Diagnostic Accuracy of Digital Photography and Image Analysis for the Measurement of Foot Conformation in the Horse

Jonathan M White BVMS CertES(Orth) MRCVS

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University of Glasgow
Division of Companion Animal Sciences
Faculty of Veterinary Medicine

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Abstract

Experimental studies have shown that foot conformation influences the forces experienced by the distal limb (Riemersma *et al.* 1996; Wilson *et al.* 1998; Willemen *et al.* 1999; Eliashar *et al.* 2004). Although some clinical studies have highlighted the importance of foot conformation as a risk factor for musculoskeletal injury (Kane *et al.* 1998; Anderson *et al.* 2004), this has not been a universal finding (Weller *et al.* 2006c). Clearly more information from large, well designed, prospective studies is needed to elucidate further the relationship between foot conformation and injury. This information would help to guide veterinary surgeons and farriers in the trimming and shoeing of horses' feet, a practice carried out regularly (usually every 4-8 weeks) during the animal's life. In order to further investigate this relationship, a tool capable of accurately, precisely and practicably collecting suitable objective data, ideally at relatively low cost, is necessary.

The variability introduced by image acquisition and subsequent analysis using digital image processing software of digital photographs of the foot has not been quantified. Similarly the level of agreement between objective measurements of foot conformation made from digital photography and digital radiography is unknown.

The objectives of this project were to assess the precision, accuracy and practicability of the entire process of obtaining measurements of horses' feet using digital photography and the digital image processing software (Metron-PX™).

For the precision study (prospective *in vivo* randomised clinical measurement study) lateral digital photographs of shod Thoroughbred racehorses were obtained twice by two veterinary surgeons working independently (Image Acquisition - IAc). Each photograph was independently analysed by the two veterinary surgeons masked to the origin of the images on two occasions using Metron-PX™ (Image Analysis - IAn). Measurements generated by the software were compared within and between operators of the software for self and non-self acquired photographs. Intra- and inter-operator agreement indices (AIs) and 95% limits of agreement (LOA) were calculated for each measurement for the IAn process alone and for the combined IAc + IAn processes for self and non-self acquired images respectively. For the accuracy study (method comparison study) measurements obtained from lateral digital photographs (index test), as in the precision study, were compared with those obtained from lateromedial radiographs (reference standard).

Agreement indices (AIs) and 95% limits of agreement were calculated for each measurement.

The results of the precision study identified excellent mean intra- and inter-operator AIs for the IAn process alone ($\geq$ 0.90 for all measurements). The mean intra- and inter-operator AIs for the combined IAc + IAn processes were $\geq$ 0.89 for all measurements with similar AIs obtained regardless of whether or not the individual whom acquired the images also analysed them. The 95% limits of agreement for hoof angle, heel height/ toe height% and coronary band angle for all comparisons were all within target values. The results of the accuracy study identified mean AIs that were $\geq$ 0.89 for all measurements. The 95% limits of agreement for heel height/ toe height% and coronary band angle were within target values.

Overall, there was excellent precision both within and between operators of the measurement process for both the image analysis process alone and the combined image acquisition and analysis processes. When the described technique is used results are comparable irrespective of whether the person whom acquired the images also analyses them. Excellent accuracy was also identified between the photographic and radiographic measurements, especially for heel height/ toe height% and coronary band angle, suggesting that these two methods may be used interchangeably for these measurements of foot conformation in the horse.

The clinical relevance of these findings is that the processes described for obtaining objective measurements of foot conformation from digital photographs and digital image processing software Metron-PX™ is practicable and produces highly precise measurements regardless of whether the same operator performs both image acquisition and analysis. Photographic measurements of heel height/ toe height% and coronary band angle closely approximate radiographic measurements such that the two techniques may be used interchangeably. Thus, digital photography and image analysis have applications in the field of clinical telemedicine and would be particularly useful to a large prospective multi-centre study investigating the relationship between foot conformation and musculoskeletal injury.

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Author’s Declaration

I, Jonathan Matthew White, declare that the work in this thesis is original, was carried out solely by myself or with due acknowledgements. It has not been submitted in any form for another degree or professional qualification.

Jonathan M White

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List of Abbreviations

AI	Agreement index
ALDDFT	Accessory ligament of the deep digital flexor tendon
BA	Bland-Altman
DDFT	Deep digital flexor tendon
IAc	Image acquisition
IAn	Image analysis
LOA	Limits of agreement
NS	Non-self acquired
S	Self acquired
SD	Standard deviation
TB	Thoroughbred
VS	Veterinary surgeon

1 Introduction

This study critically evaluated the process of obtaining objective measurements of foot conformation through the techniques of digital photography and subsequent image analysis with a commercially available digital image processing software programme (Metron-PX™)[1]. The techniques have been cited in the equine peer reviewed literature (Van Heel *et al.* 2005) but independent assessment of the precision (agreement between repeated measures) and accuracy (agreement with a reference standard) of the process has not been reported.

Experimental studies have shown that foot conformation influences the forces experienced by the distal limb (Riemersma *et al.* 1996; Wilson *et al.* 1998; Willemen *et al.* 1999; Eliashar *et al.* 2004). Although some clinical studies have highlighted the importance of foot conformation as a risk factor for musculoskeletal injury (Kane *et al.* 1998; Anderson *et al.* 2004), this has not been a universal finding (Weller *et al.* 2006c). Clearly more information from large, well designed, prospective studies is needed to further elucidate the relationship between foot conformation and injury. This information would help to guide veterinary surgeons and farriers in the trimming and shoeing of horses' feet, a practice carried out regularly (usually every 4-8 weeks) during the animal's life. In order to further investigate this relationship, a tool capable of accurately, precisely and practicably collecting suitable objective data, ideally at relatively low cost, is necessary.

Objective methods of foot measurement currently available include the use of hoof protractors, radiographic techniques, photographic techniques and 3-D motion analysis techniques. Hoof protractors give little information on overall foot dimensions and their precision and accuracy are questionable (Moleman *et al.* 2005). Measurements made from radiographs in digital format with radiopaque markers applied to the foot have been evaluated for precision and accuracy (Kummer *et al.* 2004; Rocha *et al.* 2004) and this technique is routinely used in both clinical practice and research but requires expensive equipment and has radiation safety implications for personnel involved in image acquisition. A 3-D motion analysis system has recently been shown to be useful in the measurement of whole body conformation but requires a minimum of four video cameras and custom written software, which limits its widespread adoption (Weller *et al.* 2006a). In addition, only a small number of measurements were made pertaining to foot conformation possibly as a consequence of the relatively large size of anatomical markers required.

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[1] Eponatech, Creston, California, USA

Weller *et al.* (2006a) identified increased accuracy and precision of the 3-D system over photographic techniques for whole body conformation measurements but these results may not apply to photographic techniques for foot conformation assessment as the photographs were taken at the level of the *tuber olecrani.* The precision and accuracy that would be obtained when photographs are taken at the level of the foot is likely to be greater.

In contrast to using radiography for making objective measurements of feet, digital photography does not require expensive equipment and poses no radiation hazard. This thesis describes investigations into the accuracy, precision and practicability of the entire process of obtaining digital photographs and the use of a digital image processing software programme (Metron-PX™). The study was conducted according to the guidelines of the Standards for Reporting of Diagnostic Accuracy (STARD) initiative (Bossuyt *et al.* 2003b). This initiative was developed by a group of scientists and editors to improve the quality of reporting of studies of diagnostic accuracy after a survey of studies of diagnostic accuracy published in four major medical journals between 1978 and 1993 revealed that the methodological quality was mediocre at best (Reid *et al.* 1995). The resulting statement consists of a checklist of 25 items, explanatory document and a flow diagram that authors can use to ensure that relevant information is present (Bossuyt *et al.* 2003b).

In order to obtain measurements using the Metron-PX™ software there are two major processes involved: image acquisition (IAc) and image analysis (IAn). The objective of the precision study was to estimate the precision of the individual and combined processes necessary to obtain measurements of the conformation of horses' feet, to examine the effect of different operators on the measurements made and to estimate the agreement between an automated measurement of the hairline angle and the conventional coronary band angle, the angle between a straight line adjoining the dorsal midline coronary band and the coronary band at the lateral heel and the ground surface (Eliashar *et al*. 2004)

The objective of the accuracy study was to assess the level of agreement between the measurements of foot conformation obtained from digital photography and subsequent image analysis with digital image processing software Metron-PX™ and the measurements obtained from digital radiography using a standardized radiographic technique (Kummer *et al.* 2004) and image analysis with digital image processing software (Rocha *et al.* 2004).

Potential applications of the photographic techniques involve the transmission of medical information from one location to another via telecommunication media (i.e. telemedicine). This may be of particularly beneficial when the two locations involved are distant and physical transportation of an animal to a referral centre may be problematical possibly due to excessive distance, time constraints or expenses involved. Both the veterinary and the farriery professions share the responsibility for the management of the horse’s foot and therefore techniques which allow for improved analysis of the foot and the transmission of this information between parties is advantageous for the animal’s health and welfare. Accurate and precise photographic techniques would be particularly useful to a large multi-centre prospective study investigating the relationship between foot conformation and musculoskeletal injury.

2 Literature Review

This chapter reviews the pertinent information available in the scientific literature on equine foot conformation, including consideration of the clinical significance of foot conformation in relation to its effects on distal limb biomechanics and on risk for musculoskeletal injury. As the study was concerned with the evaluation of a method for the objective assessment of foot conformation, relevant background information on the evaluation of diagnostic tests is included.

2.1 Overview of Foot Conformation

2.1.1 Definition

A recent publication aimed at standardization of podiatry terminology defined foot conformation as the shape and size of the foot determined by the shape and size of the individual structures of the foot and the spatial relationship between them (O'Grady *et al.* 2007). Foot conformation is best assessed with the animal standing evenly on all four limbs and is commonly subdivided into dorso-palmar/ plantar conformation, for assessment from the side and medio-lateral conformation, for assessment from directly in front.

2.1.1.1 Dorso-Palmar/ Plantar

Dorso-palmar/ plantar foot conformation incorporates length measurements between anatomical landmarks and the angles between them. Many measurements can be made from the side including: hoof angle; dorsal hoof length; heel angle; heel height; heel height/ toe height%; coronary band angle and support length.

2.1.1.2 Medio-Lateral

Medio-lateral foot conformation incorporates length measurements between anatomical landmarks and the angles between them. Many measurements can be made when the foot is viewed from in front including medial wall angle; medial wall length; medial wall height; lateral wall angle; lateral wall length; lateral wall height and support width.

Medio-lateral foot conformation was not assessed in this study.

2.2 Overview of Methods used to Evaluate Foot Conformation

2.2.1 Subjective Evaluation

2.2.1.1 General Foot Shape

2.2.1.1.1 Normal

When viewed from the side, it is suggested that the dorsal hoof wall should be parallel with the dorsal surface of the pastern and that the angle of the heel should approximate that of the dorsal hoof wall although it is often a few degrees less (Parks 2003). Normal or optimal hoof lengths have been suggested for approximate horse size and weight ranges (Balch *et al.* 1991) and it has previously been stated that heel length should be approximately one third of dorsal hoof wall length (Parks 2003).

2.2.1.1.2 Long toe-low heel

Descriptive term used to describe the foot which is considered to have excessive toe length relative to the conformation of the heel (O'Grady *et al.* 2007). This foot shape commonly results in a broken-back foot pastern axis (Bushe *et al.* 1987) (Figure 2-1).

2.2.1.1.3 Upright/ boxy

Descriptive term used to describe the foot which is considered to have excessive heel height and large dorsal hoof and heel angles. A markedly upright foot is described as a club foot which can be associated with a flexural deformity of the distal inter-phalangeal joint (O'Grady *et al.* 2007). This foot shape commonly results in a broken forward foot-pastern axis (O'Grady *et al.* 2007) (Figure 2-1).

2.2.1.2 Foot-Pastern Axis

The foot-pastern axis describes the relationship between the dorsal hoof angle and the angle of the dorsal surface of the pastern relative to the ground (Parks 2003). It is suggested that ideally the dorsal hoof angle and the angle of the dorsal surface of the pastern relative to the ground should be identical such that the angle between them is 180^o and the axis is considered to be straight (Parks 2003). A broken forward foot-pastern axis is defined as the situation when the dorsal hoof angle is greater than the pastern angle. A broken-back foot pastern axis is defined as the situation where the dorsal hoof angle is less than the pastern angle.

Potential problems associated with using this classification are shared by other subjective methods of assessment: difficulties in performing statistical analyses; and variability both within and between assessors of foot conformation. The latter point was exemplified in a conformational study using this method of foot assessment in Thoroughbred horses where unsatisfactory reproducibility for both fore and hind limb foot-pastern axis assessment was identified (Mawdsley *et al.* 1996).

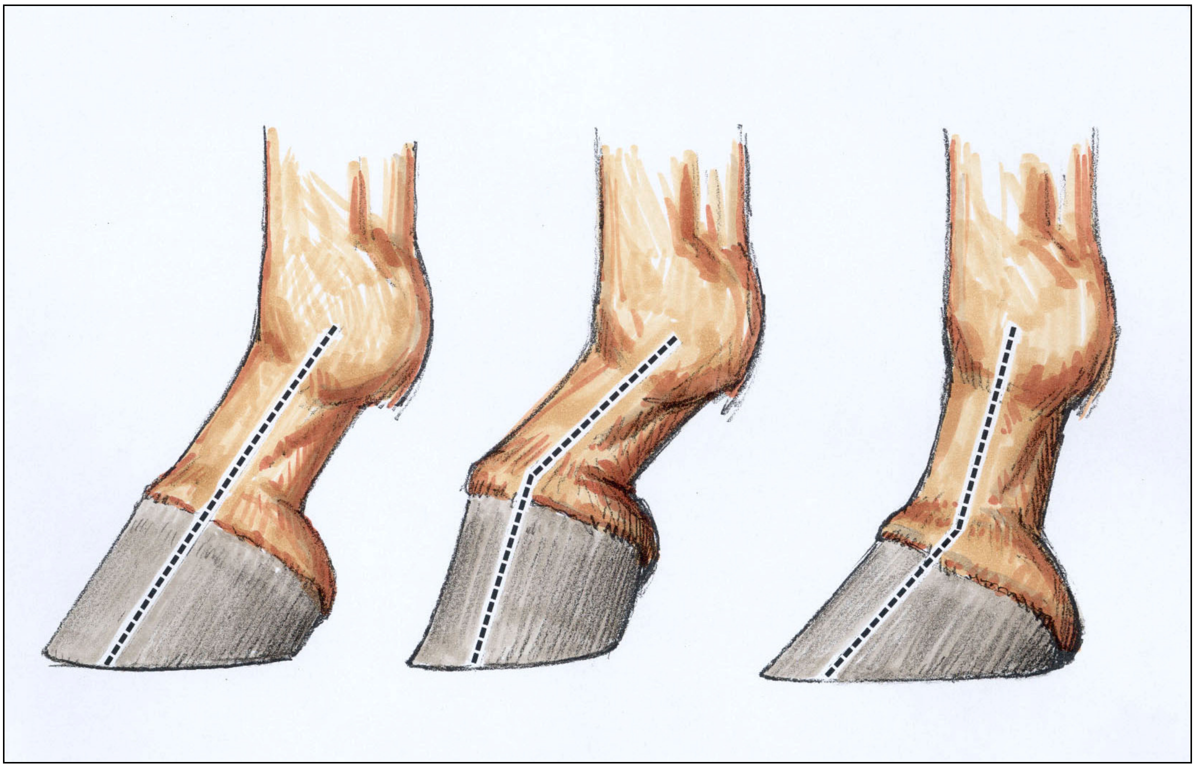

Figure 2-1 Diagram demonstrating (from left to right) normal (straight), broken forward and broken back foot-pastern axes.

