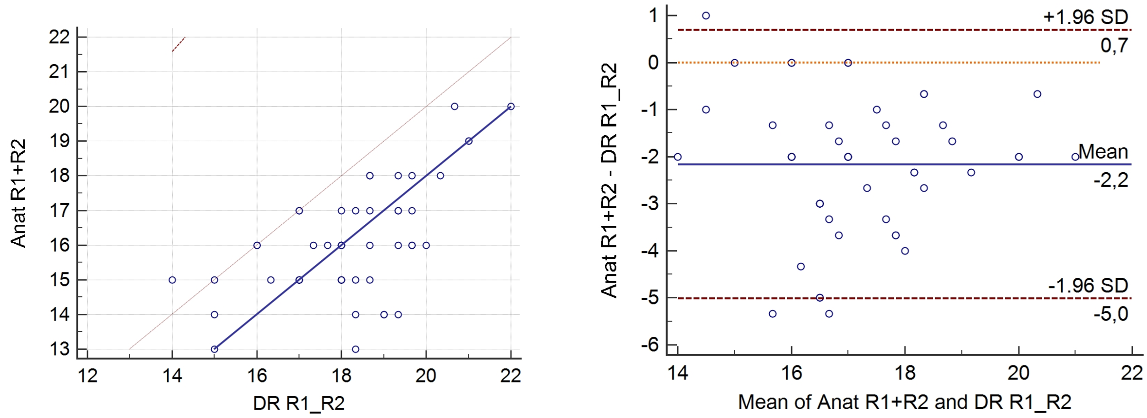


c)

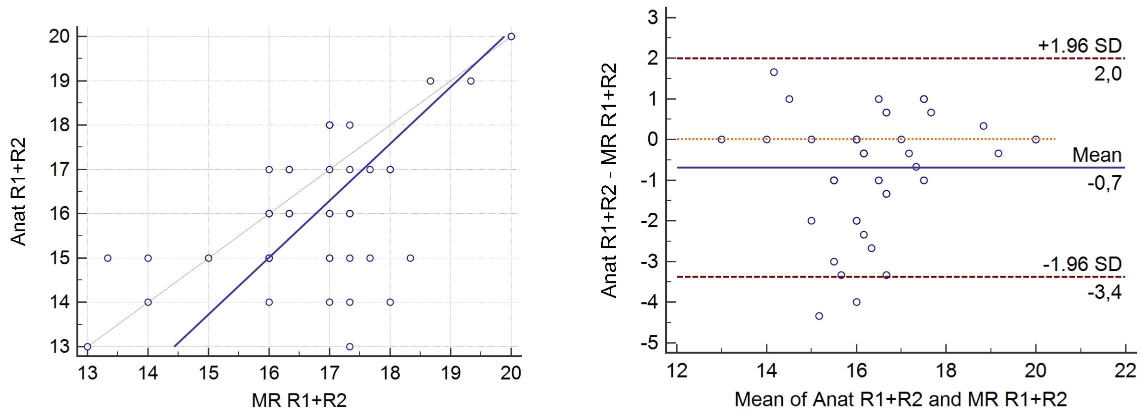

Anat R13+14
MR_R13_14
Anat R13+14 - MR_R13_14
Mean of Anat R13+14 and MR_R13_14
+1.96 SD
4,4
Mean
-4,2
-1.96 SD
-12,7

d)

Graph 3. *Regression graph and Bland-Altman Plot from the proximal dorsal hoof wall measurement: a) Anatomic vs. DR measurement; b) Anatomic vs. MR measurement*



a)



b)

14 Acknowledgements

Mein erster Dank ist an meine Eltern gerichtet, die mir nicht nur das Studium ermöglicht haben und den Start ins Berufsleben unterstützt, sondern mir auch heute noch mit Rat und Tat zur Seite stehen. Ihnen möchte ich diese Arbeit widmen.

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