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The equine diaphragm: A novel technique for repeatable ultrasound measurement

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Abstract

The diaphragm is an important respiratory muscle, playing a key role during exercise. In humans, diaphragm thickness increases in response to training and is correlated with inspiratory strength. In order to assess respiratory strength in the horse, new evaluation techniques are required and measurement of diaphragm thickness, in a non-invasive and repeatable manner, is a possible approach. The purposes of this four-part, prospective, pilot study were to develop and describe a repeatable method to measure the diaphragm thickness, using ultrasonography, in Thoroughbred racehorses. A standardized technique was developed whereby the ultrasound transducer was positioned 1 cm below a line between the cranioventral aspect of the tuber coxae and olecranon. The diaphragm thickness was measured on three occasions 1 week apart, by a single observer to determine the intraobserver repeatability, and by a second observer on one occasion to assess interobserver reproducibility. The diaphragm was observed in all intercostal spaces (ICS) from 7 to 17 on the left side, and 6 to 17 on the right side in a single horse. The thickest measurement (1.42 cm), obtained from 11 horses, was at ICS 11 on the left-side during inspiration. The narrowest measurement (0.56 cm) was obtained at ICS 16 on the right-side during expiration. There was no significant difference between the measurements obtained by a single observer on three occasions ($P < .05$). This is the first study to provide a detailed description of ultrasonographic imaging and measurement of the equine diaphragm. The novel technique developed to position the ultrasound transducer in a standardized location allowed examination and measurement of the diaphragm with good repeatability.

KEYWORDS
diaphragm thickness, horse, respiratory muscle, respiratory strength, ultrasound

1 | INTRODUCTION

The diaphragm is one of the primary respiratory muscles,[1] working principally during inspiration,[2] and has an important role in exercise. Currently there are no published techniques to measure, or assess the function of, the equine diaphragm in a clinical setting. Previously

Abbreviations: brpm, breaths per minute; ICS, intercostal space; IMT, inspiratory muscle training.

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reported methods investigating the equine diaphragm include invasive measurement of blood flow and oxygen extraction in research horses,[3] providing a global assessment of respiratory function, or have focused on the imaging diagnosis of a diaphragmatic rent.[4] A new technique for non-invasive and repeatable imaging assessment of the diaphragm in the equine athlete is required, and will enable evaluation of the response of the respiratory muscles to exercise and training.

The diaphragm is a dome-shaped structure, with a large tendinous center and a muscular periphery.[5] The oxygen requirements of the equine diaphragm during strenuous exercise are equivalent to those of the vigorously working locomotor muscles.[6] The increases in both blood flow (23-fold) and oxygen extraction (threefold) of the diaphragm, recorded during exercise evaluation in ponies, were correlated with running speed.[3] Overall, the respiratory musculature commands a similar proportion (~14-20%) of cardiac output, in both horses and humans, during maximal exercise.[7]

The diaphragm has been shown to fatigue during exercise in humans, despite the described increases in blood flow and oxygen uptake.[8] Exercise-induced diaphragmatic fatigue in humans is associated with a respiratory muscle metaboreflex,[9] whereby systemic blood flow is redistributed away from the locomotor muscles and redistributed to the diaphragm,[8,9] which hastens the development of locomotor fatigue and contributes to reduced performance.[2,9] The application of specific inspiratory muscle training (IMT) has been shown to delay the onset of this respiratory muscle metaboreflex.[10] Currently it is unknown whether this exercise-induced diaphragmatic fatigue and respiratory muscle metaboreflex occur in horses.

Several methods for assessing the strength of the respiratory musculature have been developed in humans. These include measurement of diaphragm thickness, maximal inspiratory mouth pressures, sniff nasal pressure, esophageal, gastric, and trans-diaphragmatic pressures.[11] Many of these investigations involve volitional tests and some are considered too invasive for routine clinical use in horses. Measurement of diaphragm thickness has been performed in humans using a wide range of imaging modalities including radiography, fluoroscopy, CT, MRI, and ultrasonography.[12] Diaphragm thickness, measured non-invasively using ultrasonography, is correlated with diaphragm strength in humans[13,14] and both have been shown to increase with conventional exercise training[15] and following IMT.[16] Current literature on equine diaphragm thickness is from the measurement of postmortem specimens, where examination of the costal part of the diaphragm showed the mid portion to have the greatest thickness and force generating potential (described as medial in the reference).[5]

This study is part of a larger research theme to develop and evaluate methods of assessing respiratory muscle strength in horses,[17] in order to evaluate the response of the respiratory muscles to exercise and training. The aim of this study was to establish whether a non-invasive and repeatable method of measuring diaphragm thickness by ultrasonography was possible. The objectives were to describe the locations where the equine diaphragm can be imaged ultrasonographically, to describe the normal ultrasonographic features of the equine diaphragm, to determine the location of the thickest portion of the equine diaphragm and to assess the repeatability and reproducibility of measurements of the thickest portion of the equine diaphragm.