2.2.1.2.1 Straight foot-pastern axis

A straight foot-pastern axis describes the situation where the angle that the dorsal hoof wall makes with the ground is equal to the angle that the dorsal aspect of the pastern makes with the ground. Experimental data obtained using instrumented shoes on horses moving on a treadmill at walk, trot and canter showed that forces at the toe, medial and lateral heels were collectively lowest when the hoof and pastern were aligned (Balch 1994) (Figure 2-1).

2.2.1.2.2 Broken forward foot-pastern axis

A broken forward foot-pastern axis refers to the situation where the angle that the dorsal hoof wall makes with the ground is greater than the angle the dorsal aspect of the pastern makes with the ground (O'Grady *et al.* 2007) (Figure 2-1).

2.2.1.2.3 Broken backward foot-pastern axis

A broken back foot-pastern axis describes the situation where the angle that the dorsal hoof wall makes with the ground is less than the angle the dorsal aspect of the pastern makes with the ground (O'Grady *et al.* 2007) (Figure 2-1). This abnormality has been implicated as a predisposing factor for navicular disease (Ostblom *et al.* 1982) and was recorded in 71.2% of horses with navicular disease (Wright 1993). The author of the latter study also reported the abnormality in a similar percentage of non-navicular disease lameness cases seen during a similar time period, suggesting that the prevalence of this type of foot conformation is high and not necessarily only associated with navicular disease. A more recent study investigating the variation in conformation of Thoroughbred racehorses also identified broken back foot-pastern axes in the front and hind feet in the majority of horses (Weller *et al.* 2006b)

2.2.2 Objective Evaluation

Objective evaluation of the foot offers major advantages over subjective evaluation with respect to ease of statistical analysis and reduced intra- and inter-operator variability. The ideal objective measurement tool would be precise, accurate, practicable in a clinical setting and also be affordable to the general practitioner.

2.2.2.1 Hoof Protractor Measurements

Hoof protractors, are mechanical instruments designed to measure the hoof angle, however, some are also able to measure the length of the dorsal hoof wall. Hoof protractors have been used previously in hoof and locomotion research (Bushe *et al.* 1987; Barrey 1990; Clayton 1990) and a review article stated that their use increased the accuracy and repeatability of measurements but did not report whether a direct comparison between the measurements made by different techniques was performed (Balch *et al.* 1991). A recent study however investigated the accuracy and repeatability of measurements made by four different types of hoof protractor devices and found none to be both accurate and repeatable (Moleman *et al.* 2005).

2.2.2.2 Photographic Measurements

Measurements made from photographs are commonly used in studies evaluating whole body conformation, including a limited number of foot measurements (Magnusson 1985; Holmstrom *et al.* 1990; Anderson and McIlwraith 2004) and in studies concentrating on more specific areas of interest such as the foot, when more detailed measurements are made (Kane *et al.* 1998).

Markers are commonly used in conformational studies to provide anatomical reference points which can be used during either 2-D or 3-D image analysis in order to measure segment lengths and angles (Anderson and McIlwraith 2004; Eliashar *et al.* 2004; Weller *et al.* 2006a). Although markers are commonplace in conformational studies, few reports include estimates of marker placement accuracy and repeatability. However, a recent study investigating a technique for objective measurements of whole body conformation assessed the accuracy of marker placement (Weller *et al.* 2006a) and identified excellent accuracy for landmarks distal to the elbow and stifle but poorer accuracy for more proximal landmarks. The authors suggested that bony landmarks in the proximal limb tend to be less well defined and less palpable than in the distal limb.

2.2.2.3 Video Recorded Measurements (3-D Motion Analysis)

Objective measurements of whole body conformation including some foot measurements have been performed from video recordings using multiple video cameras, anatomical markers and controlled environments (Hunt *et al.* 1999; Weller *et al.* 2006a).

The advantages of using 3-D imaging are improved representation of 3-D structures of the horse and a reduction in the error associated with photographic methods of measurement (Weller *et al.* 2006a). Three dimensional motion analysis systems are suggested to combat the error introduced from deviation in the camera-horse angle which can distort measurements and geometrical error that occur when a 3-D structure is reduced to a 2-D image (Weller *et al.* 2006a). Results of this study identified higher levels of precision with the 3-D system compared with those from photographic techniques but these results may not be directly applicable to photographic methods of the foot as the photographs were taken at the level of the *tuber olecrani* (Weller *et al.* 2006a) and likely underestimate the precision that might be obtained if photography was performed at the level of the foot.

The most recent publication identified high levels of precision and accuracy with a four video camera motion analysis system but as with other whole body conformation methods gave only a limited amount of information on foot conformation (Weller *et al.* 2006a). This may have been due to the relatively large size on the anatomical markers used (25 mm spheres).

2.2.2.4 Radiographic Measurements

Studies reporting objective measurements made from foot radiographs of groups of clinically normal horses can be found in the equine literature (Linford *et al.* 1993; Cripps and Eustace 1999b). These studies were aimed at establishing approximate normal values which may serve as comparative measurements when assessing diseased animals (Linford *et al.* 1993; Cripps and Eustace 1999a).

Measurements made from radiographs are essential in experimental research to identify centres of rotation of distal limb articulations, measure angular deviations and joint moment arms and investigate the relationship between foot conformation and the forces applied to the distal limb support structures (Eliashar *et al.* 2004).

Radiographic measurements have also been used to assess conformation of the carpus in a study investigating a possible association with carpal disease in the horse (Barr 1994).

2.3 Overview of the Effect of Dorso-Palmar/ Plantar Foot Conformation on the Distal Limb in the Horse

2.3.1 Hoof Angle

There is inconsistency in the normal ranges quoted for the hoof angles in veterinary literature with older texts quoting values of 45-50^o and 50-55^o for the front and hind feet respectively (Stashak *et al.* 2002) whilst others suggest that values of 50-54^o and 53-57^o for front and hind feet respectively are normal (Balch *et al.* 1991). One source of variation in normal hoof angle may be differences between breeds: Thoroughbred or Thoroughbred crosses having lower hoof angles (Linford *et al.* 1993; Cripps and Eustace 1999b; Anderson and McIlwraith 2004) than Warmblood horses (Van Heel *et al.* 2005; Moleman *et al.* 2006).

In vitro and *in vivo* experimental studies have investigated the biomechanical effect of alterations in the hoof angle by the application of wedges to either the toe or heel region of the foot (Bushe *et al.* 1987; Riemersma *et al.* 1996; Willemen *et al.* 1999; Crevier-Denoix *et al.* 2001; Degueurce *et al.* 2001; Viitanen *et al.* 2003). Generally, increasing hoof angle induces increased flexion in the distal and proximal inter-phalangeal joints and extension of the metacarpo-phalangeal joint with a decreasing magnitude of effect in the more proximal joints. Increasing the hoof angle also leads to a reduction in tensile force in the deep digital flexor tendon (DDFT) and consequently in the force applied to the navicular bone (Willemen *et al.* 1999; Eliashar *et al.* 2004). An *in vivo* study investigating the effects of increasing the hoof angle on the palmar support structures of the limb in ponies identified a significant reduction in the strain of the DDFT and its accessory ligament (ALDDFT) and a significant increase in the strain of the suspensory ligament, whilst changes in the tension of the superficial digital flexor tendon were minimal (Riemersma *et al.* 1996).

A clinical study investigating the effect of hoof size and shape as possible risk factors for catastrophic musculoskeletal injury in Thoroughbred racehorses, identified a significantly reduced risk of distal metacarpal condylar fracture and suspensory apparatus failure with higher hoof angles (Kane *et al.* 1998).

It is generally accepted that in the shod foot, the hoof mechanism results in continued wear of the heels whilst the toe is protected, leading to a more sloping foot shape and smaller hoof angle with increasing shoeing intervals (Barrey 1990; Back 2001). This reduction in hoof angle has recently been quantified: the average change recorded over an eight week shoeing interval was approximately a 3^{o} reduction (Van Heel *et al.* 2005; Moleman *et al.* 2006).

2.3.2 Hoof Length

Normal or optimal hoof lengths have been suggested for approximate horse size and weight ranges (Balch *et al.* 1991) but the authors state that there are many exceptions. Longer hoof lengths have been shown to induce greater strain on the dorsal hoof wall (Thomason 1998), which may predispose to hoof pathology.

As the hoof is continually growing and the toe is largely protected from frictional wear in the shod foot, excessive toe length can result if shoeing interval is excessive or the amount of foot trimmed is inadequate. A recent study quantified this increase in hoof length in a group of sound Warmblood horses with an eight week shoeing interval, where a mean increase of approximately 1.5cm was recorded (Van Heel *et al.* 2005).

The effect of gross changes in hoof length on limb kinematics was investigated by the application of 2.5 and 5.0cm pads to the hoof (Balch *et al.* 1994). Compared with unaltered hoof length, long hooves were associated with prolonged stride duration, swing duration and break over but overall stride length and stance duration were unaltered.

2.3.3 Heel Angle

Low heel angles relative to hoof angles have been associated with increased risk of catastrophic musculoskeletal injury by suspensory apparatus failure (Kane *et al.* 1998) and this trait was also found to be significantly associated with an increased risk of carpal effusion but not carpal fracture in a recent prospective clinical study (Anderson *et al.* 2004).

Lower heel angles relative to hoof angles have also been implicated in pathogenesis of navicular syndrome (Wright and Douglas 1993; O'Grady and Poupard 2001) but interestingly the ratio of heel angle: hoof angle, termed heel collapse index, and the force applied to the navicular bone during the stance phase are not correlated (Eliashar *et al.* 2004).

Under-run heels is a term commonly used in veterinary texts and a recent publication aimed at standardizing podiatry terminology describes it as the situation where the angle of the heels is considerably less than the hoof angle commonly with folding of the horn tubules under the heel bulbs (O'Grady *et al.* 2007). Unfortunately this recent definition clearly still allows for individual interpretation and therefore inconsistent use.

2.3.4 Heel Height

Heel height has not received much investigation as a direct contributor to the forces acting on the equine distal limb but may be of importance as a component of the ratio of heel height: toe height, termed the height index (Eliashar *et al.* 2004). Although probably not the primary aim of investigators involved in biomechanical research the effect of heel height has indirectly been investigated due to the large number of studies using heel or toe wedges to alter foot conformation (Bushe *et al.* 1987; Riemersma *et al.* 1996; Willemen *et al.* 1999; Crevier-Denoix *et al.* 2001; Degueurce *et al.* 2001; Viitanen *et al.* 2003). The application of heel wedges to influence hoof angle or the angle of the solar surface of the distal phalanx also has effects on the heel height of the foot. These studies have shown that heel wedges decrease the tension in the deep digital flexor tendon and the force applied to the navicular bone (Riemersma *et al.* 1996; Willemen *et al.* 1999).

2.3.5 Heel Height/ Toe Height%

Heel height/ toe height% can be described as the height index (Eliashar *et al.* 2004) expressed as a percentage. The height index was shown to have a strong negative correlation with the tensile force in the deep digital flexor tendon and the resultant force applied to the navicular bone (Eliashar *et al*. 2004). This study used pressure and force plate data in conjunction with radiographic measurements of foot conformation to calculate the tension created in the deep digital flexor tendon and the force applied to the navicular bone.

2.3.6 Coronary Band Angle

The coronary band angle was shown to have a strong positive correlation with the tension in the deep digital flexor tendon and the resultant force applied to the navicular bone (Eliashar *et al*. 2004). Although little is reported about normal values for this parameter the mean and standard deviation of the coronary band angle in the group of sound Irish draft horses used in the study were 23.5^{o} and 3.0^{o} respectively (Eliashar *et al.* 2004).

2.3.7 Support Length

There is little written in the equine literature about the dorso-palmar support length which is defined as the horizontal distance from the dorsal most to the palmar most weight-bearing surface of the foot. It can be expected that support length increases with time following trimming and shoeing as hoof length increases (Van Heel *et al.* 2005).

2.4 Overview of Methods used to Evaluate a Diagnostic Test

2.4.1 Diagnostic Test

2.4.1.1 Definition

The term “diagnostic test” refers to any method for obtaining additional information on an animal’s health status, and therefore includes methods designed to obtain information relating to an animal’s medical history or physical examination as well as diagnostic imaging modalities, laboratory tests, function tests and histopathology (Bossuyt *et al.*

2003a). The condition of interest or target condition can refer to a particular disease or to any other identifiable condition that may prompt clinical actions, such as further diagnostic testing, or the initiation, modification or termination of treatment (Bossuyt *et al.* 2003a). The condition of interest for this study was abnormal foot conformation which may predispose the musculoskeletal system to injury through single overload of critical structures or through repetitive or cumulative microdamage to critical structures leading to clinically detectable abnormalities over time (Smith *et al.* 2002).

2.4.2 Evaluation of Test Precision

Test precision is defined as the agreement between repeated measures of the same quantity with the same method. Precision is a broad term and encompasses both repeatability, agreement between measurements made on the same animals by the same operator, and reproducibility, agreement between measurements on the same animals by different operators (Holmes and Cockcroft 2008). Within (intra-) operator agreement (repeatability) can be assessed when a single operator performs repeated measurements on a set of subjects with the same method. Between (inter-) operator agreement (reproducibility) can be assessed when different operators perform repeated measurements on a set of subjects with the same method. The repeatability/ reproducibility of continuous variables can be expressed by the within subject standard deviation or by calculating the 95% limits of agreement (Bland and Altman 1986, 2003) where the range of differences that might occur in 95% individuals is calculated if repeated measures were performed.

A very precise measurement is one that is repeatable/ reproducible so that nearly the same value is obtained each time the measurement is repeated. The more precise the measurement the greater the statistical power at a given sample size. Precision is affected by random error (chance) and the greater the error the less precise the measurements become. The three main sources of error in diagnostic testing are operator variability, subject variability and instrument variability (Holmes and Cockcroft 2008).

2.4.3 Evaluation of Test Accuracy

Test accuracy can be defined as the amount of agreement between the information from a test under evaluation (index test) and those made from an accepted reference 'gold' standard test. Accuracy is an inverse function of systematic error. Systematic error is the same as bias and bias can occur due to the operator, the subject or the instruments used in the measurements. Accuracy is expressed in different ways depending on whether the measurement is on a continuous or categorical scale (Holmes and Cockcroft 2008).

3 Materials and Methods

3.1 Introduction

This chapter explains the methods used in carrying out the study, giving special emphasis to the technical specifications of the instruments and protocols used for data collection and analysis.

Diagnostic and screening tests are constantly being developed and the technology of existing tests is continuously being improved. Exaggerated and biased results from poorly designed and reported diagnostic studies can trigger their premature dissemination in general practice and lead veterinary surgeons to make incorrect management or treatment decisions (Bossuyt *et al.* 2003a). Therefore, a rigorous evaluation process of diagnostic or screening tests before introduction into clinical practice is ideal and studies to determine the diagnostic accuracy of a test are a vital part to this evaluation process (Bossuyt *et al.* 2003a).

