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Precision of ultrasonographic measurements of the equine suspensory apparatus

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Summary

This study aimed to investigate the precision of ultrasonographic measurements of the body and branches of the suspensory ligament and straight and oblique distal sesamoidean ligaments in equine fore- and hindlimbs. Ultrasonographic measurements of the horses suspensory apparatus are used for diagnostic purposes but their precision has not been assessed yet. Fourteen sound horses underwent ultrasonographic examination of all four limbs by two operators, twice. Longitudinal and transverse ultrasonographic images were used to measure the depth, width, circumference and cross-sectional area at locations determined by anatomical features. Inter- and intraoperator comparisons were made and their variability was evaluated using agreement indices and 95% limits of agreement. This method showed that the depth of the suspensory ligament branches from longitudinal images and their circumference from transverse images, and circumference of the straight sesamoidean ligament in the distal pastern were the more reliable of the measurements. All measurements of the suspensory ligament body and the oblique sesamoidean ligaments had a low reliability. The reliability of measurements of the size of the suspensory apparatus should be considered when making clinical judgements from ultrasonographic images. Particular caution should be exercised with measurements of the suspensory ligament body and the oblique distal sesamoidean ligament.

Keywords: horse / suspensory apparatus / ultrasonography / measurement / precision / diagnostic imaging

Präzision ultrasonographischer Messungen am Fesseltrageapparat des Pferdes

Ziel der Studie war es, die Präzision ultrasonographischer Messungen des Fesselträgerkörpers, der Fesselträgerschenkel und des geraden und der schrägen Gleichbeinbänder an Vorder- und Hinterbeinen des Pferdes zu untersuchen. Ultrasonographische Messungen des Fesseltrageapparats des Pferdes werden zu diagnostischen Zwecken zwar genutzt, ihre Präzision wurde aber noch nicht untersucht. In der Studie wurden 14 gesunde Pferde ultrasonographisch untersucht; zweifach an allen vier Gliedmaßen von zwei Untersuchern. Longitudinale und transversale Sonogramme wurden benutzt, um die Tiefe, die Weite, den Umfang und die Fläche an durch die Anatomie definierten Stellen zu bestimmen. Die inter- Unterschiede zwischen und von den Untersuchern (inter- and intraoperator variability – bzw. Reproduzier- und Wiederholbarkeit) wurde mittels des mean agreement index (Übereinstimmung der Mittelwerte) und 95% limits of agreement (Übereinstimmungsgrenzen) bestimmt. Die Messung der Tiefe im longitudinalen Sonogramm in den Bereichen der Fesselträgerschenkel und die Messung des Umfangs im transversalen Sonogramm in den Bereichen der Fesselträgerschenkel und des geraden Gleichbeinbandes in der distalen Fesselbeuge gehörten zu den zuverlässigeren Messungen. Alle Messungen im Bereich des Fesselträgerkörpers und der schrägen Gleichbeinbänder haben eine geringe Zuverlässigkeit, was bei klinischen Entscheidungen berücksichtigt werden muss.

Schlüsselwörter: Pferd / Fesseltrageapparat / Ultrasonographie / Größenmessung / Präzision / bildgebende Diagnostik

Introduction

The suspensory apparatus in horses consists of the suspensory ligament (SL), the distal sesamoidean ligaments (DSL) and the proximal scutum (*Barone* 1989). In the forelimb the SL arises from the distal row of the carpal bones and the proximal palmar aspect of the third metacarpal bone (MC III). In the hindlimb, it originates from the proximal plantar aspect of the third metatarsal bone (MT III) and a minor portion from the distal row of tarsal bones (*Gibson* and *Steel* 2002). Between the middle and distal third of the MC/MT III the body of the suspensory ligament divides into two branches. Before inserting on its corresponding proximal sesamoid bone (PSB), each branch sends out a thin extensor branch to the common extensor tendon. The DSLs (short, cruciate, oblique, straight) originate from the PSBs and insert in the proximal phalanx with the exception of the straight sesamoidean ligament that also inserts in the middle phalanx (*Budras* et al. 2003). The main function of the suspensory apparatus is to prevent excessive extension in the fetlock (*Denoix* 1994).