2 | MATERIALS AND METHODS

2.1 | Study design

The study was a four-part, prospective pilot design. The study was approved by the University of Bristol Animal Welfare and Ethical Review Board.

2.1.1 | Part 1: Diaphragm location, ultrasonographic features, and development of a standardized technique

A single Thoroughbred racehorse in active training for National Hunt racing was examined by observer 1 (L.F.; American College of Veterinary Sports Medicine and Rehabilitation-certified veterinary sports medicine clinician). The horse was randomly selected by the author, based on availability, and was required to be race fit and clinically healthy. Ultrasonography was performed without sedation, and the horse was restrained using a headcollar and lead rope only. During the examination the horse was stood square and still, undertaking even breathing at a rate between 6 and 14 breaths per minute (brpm).

A portable ultrasound machine (GE LOGIQ™ e R7, GE Healthcare, UK) was used for all examinations. The following standardized settings were used based on preliminary investigations to provide optimal images (*unpublished data*): 43 mm footprint linear transducer (6-13 MHz), frequency 13 MHz, harmonics on, depth 8 cm, 2 focus points (depth ~2 cm and ~4 cm), gain 46, grey map D, dynamic range 69, and frame rate of 25 frames/s. Contact was achieved by dampening the skin with isopropyl alcohol applied with a gauze swab.

Each intercostal space (ICS) was systematically interrogated from dorsal to ventral using ultrasonography to determine the region in which the muscular diaphragm could be observed. The diaphragm was identified dynamically as "the most superficial structure that was obliterated by the leading edge of the lung upon inspiration."[18] The dorsal border of the diaphragm was defined as the lung border at peak inspiration. The ventral border of the diaphragm was defined as the region of insertion of the diaphragm on the thoracic wall. A marker was placed at the dorsal and ventral border within each ICS to delineate the area in which the diaphragm could be easily observed. This was repeated for both the left and right sides and the horse photographed. Markers were only placed when the horse was subjectively breathing at an even respiratory rate (6-14 brpm) and depth. If the diaphragm was not observed, the ICS was left unmarked.

Based on the area within which the diaphragm could be observed, a standardized technique was developed using external anatomical landmarks to guide positioning of the ultrasound transducer to enable repeated observation and measurement of the diaphragm. A marker