The term test refers to any method for obtaining additional information on an animal's health status, and therefore includes information from history and physical examination, laboratory tests, imaging tests, function tests and histopathology (Bossuyt *et al.* 2003a). The condition of interest or target condition can refer to a particular disease or to any other identifiable condition that may prompt clinical actions, such as further diagnostic testing, or the initiation, modification or termination of treatment (Bossuyt *et al.* 2003a). In the context of this study the condition of interest is that of abnormal foot conformation which may predispose the musculoskeletal system to injury (Kane *et al.* 1998; Anderson *et al.* 2004) through single overload of critical structures or through repetitive or cumulative microdamage to critical structures leading to clinically detectable abnormalities over time (Smith *et al.* 2002).

Diagnostic accuracy can be expressed in many ways, including sensitivity-specificity pairs, odds ratios, likelihood ratios, and area under receiver-operator characteristic (ROC) curves but in order to obtain these estimates it would be necessary to dichotomize the data using cut-off values. Cut off values for foot conformation measurements are not currently available to separate diseased from non diseased groups.

3.2 Study Animals

3.2.1 Precision Study

Twenty shod Thoroughbred (TB) horses in race training were randomly selected from one racing yard. All horses were in routine farriery care and were at various time intervals following trimming and shoeing. All horses were sound at the walk and were able to stand evenly on all four limbs. Horses unwilling to stand still with their front feet on six centimetre high wooden blocks (Eponatech™ blocks)[2] (Figure 3-1) without sedation were excluded from the study and a replacement chosen at random from the remaining horses in the yard.

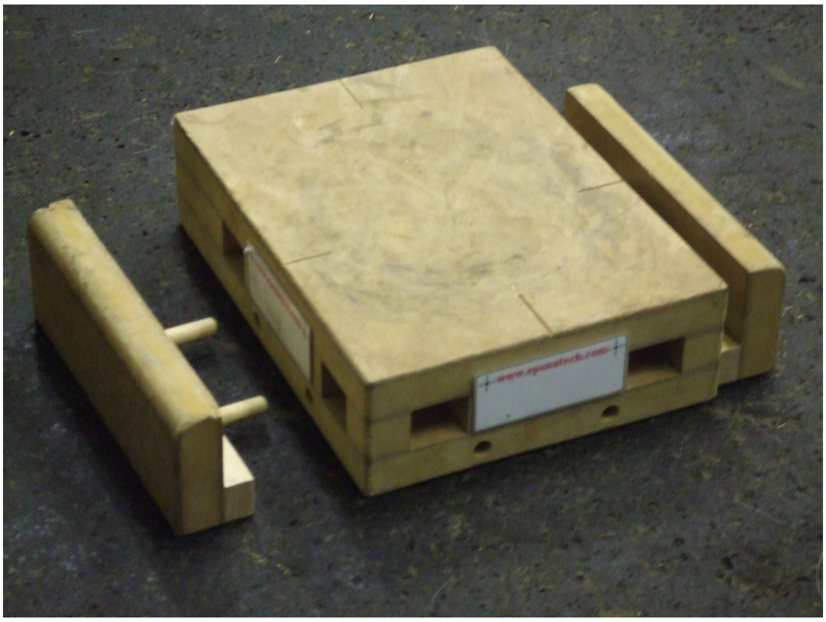

Figure 3-1 Photograph of the Eponatech Block™ used for image acquisition in both precision and accuracy studies.

3.2.2 Accuracy Study

Fifteen consecutive clinical cases undergoing lateromedial radiography of the front feet at the Weipers Centre Equine Hospital, University of Glasgow for routine or remedial farriery purposes or as part of a lameness investigation, were included in the study. The study was conducted between 1st January 2007 and 1st May 2007. Horses with feathers obscuring the coronary band were excluded from the study. Horses were sedated for radiography and underwent photography whilst under sedation.

[2] Eponatech Block™, Creston, California, USA

3.3 Ethical Approval

Both the precision and accuracy studies were approved by the University of Glasgow, Faculty of Veterinary Medicine Ethics & Welfare Committee.

3.4 Image Acquisition (IAc)

3.4.1 Marker Placement

Markers are commonly used in conformational studies to provide anatomical reference points which can be used during either two- or three-dimensional (2-D or 3-D) image analysis in order to measure segment lengths and angles (Anderson and McIlwraith 2004; Eliashar *et al.* 2004; Weller *et al.* 2006a). Although markers are commonplace in conformational studies, few reports include estimates of marker placement accuracy and repeatability. However, a recent study investigating a technique for objective measurements of whole body conformation assessed the accuracy of marker placement (Weller *et al.* 2006a) and identified excellent accuracy for landmarks distal to the elbow and stifle but poorer accuracy for more proximal landmarks. The authors suggested that bony landmarks tend to be less well defined and less palpable than in the distal limb.

3.4.1.1 Heel Markers

In this study, heel markers consisted of 3mm diameter stainless steel ball bearings attached with double sided adhesive tape to the palmar aspect of the lateral heel at the level of the coronary band and at the palmar most weight-bearing surface of the foot (Figure 3-2), determined by pushing a scalpel blade under the lateral wall in a dorsal direction between the lateral wall and the shoe, with the horse fully weight-bearing (Eliashar *et al.* 2004). The heel markers used in this study (3mm diameter metallic spheres) were smaller than those used by Eliashar and others (2004) (8mm wire) in order to provide a more focal marker for cursor placement on both radiographic and photographic images without compromising visibility.

Figure 3-2 Lateral digital photograph demonstrating the position of the heel markers used in both precision and accuracy studies (red arrows).

3.4.1.2 Dorsal Hoof Marker

A 70mm linear stainless steel marker was taped to the dorsal hoof wall with the proximal extremity at the level of the coronary band (Figure 3-3).

Figure 3-3 Photograph demonstrating the position of the linear stainless steel dorsal hoof wall marker used in the accuracy study.

3.4.2 Photographic Technique

3.4.2.1 Precision Study

Lateral digital photographs were obtained with the horses (unsedated) standing with both front feet elevated on wooden blocks in a stable with a level concrete floor (Figure 3-4). The left front foot was photographed against a white background to provide contrast with the foot (Figure 3-5). The wooden blocks contained embedded scale markers and a plaque labelled with the veterinary surgeon's (VS) number and the animal's identification number. A digital camera (Fujifilm™ Finepix Z1, 5.1 megapixels)[3] set at 3× optical zoom (maximum) was used to obtain digital photographs according to the guidelines provided by the manufacturer of Metron-PX™ (www.eponatech.com) with the heel bulbs visually aligned as is done for lateromedial radiography (Kummer *et al.* 2004) and with the camera supported at the same level as the foot using a bean bag tripod (The Pod)[4]. Two veterinary surgeons (VS1 and VS2), working independently, alternately placed markers on the heel of the foot (Section 3.4.1.1), obtained a photograph and then carefully removed all trace of the markers such that no physical evidence of their position remained on the foot. Each VS obtained two photographs of each foot in an alternating sequence (eg. VS1, VS2, VS1, VS2) with the first VS to photograph the foot chosen at random.

[3] Fuji Photo Film Co. Ltd., Tokyo, Japan

[4] The Pod™, The Pod Industries, Canada

Figure 3-4 Photograph demonstrating both fore limbs of one of the study animals elevated on the Eponatech™ blocks.

Figure 3-5 Photograph demonstrating the acquisition of lateral digital photographic images.

3.4.2.2 Accuracy Study

Lateral digital photographs were obtained as in the precision study above, however, heel markers were placed by one veterinary surgeon (resident level) on both front feet and only one image of each front foot was acquired.

3.4.3 Radiographic Technique

3.4.3.1 Accuracy Study

Lateromedial digital radiographs were obtained of the fore feet with both feet elevated on wooden blocks (Figure 3-6) with the heel markers and a dorsal hoof wall marker attached (Section 3.4.1). The imaging system comprised a gantry mounted x-ray unit (CPI Indico100 generator)[5] and computed radiography cassettes (CR MD 4.2)[6] which were digitised (CR 35X)[6] and exported as jpeg files for image analysis. Radiographs were obtained with a 100cm film focus distance (FFD) and a 8-10cm object film distance (OFD) with the x-ray beam centred 2cm distal to the coronary band at a point mid-way between the dorsal and palmar borders (Redden 2003; Kummer *et al.* 2004).

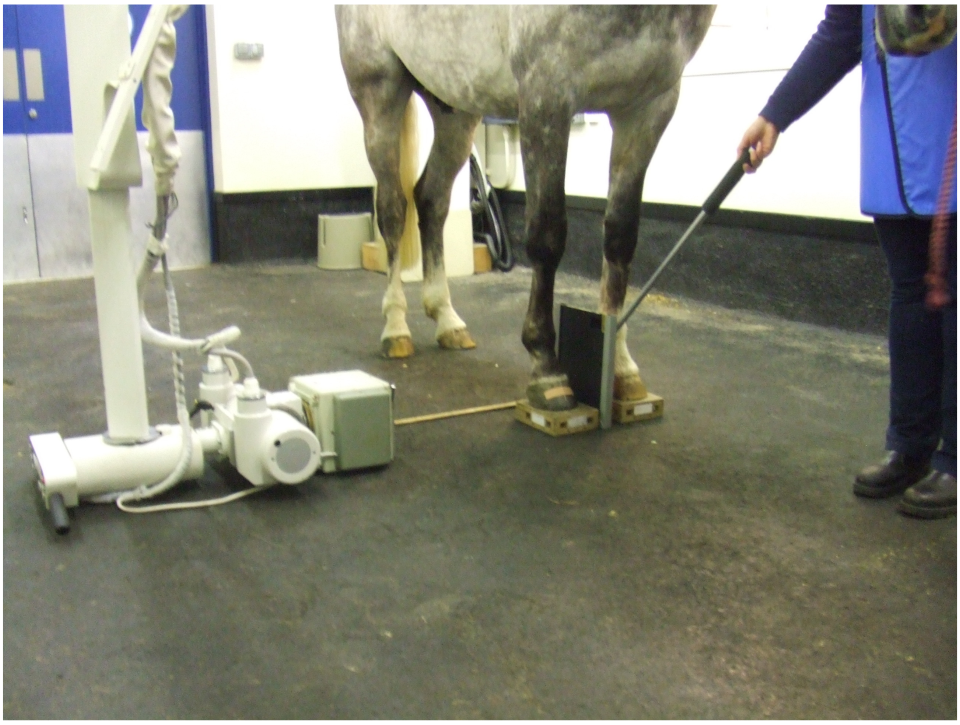

Figure 3-6 Photograph demonstrating the system for acquisition of lateromedial radiographs of the front feet.

[5] Communication & Power Industries Canada inc., Ontario, Canada

[6] Agfa-Gevaert Ltd., Brentford, Middlesex, UK

3.5 Randomisation and Concealment

All photographic and radiographic images were randomised and relabelled with a code number using Adobe Photoshop 5.0[7]. The code numbers of the images were concealed from both VS1 and VS2, such that they were unaware of either the animal's original number or the identity of the VS whom acquired the image.

3.6 Image Analysis (IAn)

3.6.1 Precision Study

Coded images were imported into Metron-PX™ version 3.0[1] and analysed independently by the two VSs (VS1 and VS2), masked to the origin of the image, using the semi-automated (guided) and free analysis function of the software on two occasions. Both VSs received a short tutorial (approximately 30 minutes) from the author on the use of the software for photographic images and used the help function of the software when necessary. Image analysis (IAn) involved the importation of jpeg files into the programme followed by a series of mouse clicks on each image using the semi-automated analysis function of the software. Firstly, a series of mouse clicks is used to define the scale marker on the Eponatech blocks™ in the image; this is followed by a series of mouse clicks to identify anatomical landmarks of the foot. The software automatically calculates seven length, angle and percentage measurements from the information obtained from the mouse clicks. An additional measurement, coronary band angle, was made using the more time consuming free analysis function of the software. This was done to assess the agreement between the automatically calculated hairline angle and that of the conventional coronary band angle (Eliashar *et al.* 2004), as would be measured from a lateromedial radiograph, in order to ascertain whether the measurements may be used interchangeably. The following lengths, angles and percentages were recorded from each analysed image: hoof angle; dorsal hoof length; heel angle; heel height; support length; heel height/ toe height%, hairline angle and coronary band angle (Figures 3-7 to 3-14).

[7] Adobe Systems Europe Ltd., Uxbridge, UK

3.6.1.1 Hoof Angle

The angle between the dorsal hoof wall and the ground surface (O'Grady *et al.* 2007). In the shod horse the ground surface is taken as the interface between the shoe and the foot.

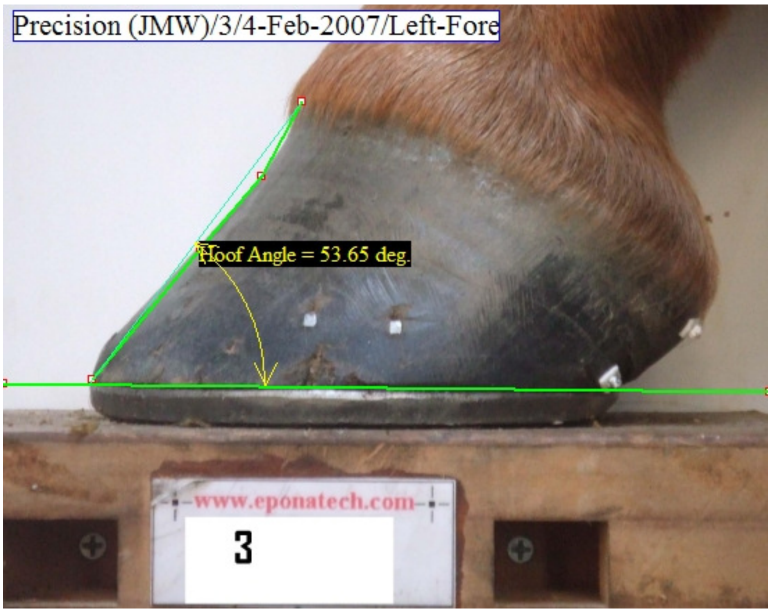

Figure 3-7 Output screen from Metron-PX™ demonstrating hoof angle measured from a lateral digital photograph.

3.6.1.2 Dorsal Hoof Length

The distance between the most distal aspect of the dorsal hoof wall and the dorsal coronary band.

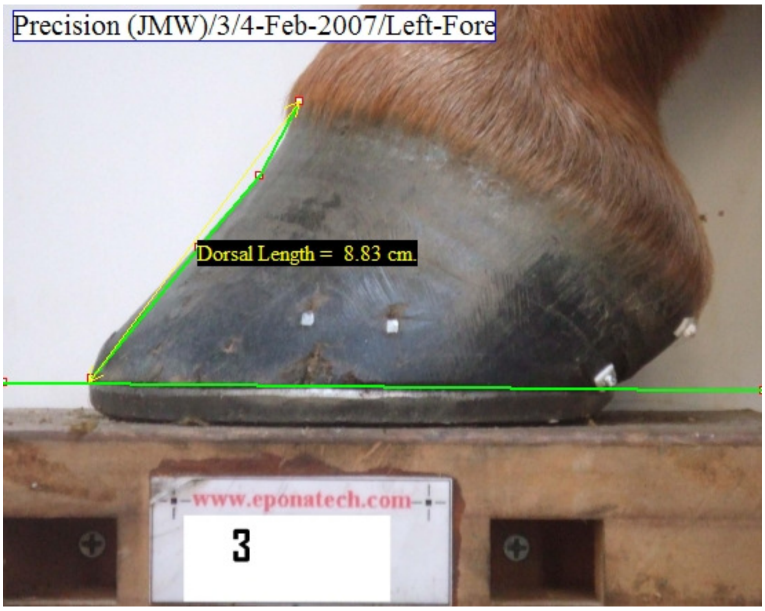

Figure 3-8 Output screen from Metron-PX™ demonstrating dorsal hoof length measured from a lateral digital photograph.