Suspensory ligament lesions are a common cause of lameness and ultrasonography is the imaging modality most frequently used to diagnose these changes (*Dyson* and *Genovese* 2010). Recently, magnetic resonance imaging studies confirmed that lesions of the DSL are an important cause of lameness localised to the pastern area (*Sampson* et al. 2007, *Smith* et al. 2008a).

Ultrasonography of tendons and ligaments is used initially to detect lesions and subsequently to monitor healing. Objective measurements such as depth, width, circumference and cross-sectional area, are commonly considered in conjunction with subjective evaluation of echogenicity (echo score) and fibre alignment (fibre alignment score) to determine lesion severity and thereby guide management during the acute and chronic phases of healing (*Smith* et al. 1994, *Reef* 1998, *Whitcomb* 2004, *Smith* 2008b, *Edinger*, 2010). The reliability with which individual parameters can be measured is an important consideration when deciding whether a change in ultrasonographic appearance is clinically significant but only limited information of this type is available (*Pickersgill* et al. 2001, *Zauscher* et al. 2012).

The aim of this study was to test the precision of ultrasonographic measurements of the body and branches of the suspensory ligament and the straight and oblique distal sesamoidean ligament in fore- and hindlimbs of horses. We hypothesized that the precision of the ultrasonographic measurements is acceptable for the branches of the SL and the straight DSL but not for the other structures of the suspensory apparatus.

Materials and methods

Horses

Fourteen horses, 8 mares and 6 geldings, were included in the study. Age ranged between 5-28 years (median 15 years), and included six Standardbreds, six Arabian mix breeds, one Warmblood and one Quarter horse. The average withers height was 156 cm (148–164 cm) and the bodyweight ranged from 360 to 545 kg (mean 466 kg). All horses underwent clinical examination (R.E.; J.Z.): none was lame or exhibited abnormalities of the suspensory apparatus. Horses were excluded from the study if evidence of disease of the suspensory apparatus was identified during the ultrasonographic examination.

Preparation of the limbs

The palmar/plantar aspect of all four limbs was clipped from the distal carpus/tarsus to the level of the heel bulbs. The clipped area was scrubbed for one minute using soap (Braunosan Vet, B. Braun Melsungen AG) and subsequently cleaned with alcohol (Hospisept, Lysoform Dr. Hans Rosemann GmbH) and covered with ultrasound gel (Sonosid, Asid Bonz GmbH).

Ultrasonographic technique

Two operators with comparable experience of musculoskeletal ultrasonography performed the examinations. Using a scanner (My Lab 5, Esaote) with a 7.5-12 MHz linear transducer a set of images of all limbs of all horses was acquired on two separate occasions at an interval of at least 24 hours and not longer than one week. The transducer was set at 12 MHz and gain at 76%. Field depth was 4 cm except when imaging the SL body in which instance a depth of 5 cm was required. The focal zone was adjusted as appropriate to the depth of the component of the suspensory apparatus being imaged. A standoff (Esaote) was used for all images except the following ones of the distal sesamoidean ligaments: longitudinal images acquired in the sagittal plane; transverse images; and longitudinal images of the oblique distal sesamoidean ligaments acquired from the palmaro/plantaro-lateral and palmaro/plantaro-medial aspects of the pastern.

The horses were fully weight bearing on all limbs and stood "squarely" during the ultrasonographic examination. To improve access to the pastern region, the horses were elevated by placing all feet on wooden blocks (23.5x7.5x15 cm).

The ultrasonographic images were saved in the scanners archive as they were acquired and recalled at the end of each examination for the operator to make the specified measurements. While the operators made the measurements, the left-hand corner of the scanner screen was obscured with tape so that the operators could not see the results. The images with the measurements were again saved in the archive, and the results were recorded in a spreadsheet once all measurements had been made. This ensured that the operators were not aware of the results during the measurement process.