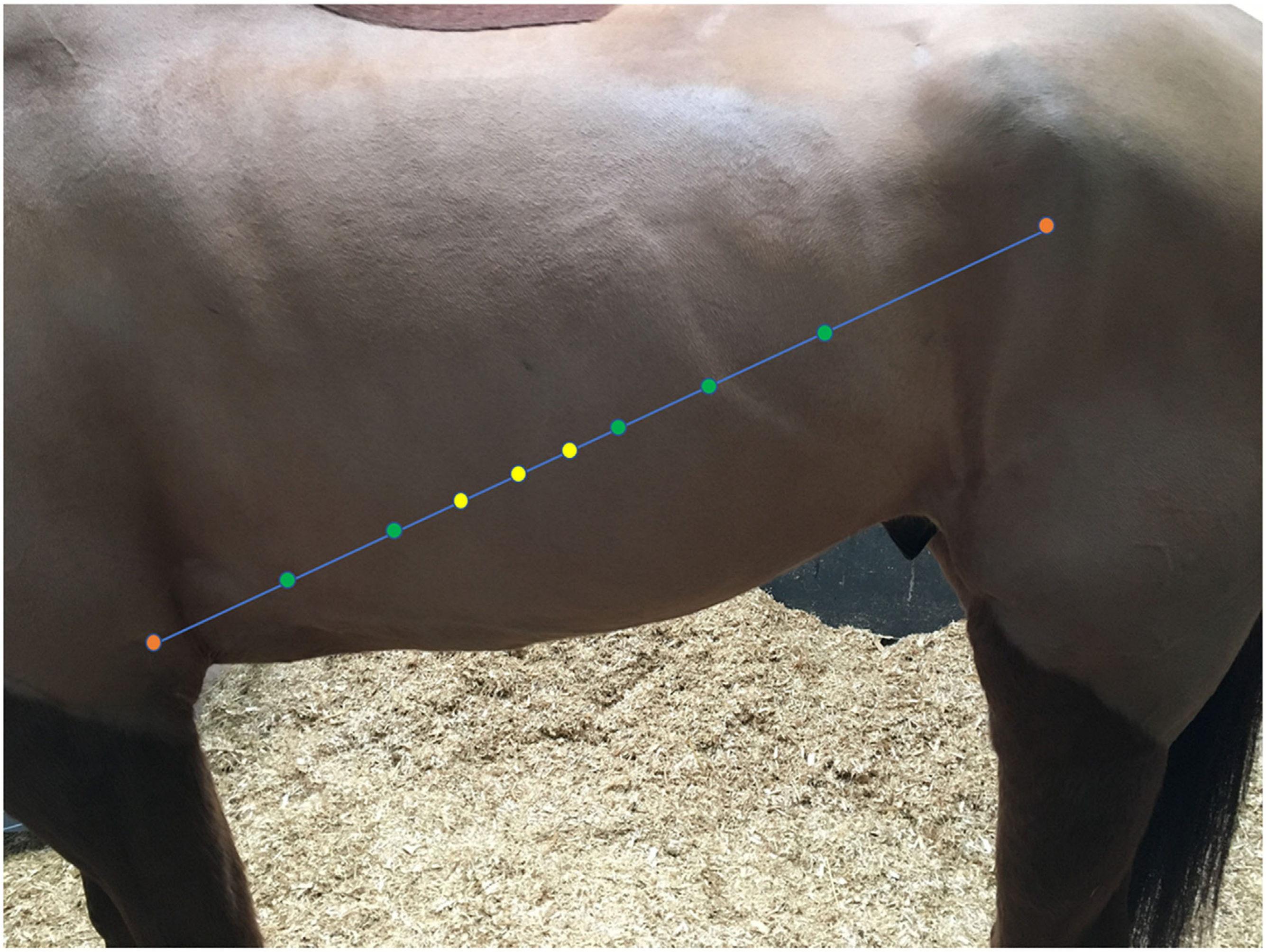

FIGURE 1 Photograph illustrating the markers placed on the horse to guide transducer placement during ultrasound examination. The orange markers are placed the cranioventral aspect of the tuber coxae, and the proximal margin of the olecranon. The green markers are placed on every second intercostal space, in a straight line between the two orange markers. The yellow markers are placed on intercostal spaces 10, 11, and 12 [Color figure can be viewed at wileyonlinelibrary.com]

was placed on the cranioventral aspect of the tuber coxae, and the proximal margin of the olecranon. Markers were then placed on every second ICS on a straight line between the two aforementioned markers on the tuber coxae and olecranon (Figure 1). A piece of string was held between the two primary markers to ensure that the secondary markers were in a straight line between the two primary markers. The ultrasound transducer was positioned in each intercostal with the top of the transducer 1 cm below the markers.

2.1.2 | Part 2: Diaphragm thickness

The ultrasonographic technique, developed in part one, was performed in a group of horses to measure the thickness of the diaphragm in the different ICSs. Eleven Thoroughbred racehorses were recruited, horses were selected for examination and inclusion by a single trainer. Subject inclusion was decided by two American College of Veterinary Sports Medicine and Rehabilitation-certified veterinary sports medicine clinicians and a European College of Veterinary Diagnostic Imaging-certified veterinary radiologist. Horses were required to be in active training for National Hunt racing, clinically healthy and considered fully fit during the study period.

Ultrasonographic examination was performed by a single observer (observer 1) and took place in the horse's stable a minimum of 2 h after routine exercise under the standardized conditions outlined in Part 1. The top of the ultrasound transducer was positioned 1 cm below the markers that were placed using the protocol developed in Part 1 (Figure 1). The transducer was held perpendicular to the skin and diaphragm,[13] and not moved during each recording. Two cine-loops were obtained from each ICS, on the left and right sides. Between each cine-loop the transducer was removed from the horse, and additional isopropyl alcohol applied. Each cine-loop was recorded prospectively for 30 s and contained a minimum of three full inspiratory and expiratory breathing cycles.[13,18] Cine-loops were only obtained in ICSs in which the diaphragm was observed. Cine-loops were rejected at the time of acquisition if the horse took a spontaneous deep breath, had an elevated (>14 brpm) or reduced (<6 brpm) respiratory rate, or moved during the recording.