3.6.1.3 Heel Angle

The angle between the palmar aspect of the lateral heel at the level of the coronary band and the most palmar weight-bearing surface of the foot (Eliashar et al. 2004).

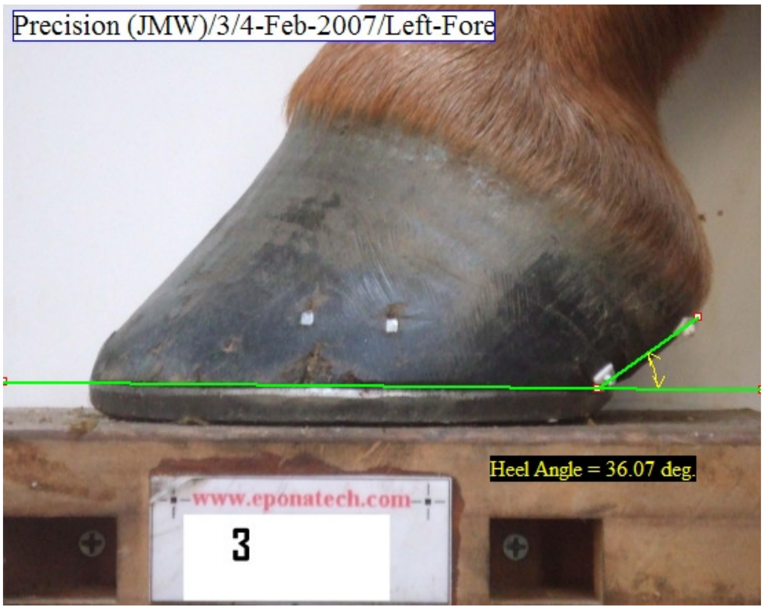

Figure 3-9 Output screen from Metron-PX™ demonstrating heel angle measured from a lateral digital photograph.

3.6.1.4 Heel Height

The vertical distance between the palmar aspect of the lateral heel at the level of the coronary band and the ground surface (Eliashar et al. 2004). In the shod horse the ground surface is taken as the interface between the shoe and the foot.

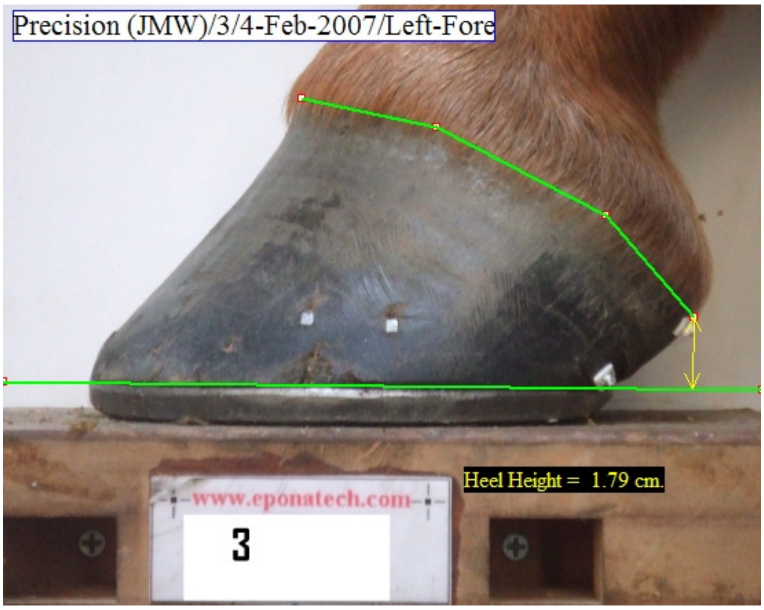

Figure 3-10 Output screen from Metron-PX™ demonstrating heel height measured from a lateral digital photograph.

3.6.1.5 Support Length

The horizontal distance between the dorsal-most and the palmar-most weight-bearing surfaces of the foot.

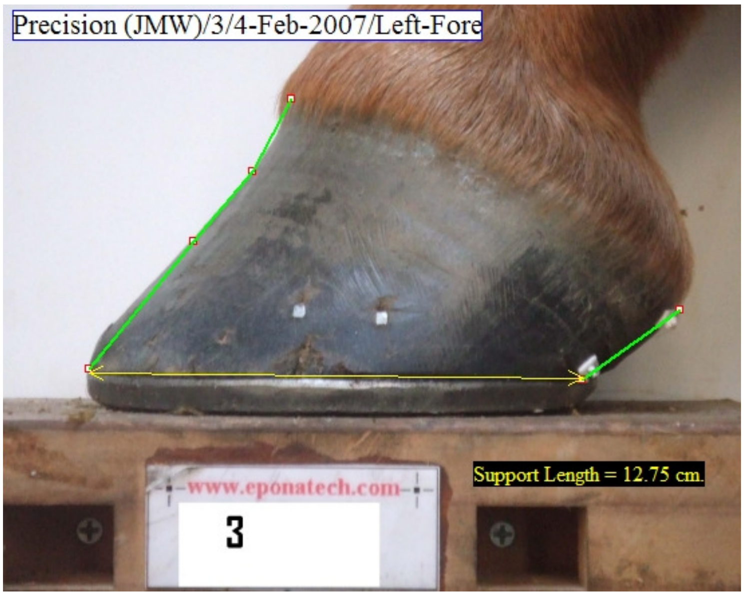

Figure 3-11 Output screen from Metron-PX™ demonstrating support length measured from a lateral digital photograph.

3.6.1.6 Heel Height/ Toe Height%

The heel height (Section 3.6.1.4) divided by the vertical distance between the dorsal coronary band and the ground surface (Eliashar *et al.* 2004) multiplied by 100. Equal to the height index (Eliashar *et al.* 2004) expressed as a percentage.

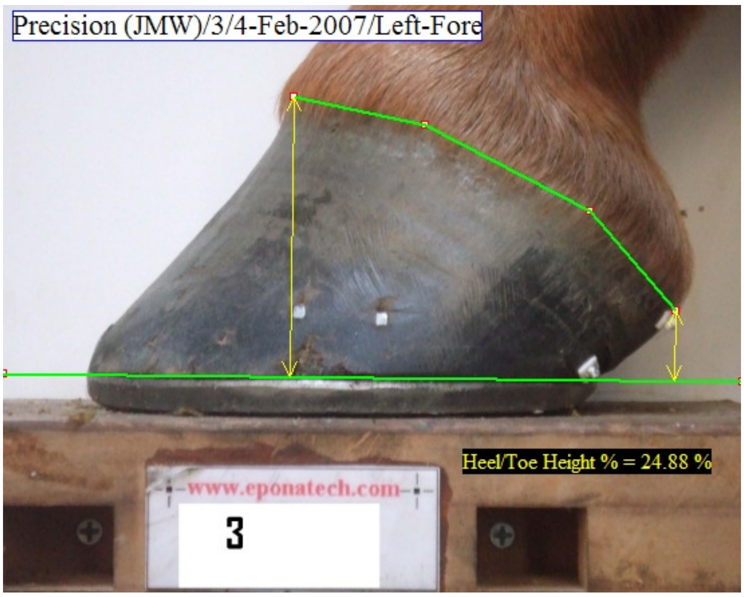

Figure 3-12 Output screen from Metron-PX™ demonstrating heel height/ toe height% calculated from a lateral digital photograph.

3.6.1.7 Hairline Angle

The angle calculated by Metron-PX™ from lateral digital photographs using four points that best approximate the shape of the coronary band. The angle is calculated by a software algorithm taking into account the lengths and angles of the three line segments made by joining the four points placed on the coronary band.

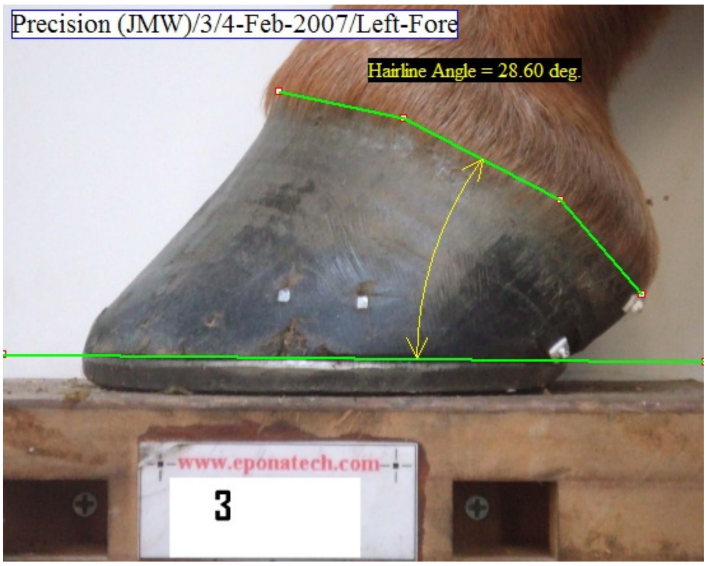

Figure 3-13 Output screen from Metron-PX™ demonstrating the hairline angle measured from a lateral digital photograph.

3.6.1.8 Coronary Band Angle

The angle between a straight line connecting the dorsal and palmar aspects of the coronary band and a line parallel to the ground surface (Eliashar *et al.* 2004).

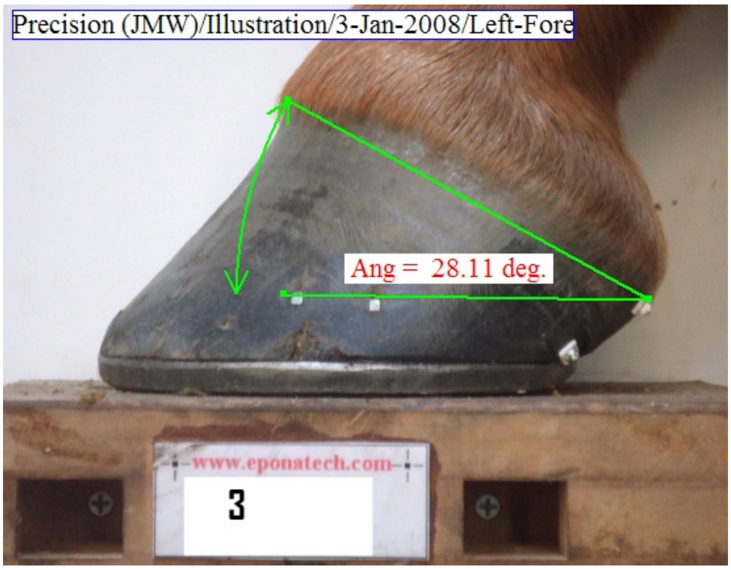

Figure 3-14 Output screen from Metron-PX™ demonstrating the coronary band angle measured from a lateral digital photograph.

3.6.2 Accuracy Study

Photographic and radiographic images were randomised and coded as for the precision study (Section 3.5). Coded photographic images were imported into Metron-PX™ version 3.0 and were analysed as for the precision study by VS1. Coded radiographic images were imported into Metron-PX™ version 3.0 and were analysed by VS1 with a combination of semi-automated (guided) and free analysis function. VS1 received a short tutorial (approximately 30 minutes) from the author on the use of the software for radiographic images and used the help function in the software when necessary. The same lengths, angles and percentages as in the precision study (Section 3.6.1) except for the hairline angle (not able to be measured radiographically) were recorded from each analysed image.

3.7 Data Analysis

3.7.1 Precision Study

Raw data from all images from each VS was directly exported from the Metron-PX™ software as a tab delimited txt file and electronically transferred to the author. The txt files were copied and pasted into blank Microsoft Word® documents and subsequently converted to tabulated form with the text to table function in the table menu of Microsoft Word 2003®. Once tabulated the raw data was copied and pasted into blank Microsoft Excel 2003® spreadsheet where all further data manipulation and calculations were performed.

Intra-and inter-operator agreement indices (AI) were calculated for the image analysis process alone using the data from the total number of images (80 images) analysed on two separate occasions by each VS. Intra- and inter-operator AIs were also calculated and for the combined image acquisition and image analysis processes for both self and non-self acquired images. Agreement indices were also calculated between the hairline angle and coronary band angle measurements within both operators to assess agreement between these measures. This was done because the hairline angle is automatically calculated by the software using the semi-automated (guided) analysis function whereas the coronary band angle (Eliashar *et al.* 2004) required the use of the free mark up function with two additional steps, making its measurement more time consuming and more prone to error.

The following equation was used to calculate AIs (Filippi *et al.* 1995):

$$AI = 1 - \left[\left| X_a - X_b \right| / \{ (X_a + X_b) / 2 \} \right]$$

Where an AI of 1 represents perfect agreement and an AI of ≥ 0.90 represents excellent agreement (Filippi *et al.* 1995; van der Vlugt-Meijer *et al.* 2006).

To calculate inter-operator agreement indices, X_a was the measurement obtained by VS1 and X_b the measurement obtained by VS2. Both first and second measurements were used for the calculations of inter-operator AIs. For the intra-operator AIs, X_a was the first measurement and X_b the second measurement obtained by the same VS. The mean and standard deviation (SD) was calculated for the inter-operator and intra-operator AIs. In addition, 95% limits of agreement (95% LOA) were calculated using the equation:

95% LOA= mean difference +/- 1.96 SD (Bland and Altman 1986, 2003).

The distribution of the differences between repeated measurements was evaluated graphically by plotting histograms (Figure 3-15) and the relationship between the differences and the mean by plotting scatter-plots (Bland-Altman plots) (Figure 3-16). These graphical representations were constructed for all analyses to check the assumptions of normally distributed data and that the magnitude of differences were independent of the mean and hence, the suitability of the 95% LOA method (Bland and Altman 1986, 2003).

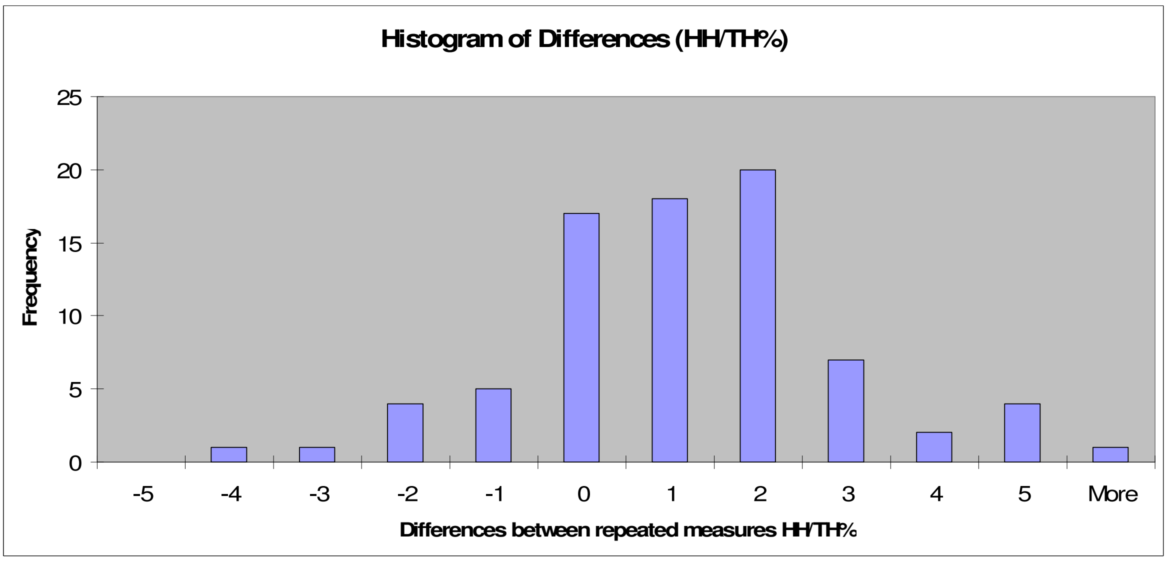

Figure 3-15 Example of histogram demonstrating approximately normal distribution of differences between repeated measures.