Ultrasonographic images and measurements

Longitudinal (Fig 1, probe location 1) and transverse (Fig 1, probe location 2; Fig 2) images were acquired of the SL body from the palmar/plantar aspect of the limb. The thickness of the SL in the longitudinal image was measured at the level of the ramus communicans between the lateral and the medial

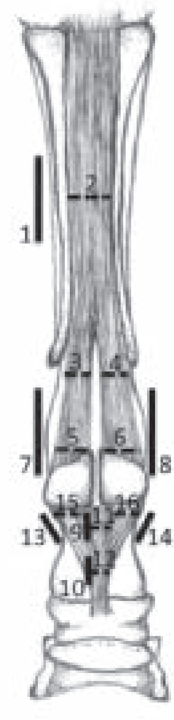

Fig. 1 Palmar /plantar view of the suspensory apparatus showing probe location for the transverse (broken line) and longitudinal (solid line) ultrasonographic images acquired in this study.
Palmare/ plantare Abbildung des Fesseltrageapparates mit eingezeichneten Schallkopfpositionen für die transversalen (unterbrochene Linie) und longitudinalen (durchgezogene Linie) Sonogramme dieser Studie.

palmar/plantar nerves. Depth, width, cross sectional area (CSA) and circumference (C) were measured in a transverse image acquired distal to the ramus communicans and slightly proximal to the bifurcation of the SL (*Schramme* et al. 2012).

The lateral and medial branches of the SL were imaged from the medial and lateral aspects of the limb, respectively (*Whitcomb* 2004, *Edinger* 2010). Two transverse (Fig 1, probe location 3–6) and one longitudinal image (Fig 1, probe location 7 and 8; Fig 3) were acquired of each branch. The distal extent of the longitudinal image included the insertion of the fibres of the SL into the PSB (*Ramzan* et al. 2012). The mediolateral depth was measured at the level of the apex of the PSB. The proximal transverse image (Fig 1, probe location 3 and 4) was located just distal to the extremity of the splint bone where the SL branches appeared oval in outline. The distal transverse image (Fig 1, probe location 5 and 6) was acquired at the level of the PSB apex and visualised the branch of the SL as triangular in outline. In both transverse images, depth, width, CSA and C were measured.

For the straight (sDSL) and oblique (oDSL) distal sesamoidean ligaments, proximal and distal longitudinal images were obtained in the sagittal plane from the palmar/plantar aspect of the pastern (Fig 1, probe location 9) and (Fig 1, probe location 10). In the proximal image the depth of the sDSL was measured at the level corresponding to the proximal border of the triangular rough area of the proximal phalanx. The oDSLs depth was measured level with the proximal border of the manica flexoria digitalis. In the distal image (Fig 4) the depth of the sDSL was measured level with the oDSLs insertion to the proximal phalanx. The depth of the oDSLs was measured at the level corresponding to the distal border of the manica flexoria digitalis.

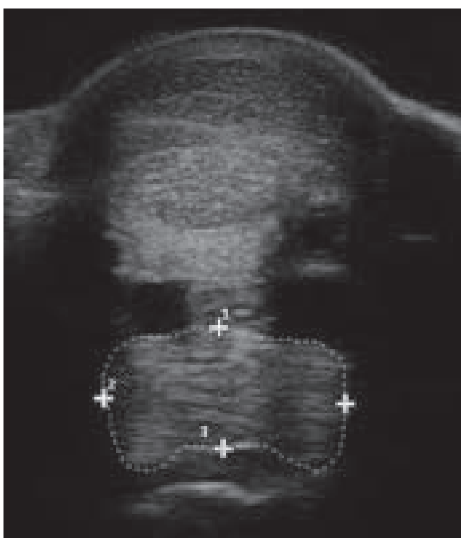

Fig. 2 Transverse image of body of the suspensory ligament (Fig 1, probe location 2) showing depth (1), width (2), cross sectional area and circumference measurements (3).
Transversales Sonogramm des Fesselträgerkörpers (Fig 1, Schallkopfposition 2) mit Messungen der Tiefe (1), Breite (2), Fläche und Umfang (3).