Data recording and analysis were performed by two American College of Veterinary Sports Medicine and Rehabilitation-certified veterinary sports medicine clinicians and a European College of Veterinary Diagnostic Imaging-certified veterinary radiologist. Measurements were performed offline at a later date. Each cine-loop was reviewed frame by frame, and still images of peak inspiration and peak expiration were obtained and saved for measurement. Measurements were performed on the ultrasound machine using the electronic calipers, with the diaphragm thickness measured from inside edge of the superficial border to the inside edge of the deep border,[13] in the middle of the screen that was denoted by an orange triangle automatically present on the ultrasound image. Measurement was only performed for images in which the diaphragm could be easily identified, with a clear distinction between inspiration and expiration.

2.1.3 | Part 3: Intraobserver repeatability

The same population as Part 2 were examined by a single observer (1) on two further occasions, each 1 week apart to assess the repeatability of obtaining images of the diaphragm using the technique described above. The three ICSs where the thickest diaphragm measurements were obtained in Part 2 (ICS 10, 11, and 12) were selected and examined on both sides of the horse (Figure 1). Markers were placed on the horse to guide ultrasound transducer placement, and three cine-loops were obtained from each ICS. Two cine-loops were obtained from each ICS during occasion 1, and three cine-loops from each ICS during occasions 2 and 3.

To assess intraobserver repeatability of the image measurement technique, a subset of 22 randomly selected cine-loops were measured on two occasions by observer 1, >28 days apart, and the results compared.

2.1.4 | Part 4: Interobserver reproducibility

Two observers performed the ultrasound examination on the same horses on occasion 3, to assess the interobserver reproducibility of the developed technique. Observer 2 (L.M.) is a European College of Veterinary Diagnostic Imaging-certified veterinary radiologist and initially underwent ~1 h of training on the developed technique. Observer 2 then worked independently, placing the markers on each horse according to the above described technique and performed ultrasound examination on the same three ICSs (ICS 10, 11, and 12) on each side, with three cine-loops obtained from each ICS. All image measurements were performed by observer 1 to allow comparison of the ultrasound technique between the two observers.

To assess the interobserver reproducibility of measurement of the cine-loops, the same subset of 22 randomly selected cine-loops obtained on occasion 3 were independently measured by observer 2, and the results compared with the measurements by observer 1. Before undertaking the measurements, observer 2 was provided with written instructions, including example images and step-by-step guidance of how to review the cine-loops frame by frame, and save still images of peak inspiration and peak expiration for measurement (Appendix S1). All measurements were performed on the ultrasound machine using the electronic calipers.

3 | STATISTICAL ANALYSIS

Selection and completion of the statistical tests were performed by two observers, an American College of Veterinary Internal Medicine-certified veterinary cardiologist and Doctor of Philosophy (PhD), and an American College of Veterinary Sports Medicine and Rehabilitation-certified veterinary sports medicine clinician and PhD candidate. The measurements were recorded in Microsoft Excel® and analyzed using SPSS® (version 24). The data were assessed for normality visually using a histogram plot and by performing a Shapiro-Wilk test. For each ICS, the mean (±standard deviation [SD]) diaphragm thickness for inspiration and expiration were calculated. Repeatability of measurements were assessed by the calculation of the coefficient of variation (CV) for the measurements obtained on each occasion (weeks 1, 2, and 3) and across all three occasions. A CV of <10% for a measurement was considered to indicate a good repeatability.[19] The measurements obtained from different cine-loops on the same occasion were compared using a paired *t*-test for the two cine-loops obtained during week 1, and a one-way repeated measures ANOVA for the three cine-loops obtained during each of weeks 2 and 3. Intraobserver comparison of measurements obtained three separate occasions (weeks 1, 2, and 3) was determined by a one-way repeated measures ANOVA. Paired *t*-tests were performed to compare the measurements from the left and right sides, to compare the image measurements obtained by observer 1 on two occasions, to compare the measurements obtained during examination on occasion 3 by observer 1 and observer 2, and to compare the measurements obtained by observer 1 and 2 on the same subset of cine-loops. The correlation between the thickness measurements obtained by observers 1 and 2 were compared with a Spearman Rho correlation. A Bland-Altman plot was subsequently created to determine the level of agreement between the measurements obtained by the two observers. Significance was set at $P < .05$.

4 | RESULTS

4.1 | Part 1: Diaphragm location, ultrasonographic features, and development of a standardized technique

In a single Thoroughbred racehorse (6 years old, gelding, 489 kg), the diaphragm was observed in all ICS from ICS 7 to 17 on the left side and ICS 6 to 17 on the right side inclusive. The dorsal and ventral borders between which the diaphragm could be observed ultrasonographically were marked (Figure 2), with the ventral border approximately following the curve of the costal arch and were roughly symmetrical on both sides.