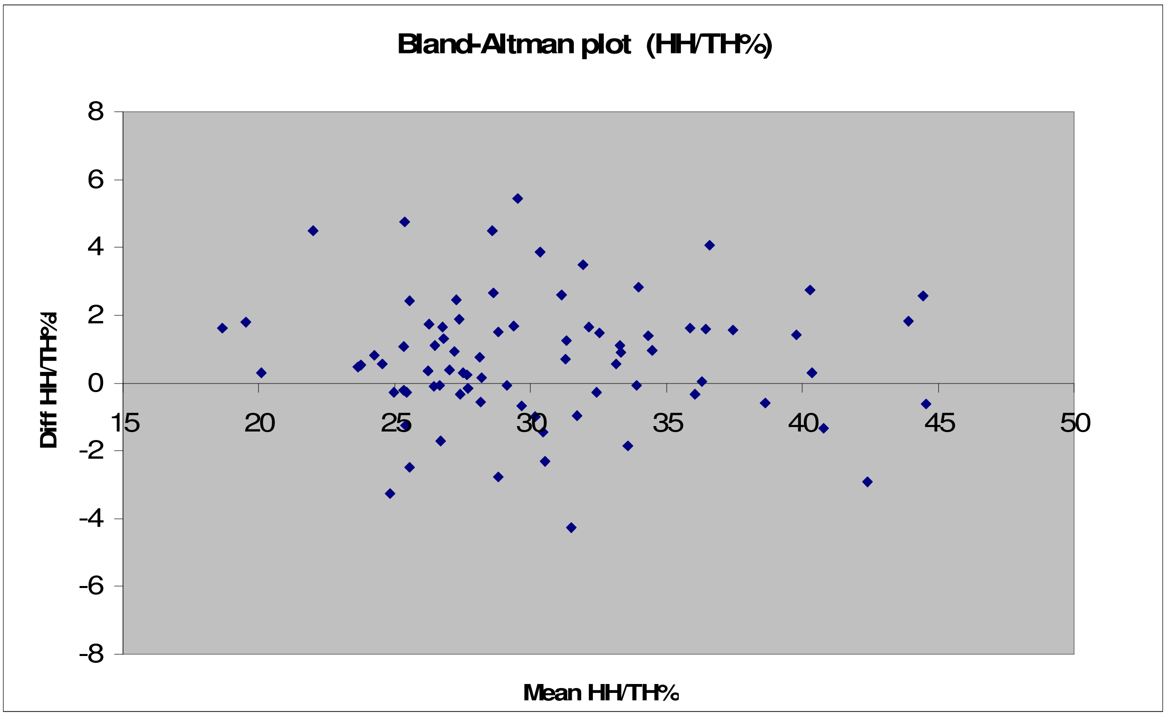

Figure 3-16 Example of scatter plot of difference against mean of repeated measures demonstrating independency of the differences from the mean values.

3.7.2 Accuracy Study

Agreement indices were calculated between the photographic and radiographic measurements using the same AI equation used in the precision study above, but substituting X_a with the radiographic measurement and X_b with the photographic measurement.

The distribution of the differences between repeated measurements was evaluated graphically by plotting histograms and the relationship between the differences and the mean by plotting scatter-plots (Bland-Altman plots). These graphical representations were constructed for all analyses to check the assumptions of normally distributed data and that the magnitude of differences were independent of the mean and hence, the suitability of the 95% LOA method (Bland and Altman 2003).

3.8 Target Values

As no data are currently available that quantify clinically significant changes in foot conformation, clinically useful target values were postulated to aid interpretation of the 95% limits of agreement. These target values were based on the smallest changes that a veterinary surgeon or farrier is likely to make to foot conformation during trimming or shoeing. These were estimated to be 5mm for length measurements, 5^{o} for angle measurements and 10% for percentage calculations. These estimates were chosen because a clinical research study such as this aims to inform clinical practice and so target values should be set to a level that would be relevant to a clinician. Smaller target values may be of little relevance to clinical practice (Holmes and Cockcroft 2008).

3.9 Summary

This chapter describes the methods used in this study of the precision and accuracy of a clinical measurement tool. The following two chapters present the results obtained with those methods.

4 Precision Study

4.1 Introduction

A very precise measurement is one that is repeatable/ reproducible with nearly the same value each time the measurement is repeated. Precision is affected by random error and the greater the error the less precise the measurements become. The three main sources of error in diagnostic testing are operator variability, subject variability and instrument variability (Holmes and Cockcroft 2008). In the context of this study, operator variability may occur at the time of marker placement, digital photography and during the image analysis process when the operator performs multiple mouse clicks at anatomical landmarks from which the software calculates lengths, angles and percentages. Subject variability would be expected to be negligible in this study given that the physical size and shape of the foot will not change due to growth or wear of the foot over the time necessary for repeated image acquisition. Instrument variability in this study pertains to the software's ability to calculate the same lengths, angles and percentages with the same image and operator input. In the context of this study the variability is assessed in the image analysis process alone and collectively in the combined image acquisition and analysis processes, with the difference between the two estimates representing the likely variability associated with the image acquisition process alone.

New (index) tests as well as gold (reference) standard tests are seldom perfect. Their precision varies, and limited precision adversely affects diagnostic accuracy (Quinn 1989). The importance of test precision to diagnostic accuracy means that the design of studies investigating the accuracy of diagnostic tests should include evaluation of the precision of the test and of the method used to do so (Bossuyt *et al.* 2003b).

Quantitative measurement of foot conformation has allowed useful information to be gathered on the correlation between foot conformation and the forces applied to the equine distal limb (Eliashar *et al.* 2004) and the clinical associations with musculoskeletal pathology (Kane *et al.* 1998; Anderson *et al.* 2004; Eliashar *et al.* 2004; Weller *et al.* 2006c). However little is known about the intra- and inter-operator agreement of the techniques utilised and agreement with other methods of quantitative foot measurement which can threaten the validity of study results.

The software evaluated in this study automatically calculates the hoof angle, dorsal hoof length, heel angle, heel height, support length, heel height/ toe height% and hairline angle following identification of scale markers and anatomical landmarks in each image. An additional measurement, the coronary band angle, was made using the more time consuming free analysis function of the software. This was done to assess the agreement between the automatically calculated hairline angle and that of the conventional coronary band angle (Eliashar *et al.* 2004), as would be measured from a lateromedial radiograph. This is of interest because the coronary band angle as represented by the angle between a single line connecting the coronary band at the dorsal toe and the lateral palmar coronary band at the heel and the ground has been shown to have a strong positive correlation with the force applied to the navicular bone (Eliashar *et al.* 2004). If high levels of agreement are identified between the two measurements they could be used interchangeably allowing for a reduction in operator time and effort.

In order to extend the existing knowledge on the effects of foot conformation on musculoskeletal pathology multi-centre prospective studies involving large study populations are needed to better identify the true relationship between foot conformation and pathology. The requirement for a large number of animals almost certainly means that multi-centre co-operation would be necessary. This would require foot conformation to be measured by multiple operators in different centres or by the same operator in the coordinating centre. In order to choose the best possible study design with the available resources knowledge of the practicability, intra- and inter-operator agreement and the effect of different veterinary surgeons involved in making the measurements is essential.

4.2 Specific Objective

In order to obtain measurements using the Metron-PX™ software there are two major processes involved: image acquisition (IAc) and image analysis (IAn). The objective of this part of the study was to estimate the precision of the individual and combined processes necessary to obtain conformational measurements of horses' feet, to examine the effect of different operators on the measurements made and to estimate the agreement between an automated measurement of the hairline angle and the conventional coronary band angle.

Image acquisition (IAc) involved two sequential processes: marker placement and digital photography (Section 3.4). The precision of the combined image acquisition process was evaluated in this study.

The objectives of this part of the study were to estimate:

1) The agreement between repeated measurements by a single operator (repeatability) for the IAn process alone and for the combined IAc + IAn processes.

2) The agreement between measurements made by different operators (reproducibility) for the IAn process alone and for the combined IAc + IAn processes.

3) The effect of the IAc process on the agreement indices (AIs) and 95% limits of agreement (LOA) from 1) and 2) above.

4) The effect of different operators performing the IAc and IAn processes (i.e. the effect of self and non-self acquisition on the AIs and 95% LOA). In order to ascertain whether it is necessary for the same person to perform both image acquisition and image analysis processes.

5) The agreement between the automated measurement for hairline angle and the conventional coronary band angle (Eliashar *et al.* 2004).

6) The practicability of the technique of image acquisition in unsedated Thoroughbreds in race training.

4.3 Materials and Methods

4.3.1 Animals

Twenty shod Thoroughbred (TB) horses in race training were randomly selected from one racing yard. All horses were in routine farriery care. Horses unwilling to stand still on low (6cm) wooden Eponatech^{TM} blocks without sedation were excluded from the study and a replacement chosen at random.

4.3.2 Image Acquisition

Lateral digital photographs were obtained with the horses (unsedated) standing with both front feet elevated on wooden blocks in a stable with a level concrete floor. The left front foot was photographed against a white background to provide contrast with the foot. The wooden blocks contained embedded scale markers and a plaque labelled with the veterinary surgeon's (VS) number and the animal's number. A digital camera (Fujifilm™ Finepix Z1, 5.1 megapixels) set at 3× optical zoom (maximum) was used to obtain digital photographs according to the guidelines provided by the manufacturer of Metron-PX™ (www.eponatech.com) with the heel bulbs visually aligned as is done for lateromedial radiography (Kummer *et al.* 2004) and with the camera supported at the same level as the foot using a bean bag tripod. Two veterinary surgeons (VS1 and VS2), working independently, alternately placed markers on the heel of the foot (Section 3.4.1.1), obtained a lateral digital photograph and then carefully removed all trace of the markers such that no physical evidence of their position remained on the foot. Each VS obtained two photographs of each foot in an alternating sequence (eg. VS1, VS2, VS1, VS2) with the first VS to photograph the foot chosen at random.

4.3.3 Image Analysis

Coded images were imported into Metron-PX™ version 3.0 and analysed independently by the two VSs on two occasions using the semi-automated (guided) analysis and free analysis function of the software. Both VSs received a short tutorial (approximately 30 minutes) from the author on the use of the software for photographic images and used the help function of the software when necessary. The following lengths, angles and percentages were recorded from each analysed image: hoof angle; dorsal hoof length; heel angle; heel height; support length; heel height/ toe height%, hairline angle and coronary band angle (Section 3.6).

4.3.4 Randomization and Concealment

All images were randomised and relabelled with a code number on two occasions using Adobe Photoshop 5.0 by the author. Code numbers were concealed from VS1 and VS2 such that they were unaware of the original animal's number or to the identity of the VS whom acquired the image.

4.3.5 Data Analysis

Intra- and inter-operator agreement indices (AIs) were calculated for the image analysis process alone using the data from the total number of images (80 images) analysed on two separate occasions and were also calculated for the combined image acquisition and image analysis processes for both self and non-self acquired images.

The following AI equation was used (Filippi *et al.* 1995; van der Vlugt-Meijer *et al.* 2006):

$$AI = 1 - \left[|X_a - X_b| \,/\, \{(X_a + X_b)/2\} \right]$$

Where an AI of 1 represents perfect agreement and an AI of ≥ 0.90 represents excellent agreement (Filippi *et al.* 1995; van der Vlugt-Meijer *et al.* 2006).

To calculate inter-operator agreement indices, X_a was the measurement obtained by VS1 and X_b the measurement obtained by VS2. Both first and second measurements were used for the calculations of inter-operator AIs. For the intra-operator AIs, X_a was the first measurement and X_b the second measurement obtained by the same VS. The mean +/- standard deviation (SD) was calculated for the inter-operator and intra-operator AIs. In addition, 95% limits of agreement (LOA) were calculated using the equation:

95% LOA= mean difference +/- 1.96 SD (Bland and Altman 1986, 2003)

Bland-Altman plots of the difference vs. mean of repeated measures were constructed for all analyses to check the assumptions of the data and the suitability of the 95% LOA method (Bland and Altman 1986, 2003) (Figure 4-1). Histograms of the differences between repeated measures were also constructed to check that the differences were from an approximately normal distribution (Figure 4-2).

4.4 Results

4.4.1 Study Animals

Horses were aged 2-12 years (median 5 years) and included 14 geldings, 3 colts and 3 fillies/ mares. One horse was excluded from the precision study because of unwillingness to stand for the repeated image acquisition process necessary for the study and another horse recruited at random.

4.4.2 Image Analysis Alone

The image analysis process for each image took approximately one minute to complete. Mean intra-operator agreement indices (AIs) for both VS1 and VS2 were > 0.90 with SDs < 0.10 for all hoof measurements recorded (Table 4-1). Mean inter-operator AIs were ≥ 0.90 with SDs < 0.10 for all hoof measurments recorded (Table 4-2). The 95% limits of agreement for hoof angle, heel height/ toe height%, hairline angle and coronary band angle were less than the target values for all intra- and inter-operator comparisons (Tables 4-1 & 4-2).

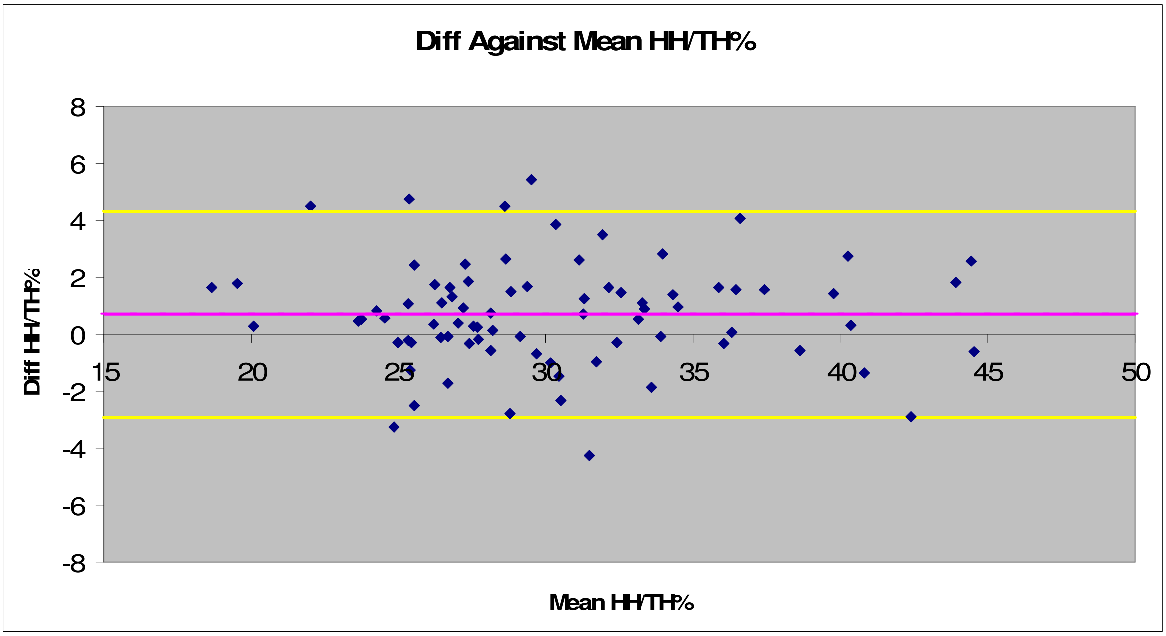

Figure 4-1 Example of Bland-Altman plot of VS1 intra-operator data for the heel height/ toe height% (HH/TH%) measurements, data points plotted are the differences between repeated measurements against the mean of the repeated measurements. The pink line represents the mean difference between repeated measurements and the yellow lines represent the upper and lower 95% limits of agreement.