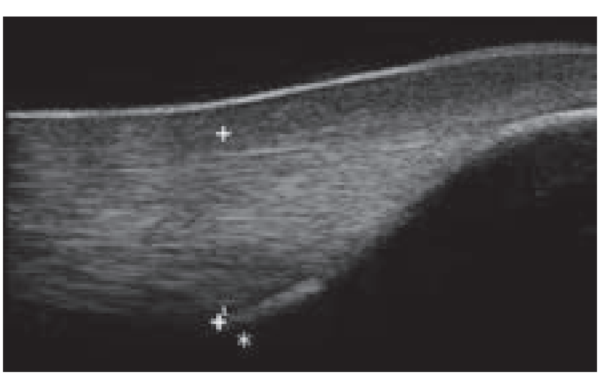

Fig. 3 Longitudinal image of branch of the suspensory ligament; images were obtained of medial and lateral branches (Fig 1, Schallkopfposition 7 and 8) and depth (1) measured at the extremity of the apex (*) of the proximal sesamoid bone.
Longitudinales Sonogramm des Fesselträgerschenkels, Sonogramme wurden vom medialen und vom lateralen Schenkel erstellt (Fig 1, Schallkopfposition 7 und 8) und die Tiefe wurde am höchsten Punkt der Apex (*) des proximalen Gleichbeins gemessen.

Two transverse images were also acquired from the palmar/plantar aspect of the pastern: the proximal image (Fig 1, probe location 11; Fig 5) where the superficial digital flexor tendon (SDFT) is still a single unit, the manica flexoria digitalis is visible, the sDSL is rectangular in outline and the oDSLs appear as a single structure between the first phalanx and the sDSL; the distal image (Fig 1, probe location 12) where the SDFT is divided into two branches and the sDSL is square in outline. The depth, width, CSA and C of the sDSL were measured in both transverse images. While in the proximal image the depth of the oDSLs was measured in midline where the two structures meet (Fig 1, probe location 11; Fig 5).

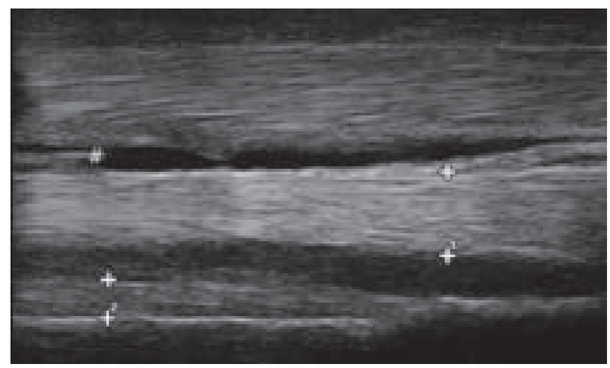

Fig. 4 Longitudinal image of distal aspect of the straight and oblique sesamoidean ligaments (Fig 1, probe location 10); depth of the straight sesamoidean ligament (1) was measured level with the distal extremity of the insertion of the oblique sesamoidean ligament to the proximal phalanx. The depths of the oblique sesamoidean ligaments (2) were measured at the level corresponding to the distal border of the manica flexoria digitalis (#).
Distales longitudinales Sonogramm des geraden und der schrägen Gleichbeinbänder (Fig.1, Schallkopfposition 10); die Tiefe des geraden Gleichbeinbandes (1) wurde in Höhe des Ansatzs der schrägen Gleichbeinbänder am proximalen Phalanx gemessen. Die Tiefe der schrägen Gleichbeinbänder (2) wurde in Höhe der distalen Grenze der manica flexoria digitalis (#) gemessen.

Further images of the oDSLs were obtained from the palmaro/plantaro-lateral and palmaro/plantaro-medial aspects of the pastern (*Whitcomb* 2004, *Carnicer* et al. 2012). The depth of the oDSL in longitudinal images (Fig 1, probe location 13 and14; Fig 6) was measured at the origin of the each ligament near the PSB base. Depth, width, CSA and C of the oDSLs were measured in transverse images (Fig 1, probe location 15 and 16) that were acquired just distal to the PSB base. In the transverse images the oDSL appeared round in outline.