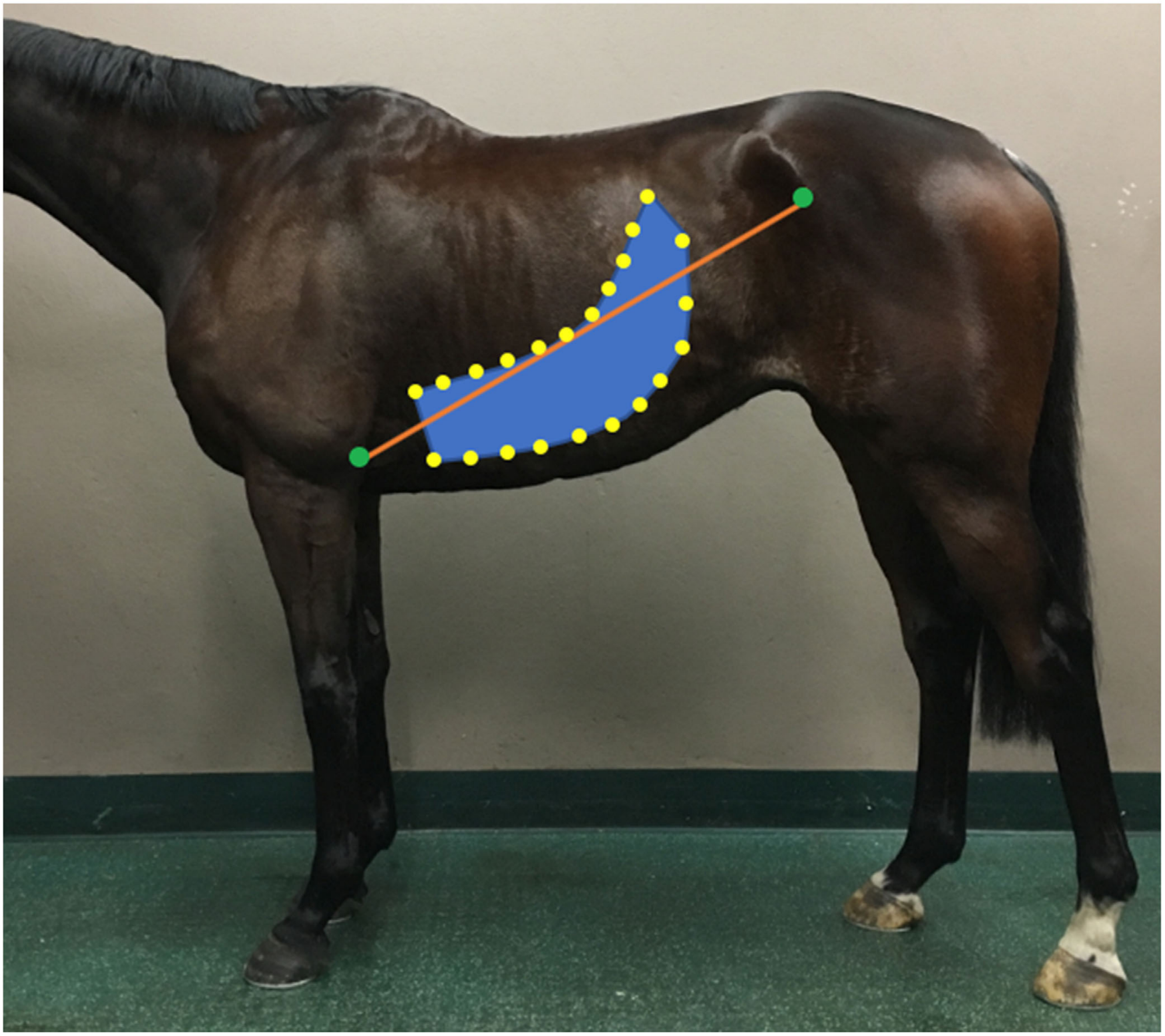

FIGURE 2 Photograph illustrates where the diaphragm can be observed ultrasonographically. The yellow markers show the dorsal and ventral borders of the diaphragm within each intercostal space. The blue area shows where the diaphragm can be observed. The green markers are placed on the cranioventral aspect of the tuber coxae, and the proximal margin of the olecranon. The orange line between the two green markers shows the method used to repeatably position the ultrasound transducer at each intercostal space to image the diaphragm [Color figure can be viewed at wileyonlinelibrary.com]

The thickness of the diaphragm was defined as the distance between the superficial and deep margins (Figure 3A,B). Within each ICS, the thickest part of the diaphragm was observed dorsally, adjacent to the lung border (Figure 4A). The superficial and deep borders of the diaphragm quickly became parallel (Figure 4B) and remain so until there is a sharp tapering before the region of insertion on the thoracic wall just proximal to the costal arch (Figure 4C,D). Commonly a blood vessel is present at the distal region of insertion of the diaphragm (Figure 4D). In addition, blood vessels can be observed within the diaphragm, most often in transverse section adjacent to the lung border (Figure 5D).