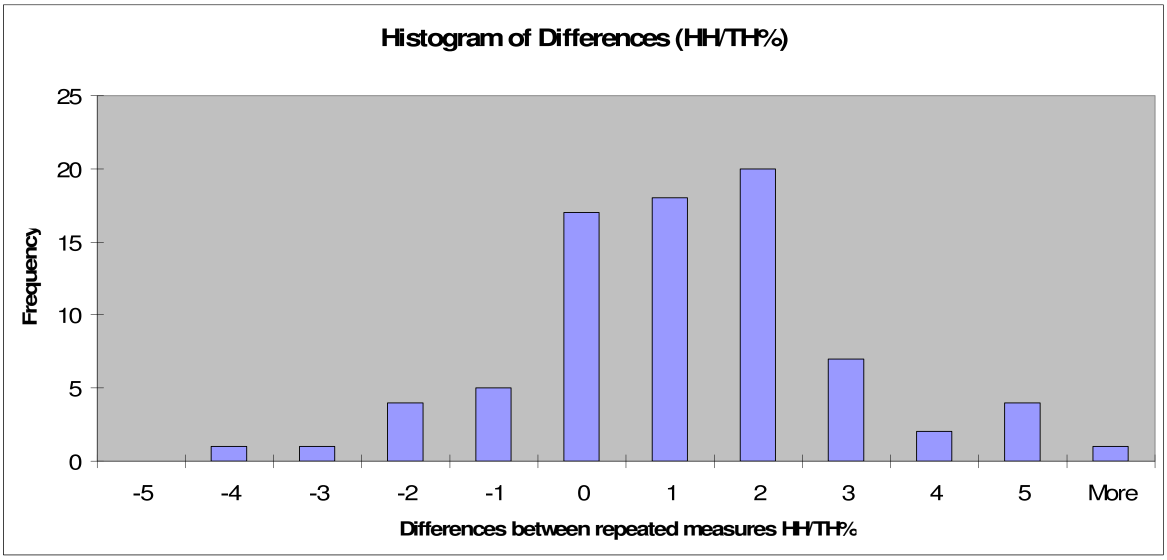

Figure 4-2 Example of histogram of differences between repeated measurements of HH/TH% by VS1 demonstrating an approximately normal distribution.

Table 4-1 Intra-operator AIs and 95% LOA of measurements for the image analysis process alone for VS1 and VS2.

INTRAOPERATOR IAn			
Hoof Measurement	**VS**	**Agreement Index (mean +/- SD)**	**95% Limits of Agreement**
Hoof angle	1	0.98 +/- 0.01	-2.47 to 1.75 (°)
	2	0.98 +/- 0.02	-1.11 to 2.95 (°)
Dorsal hoof length	1	0.97 +/- 0.03	-0.80 to 0.41 (cm)
	2	0.97 +/- 0.04	-0.85 to 0.82 (cm)
Heel angle	1	0.96 +/- 0.03	-3.17 to 3.17 (°)
	2	0.93 +/- 0.06	-6.48 to 5.89 (°)
Heel height	1	0.95 +/- 0.04	-0.27 to 0.27 (cm)
	2	0.91 +/- 0.08	-0.50 to 0.58 (cm)
Support length	1	0.99 +/- 0.02	-0.56 to 0.47 (cm)
	2	0.98 +/- 0.04	-0.77 to 0.88 (cm)
Heel height/ toe height (%)	1	0.95 +/- 0.05	-2.92 to 4.33 (%)
	2	0.92 +/- 0.08	-6.90 to 7.20 (%)
Hairline angle	1	0.95 +/- 0.03	-3.44 to 1.34 (°)
	2	0.96 +/- 0.04	-3.06 to 2.94 (°)
Coronary band angle	1	0.95 +/- 0.03	-3.37 to 1.27 (°)
	2	0.95 +/- 0.04	-3.18 to 3.30 (°)

Table 4-2 Inter-operator AIs and 95% LOA of measurements for the image analysis process alone between VS1 and VS2.

INTEROPERATOR IAn		
Hoof Measurement	**Agreement Index (mean +/- SD)**	**95% Limits of Agreement**
Hoof angle	0.98 +/- 0.01	-2.32 to 1.37 (°)
Dorsal hoof length	0.97 +/- 0.03	-0.43 to 0.73 (cm)
Heel angle	0.91 +/- 0.06	-6.90 to 1.94 (°)
Heel height	0.90 +/- 0.08	-0.55 to 0.18 (cm)
Support length	0.99 +/- 0.02	-0.54 to 0.42 (cm)
Heel height/ toe height (%)	0.90 +/- 0.08	-8.10 to 2.04 (%)
Hairline angle	0.93 +/- 0.05	-1.08 to 4.41 (°)
Coronary band angle	0.94 +/- 0.04	-1.45 to 3.90 (°)

4.4.3 Combined Image Acquisition and Image Analysis

The image acquisition process for each image took approximately two minutes to complete. Mean intra-operator agreement indices (AIs) for both VS1 and VS2 for both self (S) and non-self (NS) acquired digital photographs were ≥ 0.89 with SDs ≤ 0.10 for all hoof measurements recorded (Table 4-3). Mean inter-operator AIs for the combined process were ≥ 0.89 with SDs < 0.10 for all hoof measurements recorded (Table 4-4). The

95% limits of agreement for hoof angle, heel height/ toe height%, hairline angle and coronary band angle were less than the target values for all intra- and inter-operator comparisons (Tables 4-3 & 4-4).

Table 4-3 Intra-operator AIs and 95% LOA of measurements for the combined image acquisition and analysis processes for VS1 and VS2 for self (S) and non-self (NS) acquired images.

INTRAOPERATOR IAc + IAn			
Hoof Measurement	**(S/NS) VS**	**Agreement Index (mean +/- SD)**	**95% Limits of Agreement**
Hoof angle	S1	0.99 +/- 0.01	-1.61 to 1.90 (^{o})
	S2	0.99 +/- 0.01	-1.78 to 1.70 (^{o})
	NS1	0.99 +/- 0.01	-1.27 to 1.37 (^{o})
	NS2	0.99 +/- 0.01	-1.66 to 2.13 (^{o})
Dorsal hoof length	S1	0.97 +/- 0.02	-0.60 to 0.60 (cm)
	S2	0.98 +/- 0.01	-0.85 to 0.82 (cm)
	NS1	0.97 +/- 0.02	-0.77 to 0.65 (cm)
	NS2	0.97 +/- 0.04	-0.68 to 0.94 (cm)
Heel angle	S1	0.91 +/- 0.08	-7.17 to 7.47 (^{o})
	S2	0.91 +/- 0.05	-7.58 to 5.39 (^{o})
	NS1	0.92 +/- 0.05	-6.52 to 5.45 (^{o})
	NS2	0.89 +/- 0.07	-7.01 to 9.70 (^{o})
Heel height	S1	0.90 +/- 0.07	-0.54 to 0.43 (cm)
	S2	0.92 +/- 0.05	-0.54 to 0.36 (cm)
	NS1	0.90 +/- 0.07	-0.60 to 0.51 (cm)
	NS2	0.91 +/- 0.10	-0.47 to 0.64 (cm)
Support length	S1	0.96 +/- 0.03	-1.08 to 1.17 (cm)
	S2	0.97 +/- 0.02	-0.88 to 0.77 (cm)
	NS1	0.97 +/- 0.02	-0.81 to 0.69 (cm)
	NS2	0.96 +/- 0.05	-1.26 to 1.71 (cm)
Heel height/ toe height (%)	S1	0.90 +/- 0.06	-7.55 to 5.74 (%)
	S2	0.92 +/- 0.06	-7.47 to 5.02 (%)
	NS1	0.92 +/- 0.07	-7.36 to 6.57 (%)
	NS2	0.93 +/- 0.07	-5.27 to 6.49 (%)
Hairline angle	S1	0.94 +/- 0.04	-3.11 to 4.18 (^{o})
	S2	0.96 +/- 0.04	-2.28 to 3.26 (^{o})
	NS1	0.95 +/- 0.05	-4.03 to 3.99 (^{o})
	NS2	0.95 +/- 0.03	-2.97 to 2.89 (^{o})
Coronary band angle	S1	0.95 +/- 0.04	-3.04 to 3.72 (^{o})
	S2	0.96 +/- 0.05	-2.36 to 3.53 (^{o})
	NS1	0.95 +/- 0.05	-3.83 to 3.90 (^{o})
	NS2	0.95 +/- 0.04	-3.33 to 2.91 (^{o})

Table 4-4 Inter-operator AIs and 95% LOAs of measurements of the combined image acquisition and image analysis processes between VS1 and VS2 for self (S) and non-self (NS) acquired images.

INTEROPERATOR IAc + IAn			
Hoof Measurement	**Self / Non-Self acquisition**	**Agreement Index (mean +/- SD)**	**95% Limits of Agreement**
Hoof angle	S	0.99 +/- 0.01	-1.95 to 0.84 (°)
	NS	0.99 +/- 0.01	-2.00 to 1.23 (°)
Dorsal hoof length	S	0.98 +/- 0.02	-0.21 to 0.56 (cm)
	NS	0.97 +/- 0.02	-0.48 to 0.73 (cm)
Heel angle	S	0.90 +/- 0.06	-8.09 to 2.84 (°)
	NS	0.92 +/- 0.07	-7.08 to 2.43 (°)
Heel height	S	0.91 +/- 0.07	-0.51 to 0.13 (cm)
	NS	0.90 +/- 0.07	-0.54 to 0.17 (cm)
Support length	S	0.98 +/- 0.02	-0.73 to 0.55 (cm)
	NS	0.97 +/- 0.02	-0.83 to 0.76 (cm)
Heel height/ toe height (%)	S	0.90 +/- 0.07	-7.39 to 1.34 (%)
	NS	0.89 +/- 0.07	-8.14 to 2.14 (%)
Hairline angle	S	0.93 +/- 0.04	-0.33 to 3.74 (°)
	NS	0.93 +/- 0.04	-0.92 to 4.16 (°)
Coronary band angle	S	0.95 +/- 0.03	-0.75 to 3.20 (°)
	NS	0.94 +/- 0.03	-1.37 to 3.80 (°)

4.4.4 Agreement between Hairline Angle and Coronary Band Angle

Mean intra-operator agreement indices (AIs) for both VS1 and VS2 were ≥ 0.98 with SDs ≤ 0.01 for all comparisons (Table 4-5). Mean inter-operator AIs were ≥ 0.99 with SDs ≤ 0.01 for the comparisons (Table 4-6). The 95% limits of agreement for hairline angle and coronary band angle were well within target values for all intra- and inter-operator comparisons (Tables 4-5 & 4-6).

Table 4-5 Intra-operator AIs and 95% LOA for measurements of hairline angle and coronary band angle for the comparisons performed by both VS1 and VS2.

Intra-operator agreement between hairline angle and coronary band angle			
Comparison	**VS**	**Agreement Index (mean +/- SD)**	**95% Limits of Agreement**
Image Analysis alone	1	0.99 +/- 0.01	0.83 to -0.02 $(^{o})$
	2	0.99 +/- 0.01	0.46 to -0.54 $(^{o})$
Image Acquisition$_{(self)}$ + Analysis	1	0.98 +/- 0.01	0.83 to 0.08 $(^{o})$
	2	0.99 +/- 0.01	0.35 to -0.41 $(^{o})$
Image Acquisition$_{(non-self)}$ + Analysis	1	0.99 +/- 0.01	0.53 to 0.18 $(^{o})$
	2	0.99 +/- 0.01	0.36 to -0.45 $(^{o})$

Table 4-6 Inter-operator AIs and 95% LOA for measurements of hairline angle and coronary band angle for the comparisons performed between VS1 and VS2.

Inter-operator agreement between hairline angle and coronary band angle		
Comparison	**Agreement Index (mean +/- SD)**	**95% Limits of Agreement**
Image Analysis alone	0.99 +/- 0.01	0.52 to -0.16 $(^{o})$
Image Acquisition$_{(self)}$ + Analysis	0.99 +/- 0.01	0.51 to -0.09 $(^{o})$
Image Acquisition$_{(non-self)}$ + Analysis	0.99 +/- 0.01	0.38 to -0.07 $(^{o})$

4.5 Discussion

Excellent intra- and inter-operator agreement indices were identified for all the measurements made for the image analysis process alone when using Metron-PX™ to analyse foot conformation from lateral digital photographs. The 95% limits of agreement (LOA), however, may be more directly applicable to clinical activity as they quantify the differences that could occur between repeated measures using the described technique in 95% of feet. Unfortunately, minimum values for clinically significant differences in measurements of foot conformation have yet to be determined, making definitive interpretation of the 95% LOA data difficult. The approach chosen here was to compare the 95% LOA to the minimum change in length, angle or percentage that a veterinary surgeon or farrier is likely to make to an animal's foot conformation by the processes of trimming and/or shoeing. These were estimated to be 5mm for length measurements, 5^{o} for angle measurements and 10% for percentage calculations. When this is done, both the upper and lower 95% LOA are found to be less than these target values for hoof angle, heel height/ toe height%, hairline angle and coronary band angle for the intra-operator analyses of both veterinary surgeons (VSs) as well as for the inter-operator analysis between VSs. Both upper and lower 95% LOA were also less than the target values for intra-operator VS1 heel angle and heel height measurements whilst either the upper or lower 95% LOA were less than the target values for intra-operator VS1 dorsal hoof length and support length measurements and for inter-operator dorsal hoof length, support length, heel height and heel angle measurements. The 95% LOA for the remaining intra-operator analyses approached the target values. The results suggest that the image analysis process alone did not introduce unacceptable levels variation within or between operators of the software.

Excellent or near excellent intra- and inter-operator agreement indices were also identified for the combined image acquisition and image analysis processes. The upper and lower 95% LOA were less than the target values for all intra-operator and inter-operator comparisons of the hoof angle, heel height/ toe height%, hairline angle and coronary band angle whilst either the upper or lower 95% LOA were less than the target values for intra-operator heel height measurements and for inter-operator heel height, heel angle and dorsal hoof length measurements. These findings suggest that the combined processes necessary to obtain measurements of foot conformation with the software also did not introduce unacceptable levels of variation either within or between operators of the software. Despite excellent mean AIs (0.96 - 0.98) for intra-operator dorsal hoof length and support length measurements, the calculated 95% LOA were greater than our predetermined target values for length measurements (5mm). It is probable that given the magnitude of these two measurements and the magnitude of changes that occur at the toe region during routine trimming and shoeing (Kummer *et al.* 2006) compared to, for example heel height measurements, that greater target lengths would be acceptable for these two measurements. In addition, similar mean AIs and 95% LOA were obtained for the combined process regardless of whether or not the individual whom acquired the lateral digital photographs of the foot also analysed them. The implication of this finding is that images acquired by one VS can be analysed by a second VS with results comparable to image analysis performed by the first VS. Thus the software would appear to have a potential application in telemedicine by facilitating the remote analysis of images to provide an expert opinion on foot conformation, and additionally may be useful in multi-centre prospective studies where images may be acquired using the standardised technique by veterinary surgeons at separate centres and the images analysed by primary investigators at the co-ordinating centre.