Data analysis

Repeatability

Inter- and intraoperator agreement indices (AI) were calculated for the 46 different parameters that were measured in each limb and in each session. Each operator made 5152 measurements (46 different parameters measured on two occasions in 28 fore- and 28 hindlimbs, respectively). The following definition of the AI equation was used (*Filippi* et al. 1995, *van der Vlugt-Meijer* et al. 2006, *White* et al. 2008):

$$AI = 1 -[|Xa - Xb|/\{(Xa + Xb)/2\}].$$

For calculation of the interoperator AIs, Xa was the mean of the measurements made by the first operator, and Xb the mean of the measurements made by the second operator. For the calculation of the intraoperator AIs, Xa was the first measurement and Xb was the second measurement made by the same operator. The mean and standard deviation was calculated for the inter- and intraoperator AIs for fore- and hindlimb measurements.

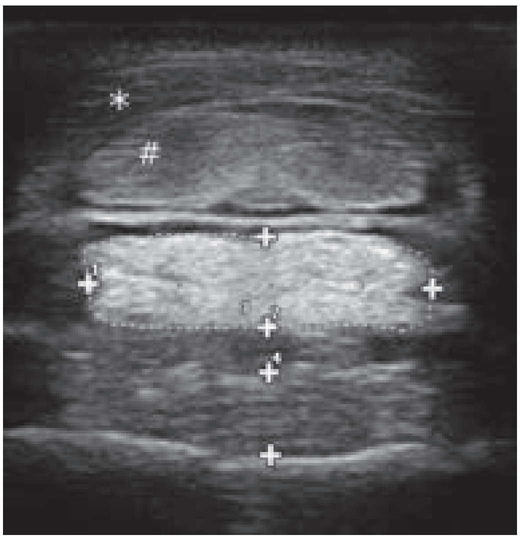

Fig. 5 Transverse image of proximal aspect of the straight and oblique distal sesamoidean ligaments obtained where the superficial digital flexor tendon (SDFT, *) is still a single unit and the deep digital flexor tendon (DDFT, #) is bilobed (Fig 1, probe location 11). Width (1), depth (2), cross sectional area and circumference (3) of the straight sesamoidean ligament, which is rectangular in outline at this level, were measured as illustrated. The oblique sesamoidean ligaments appear as one structure and depth (4) was measured where the two meet in midline.
Proximales transversales Sonogramm des geraden und der schrägen Gleichbeinbänder; das Bild wurde dort erstellt, wo die oberflächliche Beugesehne (*) noch eine einheitliche Struktur hat und die tiefe Beugesehne (#) eine bohnenähnliche Form annimmt (Fig. 1, Schallkopfposition 11). Breite (1), Tiefe (2), Fläche und Umfang (3) des geraden Gleichbeinbandes, das in dieser Höhe rechteckig ist, wurden wie gezeigt gemessen. Die Tiefe der geraden Gleichbeinbänder (4), die hier als eine einheitliche Struktur erscheinen, wurde in der Mitte, wo sie aufeinanderstoßen gemessen.

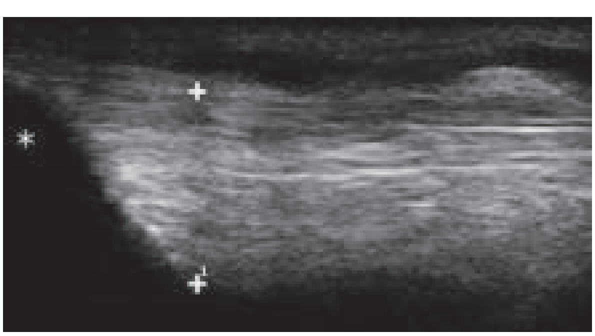

Fig. 6 Longitudinal image of medial and lateral oblique distal sesamoidean ligaments (Fig 1, probe location 13 and 14). Ligament depth (1) was measured near the origin at the base of the sesamoid bone.
Longitudinales Sonogramm der medialen und lateralen schrägen Gleichbeinbänder (Fig 1, Schallkopfposition 13 und 14). Die Bandtiefe (1) wurde nahe am Ursprung an der Basis des Gleichbeins gemessen.