During inspiration the diaphragm flattens, at the zone of apposition (where the ultrasound probe is positioned) this is seen as the diaphragm moving ventrally and caudally, which is characterized by the fibers moving across the screen from dorsal to ventral. During expiration the diaphragm becomes more dome shaped, at the zone of apposition this is seen as moving dorsally and cranially, which is characterized by the fibers moving across the screen from ventral to dorsal. Peak inspiration was defined as the point where the diaphragm fibers change from moving ventrally to moving dorsally, and the thickness of the diaphragm usually increases (Figure 5A-D). The lung border may be observed in images during peak inspiration. Peak

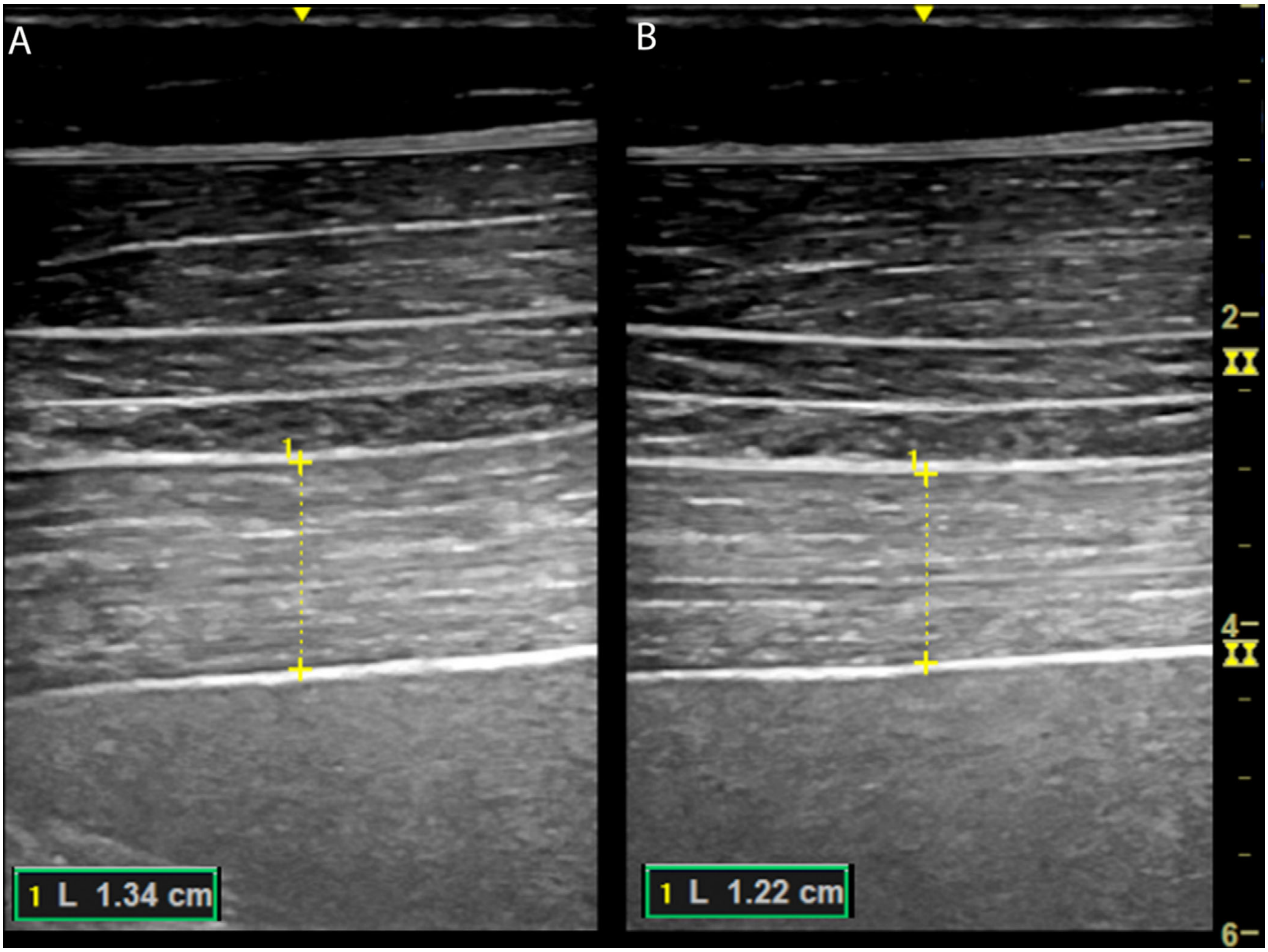

FIGURE 3 A,B, Longitudinal ultrasound images, obtained from the 10th intercostal space on the left side, demonstrate the measurement of the diaphragm during inspiration (A) and expiration (B). Measurements were performed from inside edge of the superficial border to inside edge of the deep border in the middle of the screen, measuring across the shortest distance. Dorsal is to the left of the images; images obtained with a variable frequency linear transducer set at 13 MHz [Color figure can be viewed at wileyonlinelibrary.com]