Excellent mean agreement indices were identified for all intra- and inter-operator comparisons between the hairline angle and the coronary band angle with AIs $\geq$ 0.98 with

SDs ≤ 0.01. The 95% LOA indicate that in 95% of the cases measures of the hairline angle and the coronary band angle will lie within one degree of each other when performed by the same or different operators (Tables 4-5 & 4-6). This finding is particularly useful as the hairline angle is quickly and easily calculated from the semi-automated (guided) analysis function of the software whereas the free analysis function is required to calculate the conventional coronary band angle (Eliashar *et al.* 2004) which is more time consuming. This requires extra steps by the operator which may become very tedious and prone to operator fatigue and subsequent error if many images have to be analysed as would be the case in a large prospective multi-centre study.

Interestingly, intra-operator agreement for the image analysis process alone did not appear to be directly related to the amount of post-graduate experience of the VS as VS1 (resident level) attained equivalent or slightly higher AIs for the IAn process alone than VS2 (lecturer). The study also identified similar intra-operator mean agreement indices for the combined image acquisition and image analysis processes. Although the study was confined to just two operators the results suggest that highly repeatable and reproducible results can be achieved by the equine practitioners with a wide range in experience.

The study demonstrated that the steps required to make measurements from lateral digital photographs of the fore feet of horses were easily and rapidly accomplished in a clinical setting. Only one horse was excluded from the precision study due to lack of compliance with the image acquisition process. All other horses, including the Thoroughbred racehorses, which were not sedated, tolerated marker placement and photography whilst standing on wooden blocks on multiple occasions. On average the time taken to place the heel markers, position the feet on blocks and acquire a lateral digital photograph was approximately 1.5-2 minutes. As a result the acquisition and analysis of images of both front feet could be performed in 5-6 minutes.

Heel markers were used in the study for two reasons: heel markers were shown to reduce the variation in measurements involving the heel region in both shod and unshod horses in pilot studies at the University of Glasgow (White 2006); and radiopaque markers at predetermined anatomical sites were used in a related study which investigated the relationship between measurements of foot conformation made from radiographs and the tension in the DDFT and the force applied to the navicular bone (Eliashar *et al.* 2004). However, the heel markers used in this study (3mm diameter spheres) were smaller than those used by Eliashar and others (2004) (8mm wire) to provide a more focal marker for cursor placement on both radiographic and photographic images without compromising visibility.

This study focused on measurements made from lateral photographs and lateromedial radiographs of the feet because static measurements and the static appearance of the foot in a dorso-palmar plane are used commonly by the authors to guide trimming and shoeing of the foot. The software is also capable of making objective measurements from dorsal photographs and dorso-palmar radiographs but were not critically assessed in this study because dynamic foot balance, i.e. how the foot strikes the ground during motion, is used more frequently by the authors than static foot balance or foot dimensions in decision making about trimming and shoeing in a medio-lateral plane.

In conclusion, the findings of this study indicate that the entire process necessary for making measurements of foot conformation in the horse using the software Metron-PX™, is both practicable and produces measurements with excellent precision, especially for measurement of hoof angle, heel height/ toe height%, hairline angle and coronary band angle. It is probable that the limits of agreement for the other measurements recorded are also of acceptable precision but further work is required to aid in the definition of clinically significant differences in these measurements. As such the software has applications in both clinical practice and as a research tool.

5 Diagnostic Accuracy Study

5.1 Introduction

Studies of diagnostic accuracy have a common basic structure where one or more tests are evaluated with the purpose of detecting or predicting a target condition. The target condition can refer to a particular disease, a disease stage, a health status, or any other identifiable condition within a patient, such as staging a disease already known to be present, or a health condition that should prompt clinical action such as the initiation, modification, or termination of treatment (Bossuyt *et al.* 2003b). This part of the study is concerned with evaluating whether or not the measurements of foot conformation can be compared irrespective of the method used - specifically whether digital photography and radiography may be used interchangeably.

In diagnostic accuracy studies, the test under evaluation, referred to as the index test, is applied to a series of subjects. The results obtained with the index test are then compared with the results of reference standard, obtained in the same subjects. In this framework, the reference standard is the best available method for establishing the presence or absence of the target condition (Bossuyt *et al.* 2003b).

The term accuracy thus refers to the amount of agreement between the results from the index test and those from the reference standard. Diagnostic accuracy can be expressed in many ways depending on whether the measurement is on a continuous or categorical scale (Holmes and Cockcroft 2008). It has been suggested that the accuracy of continuous measurements may be assessed by correlation (Holmes and Cockcroft 2008), but it is noteworthy that correlation is blind to the possibility of bias and should be used only where there should be no consistent bias present for example when dealing with intra-observer variation using the same method of measurement. But when comparing two different methods of measurement, there may well be a consistent bias, and the correlation coefficient could be quite misleading (Bland and Altman 2003). The 95% limits of agreement (LOA) method avoids this potentially misleading approach of assessing methods of measurement (Bland and Altman 1986, 2003). How small the limits of agreement should be to support the conclusion that the methods agree sufficiently is a clinical, rather than a statistical decision, and was made in advance of the analysis. If the test provides results on a categorical scale, sensitivity-specificity pairs, likelihood ratios, diagnostic odds ratios and areas under receiver–operator characteristic (ROC) curves may be used and compared to the reference standard test (Bossuyt *et al.* 2003b).

5.2 Specific Objective

The objective of this section of the study was to assess the level of agreement between the measurements of foot conformation obtained from digital photography and subsequent image analysis with digital image processing software and the measurements obtained from digital radiography using a standardized radiographic technique (Kummer *et al.* 2004) and image analysis with digital image processing software (Rocha *et al.* 2004).

The reference standard used for the study was radiography and subsequent measurement with digital processing software because these techniques have received independent analysis of their precision and accuracy (Kummer *et al.* 2004; Rocha *et al.* 2004) and because of their widespread availability and frequent use in diagnosing and guiding the treatment of abnormalities of foot conformation and lameness.

If the measurements are found to agree sufficiently it would suggest the two methods may be used interchangeably in certain situations, i.e. those where it is not necessary to image the bony structures of the foot. The use of digital photography rather than radiography could reduce the cost to the client, the radiation risk to the veterinary personnel, and may be used in situations where radiographic equipment is unavailable, e.g. at the time of trimming and shoeing by a farrier. The greater availability of photographic compared with radiographic equipment could be utilised in the transmission of information for expert opinion or during follow up examinations between veterinary surgeons and referral centres, i.e. telemedicine, possibly saving time and the expense of the physical transportation of the animal.

5.3 Materials and Methods

5.3.1 Animals

Fifteen consecutive clinical cases undergoing lateromedial radiography of the front feet at the Weipers Centre Equine Hospital, for routine/ remedial farriery purposes or as part of a lameness investigation, were included in the study. The study was conducted between 1st January 2007 and 1st May 2007. Horses with feathers obscuring the coronary band were excluded from the study. Horses were sedated for radiography and underwent photography during the same sedation period.

5.3.2 Image Acquisition (digital photographs)

Lateral digital photographs were obtained of both front feet using the technique described in the Materials and Methods chapter (Section 3.4) by a senior clinical scholar (resident) at the Weipers Centre Equine Hospital. Image acquisition of digital photographs of the front feet formed the first part of the index test (Bossuyt *et al.* 2003).

5.3.3 Image Acquisition (radiographs)

Lateromedial digital radiographs were obtained of the front feet with both feet elevated on wooden blocks. Heel markers (Section 3.4.1.1) and a 7cm stainless steel wire dorsal hoof wall marker with its proximal extremity at the level of the coronary band were attached to the feet (Section 3.4.1.2). Computed radiographs were obtained with a gantry mounted x-ray unit (CPI Indico100 generator) and digital cassettes (CR MD 4.2) which were digitised (CR 35X) and exported as jpeg files for image analysis. Radiographs were obtained with a 100cm film focus distance (FFD) and a 8-10cm object film distance (OFD) with the x-ray beam centred 2 cm distal to the coronary band at a point mid-way between the dorsal and palmar borders (Kummer *et al.* 2004).

5.3.4 Randomization and Concealment

Photographic and radiographic images were randomised and coded on one occasion as described in the Materials and Methods Chapter (Section 3.5).

5.3.5 Image Analysis (digital photographs)

Coded photographic images were imported into Metron-PX™ version 3.0 as jpeg files and were analysed by one veterinary surgeon (VS1) with a combination of semi-automated (guided) and free analysis functions of the image processing software as for the precision study (Section 3.6.1). VS1 received a short tutorial (approximately 30 minutes) from the author on the use of the software for photographic images and used the help function in the software when necessary. The following lengths, angles and percentages were recorded from each analysed image: hoof angle; dorsal hoof length; heel angle; heel height; support length; heel height/ toe height% and coronary band angle.

5.3.6 Image Analysis (radiographs)

Coded radiographic images were imported into Metron-PX™ version 3.0 as jpeg files and were analysed by one veterinary surgeon (VS1) with a combination of semi-automated (guided) and free analysis function (Section 3.6.2). Image analysis involved performing a series of mouse clicks to define scale markers on the Eponatech block™ in the image followed by multiple mouse clicks on anatomical landmarks of the foot (Section 3.6.2). VS1 received a short tutorial (approximately 30 minutes) from the author on the use of the software for radiographic images and used the help function in the software when necessary. The following lengths, angles and percentages were recorded from each analysed image: hoof angle; dorsal hoof length; heel angle; heel height; support length; heel height/ toe height% and coronary band angle.

5.3.7 Data Analysis

Agreement indices (AIs) were calculated between the photographic and radiographic measurements using the same AI equation used in the precision study (Section 4.3.5).

The following AI equation was used (Filippi *et al.* 1995; van der Vlugt-Meijer *et al.* 2006):

$$\mathrm{AI} = 1 - \left[|X_a - X_b| \,/\, \{(X_a + X_b)/2\} \right]$$

Where an AI of 1 represents perfect agreement and an AI of ≥ 0.90 represents excellent agreement (Filippi *et al.* 1995; van der Vlugt-Meijer *et al.* 2006).

To calculate the agreement indices, X_a was the radiographic measurement and X_b the photographic measurement. The mean +/- standard deviation (SD) was calculated for the AIs. In addition, 95% limits of agreement (LOA) were calculated using the equation:

95% LOA= mean difference +/- 1.96 SD (Bland and Altman 1986, 2003)

Bland-Altman plots of the difference vs. mean of repeated measures were constructed for all analyses to check the assumptions of the data and the suitability of the 95% LOA method (Bland and Altman 1986, 2003) (Figure 5-1). Histograms of the differences between repeated measures were also constructed to check that the differences were from an approximately normal distribution (Figure 5-2).

5.3.8 Target Values

As no data are currently available that quantify clinically significant changes in foot conformation, clinically useful target values were proposed to aid interpretation of the 95% limits of agreement. These values were based on the smallest changes that a veterinary surgeon or farrier are likely to make to foot conformation during trimming and shoeing. These were estimated to be 5mm for length measurements, 5^o for angle measurements and 10% for percentage calculations. These estimates were chosen because clinical research studies such as this aims to inform clinical practice and so targets values were set to a level that would be relevant to a clinician. Smaller target values are likely to be of little relevance to clinical practice (Holmes and Cockcroft 2008).

5.4 Results

5.4.1 Study Animals

Horses were aged 1-18 years (median 11 years) and included 5 geldings and 10 fillies/ mares.

5.4.2 Accuracy Study

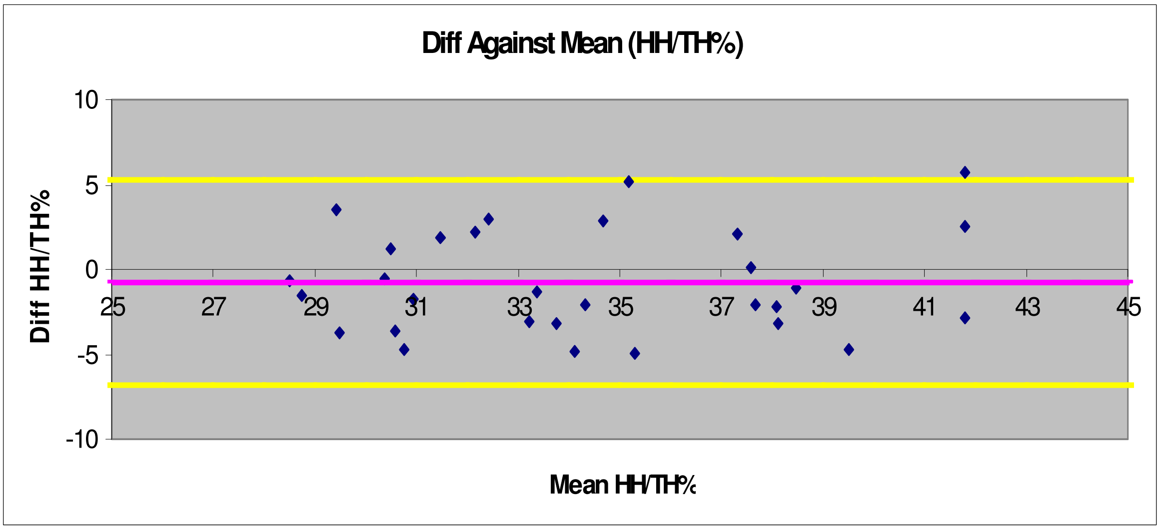

Figure 5-1 Example of Bland-Altman plot of accuracy data for the heel height/ toe height% (HH/TH%) measurements, data points plotted are the differences between radiographic and photographic methods of measurement against the mean of the measurements. The pink line represents the mean difference between methods of measurement and the yellow lines represent the upper and lower 95% limits of agreement.

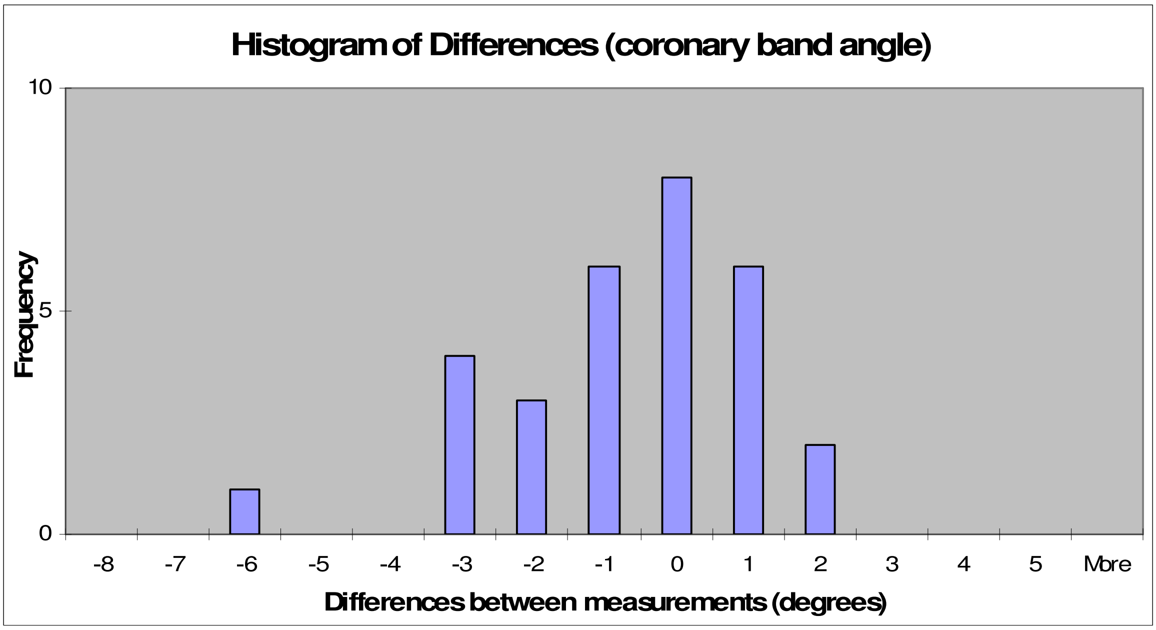

Figure 5-2 Example of histogram of differences between radiographic and photographic methods of measurement for coronary band angle demonstrating an approximately normal distribution of differences.