An AI of 1 is considered a perfect agreement and AIs ≥ 0.90 represent excellent agreement (*van der Vlugt-Meijer* et al. 2006, *White* et al. 2008). An AI of 0.90 indicates that the absolute difference between two measurements amounts to 10% with respect to the mean of the two measurements.

In addition, 95% limits of agreement (LOA) were calculated with LOA being the mean difference±1.96 s.d. (*Bland* and *Altman* 1986, *Bland* and *Altman* 2003). To check the suitability of the 95% LOA method, Bland–Altman plots of differences vs. mean of repeated measurements were constructed to evaluate the distribution of the differences (*Bland* and *Altman* 1986, *Bland* and *Altman* 2003, *White* et al. 2008). The LOA are an estimate of the potential range of difference for a wider population between the measurements of two observers or the first and the second measurement of one observer, respectively. As no data for clinically significant enlargement of the suspensory apparatus have been validated, the 95% LOA were compared to target values (Table 1) that represented approximately 20 % of the mean values acquired in earlier and this study (*Redding* 1993, *Çelimli* et al. 2004, *Boehart* et al. 2010a, *Boehart* et al. 2010b, *Carnicer* et al. 2012).

Results

The inter- and intraoperator variability of ultrasonographic measurements of the suspensory apparatus in the 14 horses used in this study are summarized in Table 1. Single AIs for the branches of the SL and oDSL are reported because there was no difference in the mean index when calculated for separate or pooled lateral and medial indices. Mean AIs ≥ 0.90 are marked "+" in the table, whereas a mean AIs <0.90 are marked "-". Limits of Agreement (LOA) that were within the target values are indicated by "+"; those that were outside by "-".

All intra- and interoperator AIs were ≥ 0.9 and LOA were within target values for three measurements of the nine investigated parameters of the SL branches. These were depth and proximal width in the longitudinal ultrasonographic image and distal circumference in the transverse image. In comparison, the same criteria were fulfilled only by one parameter of ten for the sDSL, i.e. the measurement of distal circumference in the transverse image. The same criteria were not fulfilled by all five parameters for the SL body and by all eight for the oDSL.

AIs for proximal depth, proximal width and distal width of the SL branches measured in transverse images were ≥ 0.9 but LOA were outside target values for these parameters. The same pattern of results was seen for the sDSL for proximal depth, width, cross sectional area and circumference in the transverse image and distal width in the transverse image, for depth of the SL body in the transverse image, and for circumference of the oDSL in the transverse image obtained