expiration was defined as the point where the diaphragm fibers change from moving dorsally to ventrally, and the thickness of the diaphragm usually decreases (Figure 6A-D).

The location of the lung border at peak inspiration and peak expiration varied, on a breath-by-breath bases, due to differences in tidal volume. Peak inspiration was almost always associated with the greatest thickness of the diaphragm, and peak expiration almost always the smallest thickness of the diaphragm.

4.2 | Part 2: Diaphragm thickness

Eleven Thoroughbred racehorses (eleven geldings) with a mean (±SD) age of 6.1 ± 1.6 years, and a mean (±SD) weight of 503.9 ± 50.0 kg were examined. Using the standardized technique developed in Part 1, the diaphragm could be observed with ultrasound at ICS 8-15 on the left and right sides in all 11 horses. The diaphragm could be observed in more cranial and caudal ICSs in some horses. The mean thickness measurements, minimum to maximum range, and the mean CV for each ICS on both sides are shown in Table 1. Occasionally the ventral lung border entered the image plane during inspiration but this only impeded measurement infrequently (<1% of the time).

4.3 | Part 3: Intraobserver repeatability

Using the standardized technique, markers were placed on ICS 10, 11, and 12 (where the thickest measurements were obtained in part 2), on each side, to guide ultrasound transducer placement (Figure 1). Eleven horses were measured during occasion 1, one horse was lost from the study on occasion 2, and a further horse was lost from the study on occasion 3, leading to 10 and nine horses being measured on these occasions, respectively.

For the examinations during occasion 1, there was no significant difference for the measurements obtained from the first and second cineloops for each ICS ($P > .05$) except one. At ICS 11 on the right side,

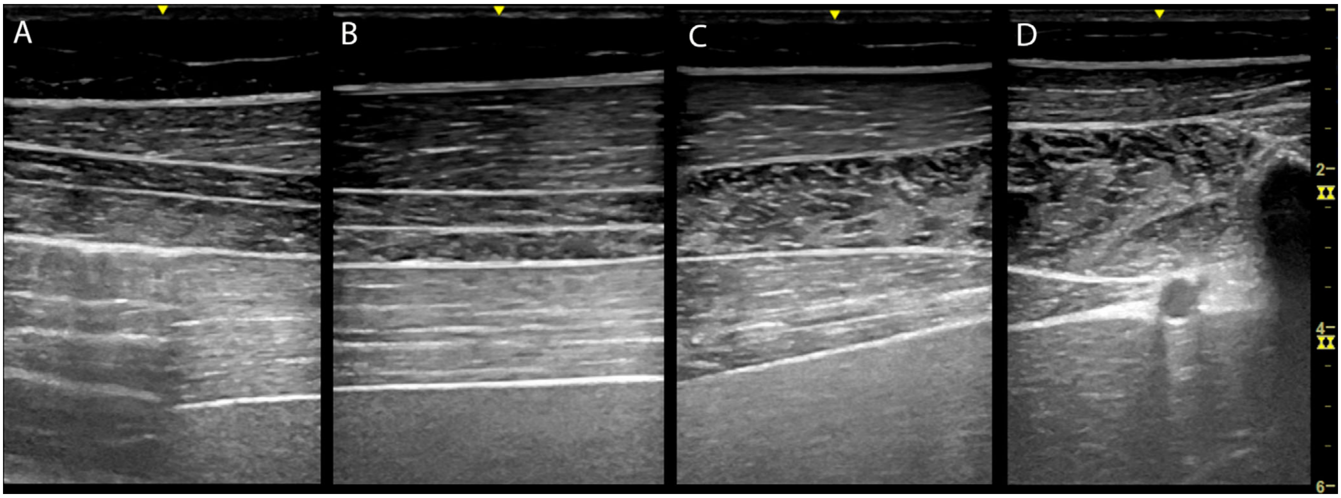

FIGURE 4 Series of longitudinal ultrasound images obtained from the 10th intercostal space on the left, from dorsal to ventral. Panel (A) shows the lung border where the diaphragm is thickest. Panel (B) shows the parallel superficial and deep borders of the diaphragm. Panel (C) shows the thickness of the diaphragm tapering. Panel (D) shows the region of insertion of the diaphragm onto the thoracic wall, just proximal to a blood vessel and the costal cartilage of the 11th rib. Dorsal is to the left of the images; images obtained with a variable frequency linear transducer set at 13 MHz [Color figure can be viewed at wileyonlinelibrary.com]