Mean AIs between photographic and radiographic measurements were ≥ 0.89 with SDs < 0.10 for all seven hoof measurements recorded (Table 5-1). The 95% LOA for heel height/ toe height% and coronary band angle were less than the target values for the method comparison (Table 5-1).

Table 5-1 Agreement indices, differences and 95% LOA between photographic measurements (index test) and radiographic measurements (reference standard).

ACCURACY			
Hoof Measurement	**Agreement Index (mean +/- SD)**	**Differences (rad-photo) (mean +/- SD)**	**95% Limits of Agreement**
Hoof angle	0.95 +/- 0.04	-2.42 +/- 1.99 (°)	-6.31 to 1.48 (°)
Dorsal hoof length	0.95 +/- 0.05	-0.12 +/- 0.74 (cm)	-1.57 to 1.32 (cm)
Heel angle	0.89 +/- 0.09	2.22 +/- 4.62 (cm)	-6.83 to 11.28 (°)
Heel height	0.89 +/- 0.08	-0.18 +/- 0.31 (cm)	-0.79 to 0.42 (cm)
Support length	0.95 +/- 0.06	0.36 +/- 0.90 (cm)	-1.40 to 2.13 (cm)
Heel/ toe height (%)	0.92 +/- 0.04	-0.74 +/- 3.07 (%)	-6.76 to 5.29 (%)
Coronary band angle	0.94 +/- 0.06	-1.21 +/- 1.69 (°)	-4.52 to 2.10 (°)

5.5 Discussion

Excellent mean agreement indices (AIs), i.e. ≥ 0.90, with small standard deviations were identified between the photographic and radiographic measurements for hoof angle, dorsal hoof length, support length, heel height/ toe height% and coronary band angle. In addition, the mean AIs for heel angle and heel height were close to the 0.90 value (mean AIs of

0.89) also with small standard deviations. The upper and lower 95% LOA were less than the target values for heel height/ toe height% and coronary band angle. The upper 95% limits of agreement (LOA) were less than target values for hoof angle and heel height. The high levels of agreement and acceptable limits of agreement between heel height/ toe height% and the coronary band angle measurements suggest that the radiographic and photographic methods may be used interchangeably for the measurement of these two parameters, which are clinically important given their strong correlations with the tensile force in the deep digital flexor tendon and the force applied to the navicular bone (Eliashar *et al.* 2004). It is also probable that the two methods may also be used interchangeably for hoof angle and heel height measurements as the 95% LOA for both of these measurements were close to target values.

Neither the upper nor lower 95% limits of agreement were within the predetermined targets values for heel angle, dorsal hoof length or support length. The results showed that for heel angle measurements the radiographic method recorded angles that were approximately two degrees greater than those from the photographic method, i.e. there was consistent bias (Table 5-1). This bias is likely to have been the result of operator variability in identification of the palmar most aspect of the lateral heel between radiographic and photographic images. This occurs because the soft tissues of the heel region tended to be relatively overexposed in radiographs leading to the appearance of a “floating” radiopaque marker (i.e. the marker did not appear to be in direct contact with the soft tissues of the heel) which may have lead to the operator rejecting this marker and selecting another point further dorsally on the image. Marker identification in radiographic images could be improved by enhancing the ability to alter contrast and brightness within the software programme and may be avoided when measurements are made with the computed or digital radiographic system with which the image was acquired. Alternatively, stipulating that the operator rigidly adhere to picking the marker at the lateral heel during image analysis might improve the levels of agreement for heel angle measurements between photographic and radiographic measurements. For dorsal hoof length and support length measurements there was no consistent bias apparent with the mean differences between the radiographic and photographic measurement being -0.12cm and 0.36cm respectively (Table 5-1) but with relatively large standard deviation values of 0.74cm and 0.90cm respectively. The value of the 95% LOA method is highlighted because despite excellent mean AIs for dorsal hoof length and support length measurements (both 0.95), the calculated 95% LOA were greater (approximately 2-4 times greater) than the predetermined target values for length measurements (Table 5-1). However, given the magnitude of changes that occur at the toe region during routine trimming and shoeing practices (Kummer *et al.* 2006) which directly influences both dorsal hoof length and support length measurements it is probable that greater target lengths would be acceptable. The greater 95% LOA between radiographic and photographic measurements for dorsal hoof length and support length is likely to have been a result of operator variability and may reflect difficulties in identifying the dorso-distal extent of the toe in radiographs due to relative overexposure, as this point is common to both measurements. Placing a radiopaque marker at this point and rigidly selecting it during image analysis might result in increased agreement. Enhancing the ability to alter contrast and brightness during the guided analysis of radiographic images would also improve identification of the dorso-distal tip of the toe.

Ideally for method comparison studies, such as the accuracy study reported here, each method should be performed twice on each subject, so that limits of agreement within methods as well as between methods separately may be determined (Bland and Altman 2003). Thus for measurements made from radiographs a second radiograph would have been required. This was not done because there was no clinical justification for the additional exposure to radiation that would result. However, information on the precision of radiographic measurements using Metron-PX™ has been published (Kummer *et al.* 2004; Rocha *et al.* 2004). The agreement between repeated measurements made from photographs was determined in the precision study (Chapter 4).

In conclusion, the results of the accuracy study suggest that for the measurement of heel height/ toe height% and coronary band angle it is not important whether the measurements are obtained using the radiographic or photographic methods described and can be used interchangeably. The same is also probably true for hoof angle and heel height measurements given their close proximity to target values. Care should be exercised however with the measurement of heel angle, dorsal hoof length and support length due to appreciably larger 95% LOA than the predetermined target values but agreement may be improved by instructing the operator to rigidly select the lateral heel marker in radiographic images, the provision of a marker at the dorsal-distal extent of the toe and through manipulation of contrast and brightness on radiographic images during image analysis.

6 General Discussion and Conclusions

High levels of intra- and inter-operator agreement were identified for the image analysis process alone and for the combined image acquisition and image analysis processes for all the measurements of foot conformation recorded. Excellent or near excellent mean agreement indices (AIs) were identified for all measurements and the upper and lower 95% limits of agreement (LOA) were less than the predetermined target values for hoof angle, heel height/ toe height% and coronary band angle for all the intra-operator analyses of both veterinary surgeons (VSs) as well as for inter-operator analysis between VSs. The 95% LOA were close to or less than the target values for all other measurements for the analyses. The results of the precision study suggest that the combined processes necessary to obtain measurements of foot conformation with the software did not introduce unacceptable levels of variation either within or between operators of the software. The results of the precision study also suggest that the image analysis (IAn) process introduced the majority of the variation both within and between operators because the AIs and 95% LOA for the IAn process alone were very similar to those for the combined IAc and IAn processes.

Similar mean AIs and 95% LOA were obtained for the combined processes regardless of whether or not the individual whom acquired the lateral digital photographs of the foot also analysed them. The implication of this finding is that images acquired by one VS can be analysed by a second VS with results comparable to image analysis performed by the first VS. Thus the technique described here has potential applications in telemedicine by facilitating the remote analysis of images to provide an expert opinion on foot conformation but may also be particularly useful in prospective multi-centre longitudinal studies investigating the relationship between foot conformation and musculoskeletal injury as a multi-centre design would be required to achieve the large sample numbers required for sufficient statistical power.

The precision study identified that the quicker and easier semi-automated hairline angle calculation closely approximates the more time consuming and tedious free calculation of the conventional coronary band angle in photographic images and thus can be used interchangeably. The use of the hairline angle as an approximation of coronary band angle would lead to a marked reduction in operator time and fatigue when performing image analysis on multiple photographic images and could lead to a reduction in operator error.

For the image acquisition process used in this study, the camera lens was aligned visually by each operator to be perpendicular to the bulbs of the heel as it is the method most likely to be used in a clinical setting. Any variation in measurements arising because of a failure to achieve perfect alignment was not specifically determined but contributed to the estimates of variation involved in the entire image acquisition process. The results showed that the variation introduced by the image acquisition process was less than that introduced by the image analysis process.

The study highlights the suitability of the 95% LOA method for agreement studies as despite excellent mean AIs for some parameters e.g. intra-operator dorsal hoof length and support length measurements for both VSs (0.96 - 0.98), the calculated 95% LOA were greater than the target values for length measurements (5mm). However, for these two parameters in particular, the magnitude of changes that occur at the toe region during routine trimming and shoeing (Kummer *et al.* 2006) make it probable that greater target lengths would be acceptable.

The levels of agreement and acceptable limits of agreement between heel height/ toe height% and the coronary band angle measurements suggest that the radiographic and photographic techniques may be used interchangeably for the measurement of these two parameters, which are clinically important given their strong correlations with the tension in the DDFT and the force applied to the navicular bone (Eliashar *et al.* 2004). It is also probable that the techniques may also be used interchangeably for hoof angle and heel height given that their 95% LOA were close to the target values.

Interestingly, intra-operator agreement for the image analysis process did not appear to be directly related to the amount of post-graduate experience of the VS as VS1 (resident level) attained equivalent or slightly higher AIs for the image analysis (IAn) process alone than VS2 (lecturer). The study also identified similar intra-operator mean agreement indices for the combined image acquisition and image analysis processes. Although the study was confined to just two operators the results suggest that highly repeatable/ reproducible results can be achieved by equine practitioners with varying levels of experience.

The study demonstrated that the steps required to make measurements from lateral digital photographs of the fore feet of horses were easily and rapidly accomplished in a clinical setting with only one horse being excluded from the precision study due to lack of compliance with the repeated image acquisition process. All other horses, including the unsedated Thoroughbred racehorses, tolerated repeated marker placement and photography.

There are other software programmes capable of making measurements from digital photographs (e.g. Image J, NIH image systems) but direct comparison between these and the Metron-PX™ system was not the aim of this study. However, the system used in this study has a number of strengths (purpose-designed programme for veterinarians and farriers, user friendly interface, semi-automated analysis, help function, purpose-designed blocks with scale markers, online support, archiving and data export functions) which contribute to ease and speed of use and could make the system attractive to practitioners as well as researchers. In conclusion, the findings of this study indicate that the entire process necessary for making measurements of foot conformation in the horse from digital photographs using adhesive heel markers and Metron-PX™ software is both practicable and produces measurements with excellent precision, especially for measurement of hoof angle, heel height/ toe height%, hairline angle and coronary band angle, and excellent accuracy for measurement of heel height/ toe height% and coronary band angle. It is not essential that the same individual to perform both image acquisition and image analysis processes which lends itself to the use of the technique in telemedicine and in research (prospective multi-centre studies). It is probable that the limits of agreement for the other measurements recorded also reflect acceptable precision and accuracy but further work is required to define clinically significant differences in these measurements. As such the software has applications in both clinical practice and as a research tool.

Appendices

Appendix 1- Client information sheet and consent form

Weipers Centre for Equine Welfare
University of Glasgow Veterinary School

UNIVERSITY *of* GLASGOW

INFORMATION SHEET

TITLE OF INVESTIGATION: Assessment of the diagnostic accuracy and the intra-observer and inter-observer variability of Hoof Measurements made by Veterinary Surgeons using a commercial software program (Metron-PX ®).

The Weipers Centre for Equine Welfare at the University of Glasgow are conducting research into the accuracy of computer based software for the measurement of the equine hoof. Achieving foot balance in an attempt to prevent and treat lameness has been the aim of farriers and veterinary surgeons since the horse was domesticated. Scientific evidence suggests that certain types of foot conformation are associated with increased loading of the limb. As a result of repetitive overload a number of tissues (bones, tendons and ligaments) may sustain injury.

Current methods employed for the measurement of hoof angles and lengths involve the use of hoof protractors, and either conventional or digital radiography using radiopaque markers on the foot. There are a variety of commercially available hoof protractors, which give information primarily on the dorsal hoof wall angle. The results of a recently published paper in a peer reviewed journal revealed that none of four types of hoof protractors was both reliable and accurate (Moleman et al 2005). Radiography with radiopaque markers placed on the foot is required to measure additional angles and lengths which have been shown experimentally to contribute to foot balance, e.g. heel height, toe height, coronary band angle.

You have been invited to volunteer your horse to participate in a research study looking at the ability of Metron-PX to accurately measure hoof angles and lengths from digital photographs of your horses' feet. In order to help you to understand what the investigation is about, please read the following information carefully. If there are any points that need further explanation, please ask a member of the research team. It is important that you understand what you are volunteering to do and are completely happy with all the information before you sign this form.

What is the purpose of the study? The purpose of this study is to investigate the accuracy of the software and investigate the intra-observer and inter-observer variability in the measurements of hoof angles and lengths obtained from lateral digital photographs of horses' feet.

Why has my animal been chosen? We are currently recruiting participants in this investigation from horses presenting at Glasgow University Veterinary School and Thoroughbred horses in training.

Do I have to take part? It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and you will be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason.

Appendix 1 Cont'd- Client information sheet and consent form

What will happen to my horse if I take part? If you decide to take part a veterinary surgeon will place your horses' feet on low (approx. 6cm) wooden blocks (much the same as for foot radiography) and lateral digital photographs taken using standard technique. Under no circumstances will the treatment of your horse altered in any way because of your agreement to participate in this study. It is important that you understand that radiographs will **NOT** be taken of your horse unless there is a clinical indication to do so.

What are the possible disadvantages and risks of taking part? There are no disadvantages of taking part in this study. The procedure is non-invasive, non-painful. The procedure and images of your horses' feet will be for purpose of this study and will be performed free of charge, however all medical and surgical veterinary procedures undertaken during hospitalization of your horse will be charged as per normal.

What are the possible benefits of taking part? The results of this study will provide valuable information on the ability of the software to accurately measure hoof angles and lengths and to quantify the variation within and between veterinary surgeons using the software. Your participation in this study will help us assess whether this form of measurement may be useful in a clinical situation. If the degree of variability is within acceptable limits one possible application for this software could be to allow veterinarians and farriers to seek advice from referral centres, such as the Weipers Centre, on how to trim horses' feet by the transmission of photographs via e-mail (telemedicine/telefarriery). This may reduce the necessity for transportation of horses to the Centre. If you are interested in further information, the research team will take the time to explain the results to you.

Will my taking part in this study be kept confidential? All information which is collected about you and your horse during the course of the research will be kept strictly confidential. All data that we collect is anonymous, so neither you nor your horse can be identified.

What will happen to the results of the research study? Results will be published in a peer-reviewed scientific journal once the study is completed. You or your horse will not be identified in any publication.

Appendix 1 Cont'd- Client information sheet and consent form

Assessment of the diagnostic accuracy of Metron-PX ® and the intra- and inter-obser variability of veterinary surgeons using Metron-PX ® for the measurement of hoof parameters in horses.

Consent Form

I ....

give my consent to the clinical studies which are outlined above, the aim, procedures and possible consequences of which have been outlined to me

Signature ....

Date ....

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