Table 1 Intra- and interoperator agreement indices and 95% limits of agreement for measurements of the fore- and hindlimb													
				Forelimb					Hindlimb				
				AI		LOA			AI		LOA		
Measurement	LOA Target Value	Scan	Probe Location (Fig. 1)	Inter	Intra	Inter	Intra		Inter	Intra	Inter	Intra
Body of Suspensory Ligament												
Depth	1.8 mm	L	1	+	+ +	+	- -		+	+ +	-	+ -
Depth	1.6 mm	T	2	-	+ -	-	- -		-	- -	-	- -
Width	2.8 mm	T	2	+	- -	-	- -		+	+ +	+	- -
CSA	16 mm²	T	2	-	- -	-	- -		-	- -	-	- -
C	8.4 mm	T	2	+	+ -	+	- -	-	+	+ +	-	- +
Branches of Suspensory Ligament												
Depth	3.4 mm	L	7 and 8	+	+ +	+	+ +	+	+	+ +	+	+ +
Prox. depth	2.2 mm	T	3 and 4	+	+ +	+	- +	+	+	+ +	+	- +
Prox. width	2.2 mm	T	3 and 4	+	+ +	+	+ -	-	+	+ +	+	+ +
Prox. CSA	14.9 mm²	T	3 and 4	-	- -	-	- -	-	+	- +	-	- -
Prox. C	6.2 mm	T	3 and 4	+	+ +	+	+ +		+	+ +	+	+ +
Distal depth	2.7 mm	T	5 and 6	-	+ +	+	+ -		+	+ +	+	+ -
Distal width	2.1 mm	T	5 and 6	+	+ +	-	- -		+	+ +	-	- -
Distal CSA	22 mm²	T	5 and 6	+	+ -	-	- -		+	+ -	-	- -
Distal C	9.0 mm	T	5 and 6	+	+ +	+	+ +		+	+ +	+	+ +
Straight Distal Sesamoidean Ligament												
Prox. depth	1.0 mm	L	9	+	+ +	-	- -		+	- -	-	- -
Distal depth	1.4 mm	L	10	+	+ +	+	+ +		+	- +	-	- +
Prox. depth	1.3 mm	T	11	+	+ +	+	+ -		+	+ +	+	+ +
Prox. width	2.9 mm	T	11	+	+ +	+	+ +		+	+ +	+	+ -
Prox. CSA	12.2 mm²	T	11	+	+ +	-	+ -		+	+ +	-	- +
Prox. C	7.0 mm	T	11	+	+ +	+	+ +		+	+ +	+	+ -
Distal depth	1.4 mm	T	12	+	+ +	-	- +		+	+ +	-	+ -
Distal width	2.3 mm	T	12	+	+ +	+	+ -		+	+ +	+	+ +
Distal CSA	14 mm²	T	12	-	+ +	-	- -		-	- -	-	- -
Distal C	6.8 mm	T	12	+	+ +	+	+ +		+	+ +	+	+ +
Oblique Distal Sesamoidean Ligament – palmar/plantar												
Prox. depth	0.7 mm	L	9	-	- -	-	- -		-	- -	-	- -
Distal depth	0.6 mm	L	10	-	- -	-	- -		-	- -	-	- -
Thickness	0.8 mm	T	11	-	- -	-	- -		-	+ -	-	- -
Oblique Distal Sesamoidean Ligament – palmaro/plantaro-lateral and palmaro/plantaro-medial												
Depth	2.2 mm	L	13 and 14	+	+ +	+	- -		+	- +	-	- -
Depth	2.4 mm	T	15 and 16	+	- -	-	- -		-	- +	-	- -
Width	2.4 mm	T	15 and 16	-	- -	-	- -		-	- -	-	- -
CSA	20 mm²	T	15 and 16	-	- -	-	- -		-	- -	-	- -
C	7.4 mm	T	15 and 16	+	+ +	+	+ +		+	+ +	-	- -

AI=agreement index; 95% LOA=limits of agreement; Inter=interoperator; Intra=intraoperator
+=AI ≥ 0.90 or LOA were within the target values; -=AI <0.90 or LOA were not within the target value;
CSA=cross sectional area; C=circumference; L=longitudinal ultrasound image; T=transversal ultrasound image

from the palmaro/plantaro-lateral and palmaro/plantaro-medial aspects of the pastern.

No trend was seen in differences between intra- and interobserver agreement or between measurements of the same component of the suspensory apparatus in fore and hindlimbs.

Discussion

The results of this study show that ultrasonographic measurements of the branches of the suspensory ligament in particular but also the straight distal sesamoidean ligament can be made with a high level of precision. In contrast, none of the measurements of the body of the suspensory ligament and the oblique distal sesamoidean ligaments show equivalent precision. The precision of measurement of the proximal suspensory ligament has been described (*Zauscher* et al. 2012) but there are no similar reports relating to other components of the suspensory apparatus.

For the SL branches, intra- and interoperator AIs were excellent and 95% LOA within target values for three parameters, and AIs were excellent but LOA outside target values for a further three. In the absence of data to define the magnitude of clinically significant changes in size of the components of the suspensory apparatus examined, the setting of the target value for the 95% LOA (20% of the mean measurement) was based on the authors' clinical experience. Although this approach has value in indicating the clinical relevance of the level of agreement achieved between measurements, it is recognised that the target values may be inaccurate in a number of instances. More accurate target values, derived from higher quality evidence for clinically significant changes in size of the suspensory apparatus, would be likely to alter the results of comparison of LOA with target values for a number of parameters. In this regard, the parameters for which measurements exhibited excellent AIs but LOA were outside current target values, such as proximal depth, proximal width and distal width of the SL branches in transverse ultrasonographic images, would be of particular interest.

Distal circumference of the sDSL in the transverse image was the only parameter that was measured in the components of the suspensory apparatus studied other than the SL branches for which intra- and interoperator AIs were excellent and LOA within target values. However, the measurement of five parameters in the sDSL and one each in the SL body and oDSL exhibited excellent AIs but LOA were outside the target values set. Irrespective of whether or not these parameters are taken into account, the precision of ultrasonographic measurements of the SL branches was found in general terms to be greater than those of the sDSL. In comparison, the measurements of the SL body and of the oDSL were imprecise.

The levels of precision achieved for measurement of the SL branches were likely to have been related to the features used to locate the images and image quality. The mixed sizes and phenotypes of the population of horses used in this study required the use of anatomical features to identify each image location separately to ensure that the equivalent measurements were made in the different horses, rather than using a fixed distance from a single feature, for example, the accessory carpal bone or calcaneal tuber. This however, mimicked clinical practice. The features available for the SL branches (distal extremity of splint bone, apex of proximal sesamoid bone) were easy to identify, which would have improved the reliability of imaging compared to using landmarks that required an element of judgement to determine the exact image location, for example, the point of bifurcation of the SL body.

The absence of discrete overlying anatomical structures meant that edge shadowing did not hinder the visualisation of the medial and lateral margins of the SL branches. In contrast, production of this artefact by the overlying superficial and deep digital flexor tendons and the accessory ligament of the deep digital flexor tendon increased the difficulty with which the margins of the SL body could be visualised; the palmar digital neurovascular structures had a similar effect for the oDSL. On the other hand, the superficial location of the SL branches facilitated imaging because limb topography provided a good guide for transverse and longitudinal probe placement and therefore helped minimise off incidence artefacts and their effect on image quality. Off incidence artefacts particularly affected oDSL image quality because of the limited topographical references for these structures and their angled path relative to the limb axis. These effects were seen when making measurements from the longitudinal and transverse images obtained in the sagittal plane of the limb (proximal and distal depth from a longitudinal image, distal depth from a transverse image).

The precision of measuring the depth, circumference and cross-sectional area of the SL body was likely to have been affected by the difficulty experienced in defining the dorsal margin of the ligament in the transverse image. The reasons for this are not clear but may include the presence of soft tissues of similar acoustic impedance at this margin and heterogeneous echogenicity of the ligament due to the presence of muscle and adipose tissue amongst the collagen fibres (*Souza* et al. 2010, *Schramme* et al. 2012).

The absence of a trend for differences in agreement between repeated measurements made by the same operator compared to measurements made by different operators was a finding that will be of interest to clinicians because it suggests that measurements made by different individuals may be compared without introducing bias (for example, when monitoring a patient), at least if the imaging protocol used in this study is followed. This is a preliminary observation however and requires confirmation by means of a larger study, which could also usefully investigate whether agreement is affected by operator experience or the presence of lesions of the suspensory apparatus. It should be noted that particular care was taken to standardise positioning of the horses to minimise the influence of changes in load on morphology of the suspensory apparatus (*Reef* 1998). The similarity in agreement irrespective of whether measurements where obtained from fore or hindlimbs was anticipated, given the similarities in anatomy of the SL body and branches, sDSL and oDSLs and imaging practicalities for these limbs.

Circumference and cross-sectional area were generated from a single tracing and therefore the better agreement of measurements of circumference compared to area simply reflected their mathematical relationship.

Conclusion

The use of a mixed population of horses and of only two operators are aspects of an experimental design that may have affected the results obtained in this study, and therefore the conclusions made should be regarded as preliminary, pending the conduct of studies utilising more uniform horse populations and greater numbers of operators. Nevertheless it was evident that the precision of measurements of the SL body, SL branches, sDSL and oDSL from ultrasonographic images varies between structures and between parameters, and that measurements made by different operators can be regarded as similarly precise. Measurements of selected parameters of the SL branches and the sDSL were sufficiently precise to support their clinical use for identification of pathological change. It is recommended that caution is exercised in interpreting the other parameters because of the level of variability encountered when using ultrasonography for measuring them.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

The study is part of the doctoral theses of Johanna M. Zauscher, which is still in progress.

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