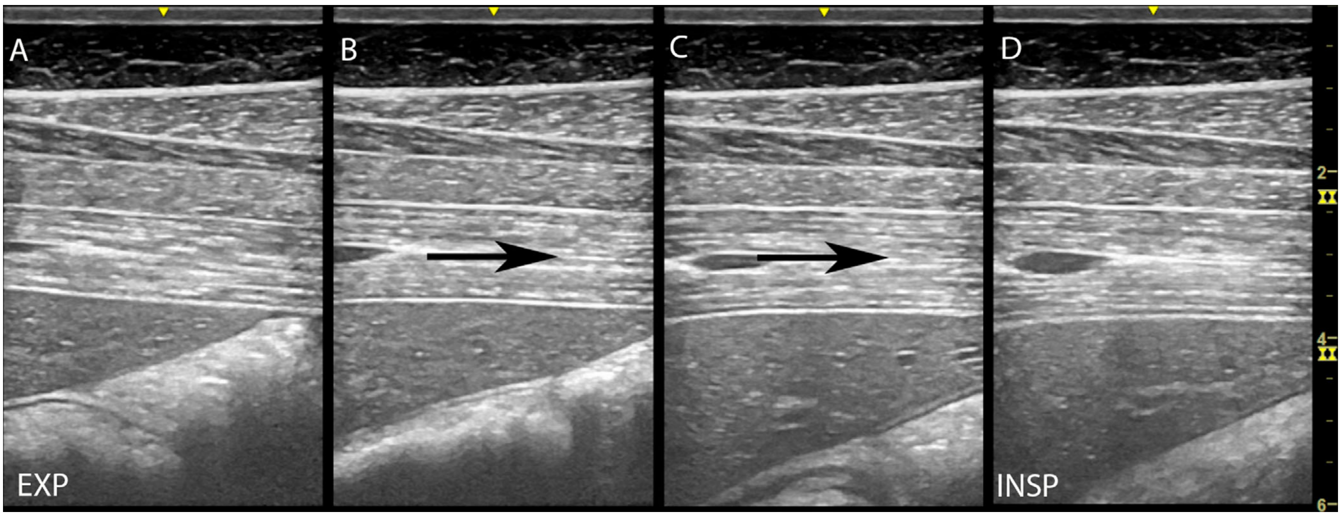

FIGURE 5 Inspiration series of longitudinal ultrasound images, obtained from the 12th intercostal space on the right side, showing the changes in the diaphragm observed during inspiration. The muscle fibers of the diaphragm move from dorsal to ventral, from the left to the right side of the screen, in the direction of the arrows and the thickness of the diaphragm usually increases. When the fiber movement changes direction, this is peak inspiration. Panel (A) shows the diaphragm measurement at peak expiration. Panels (B and C) show the direction of the muscle fiber movement during inspiration. Panel (D) shows the diaphragm measurement at peak inspiration. The time difference between each image is ~1 s, with the entire duration of inspiration lasting 3 s. Dorsal is to the left of the image; images obtained with a variable frequency linear transducer set at 13 MHz [Color figure can be viewed at wileyonlinelibrary.com]

there was a significant difference between the expiratory measurements between the first (Mean = 1.14 cm [±SD = 0.11]) and second (1.07 cm [±0.09]) cine-loops ($P = .012$). There was no significant difference between the measurements from the three cine-loops obtained from each ICS during examination on occasions 2 and 3 ($P > .05$).

Full repeatability results are shown in Tables 1 and 2. The CV for the measurements obtained during each of the three occasions, were <10% except for the right ICS 15 during inspiration during occasion 1 (CV = 10.82%) (Table 1) and the right ICS 12 during inspiration on occasion 2 (CV = 10.88%; Table 2). The CV across all three occasions were <10% for six of 12 locations and <12.5% for the remaining six of 12 locations during inspiration and expiration at ICSs 10, 11, and 12 on the left and right sides. For the nine horses examined on all three occasions, there was no significant difference between the thickness measurements obtained on each occasion ($P > .05$).

The full results comparing the left and right sides are shown in Table 3. The measurements obtained from the left side were

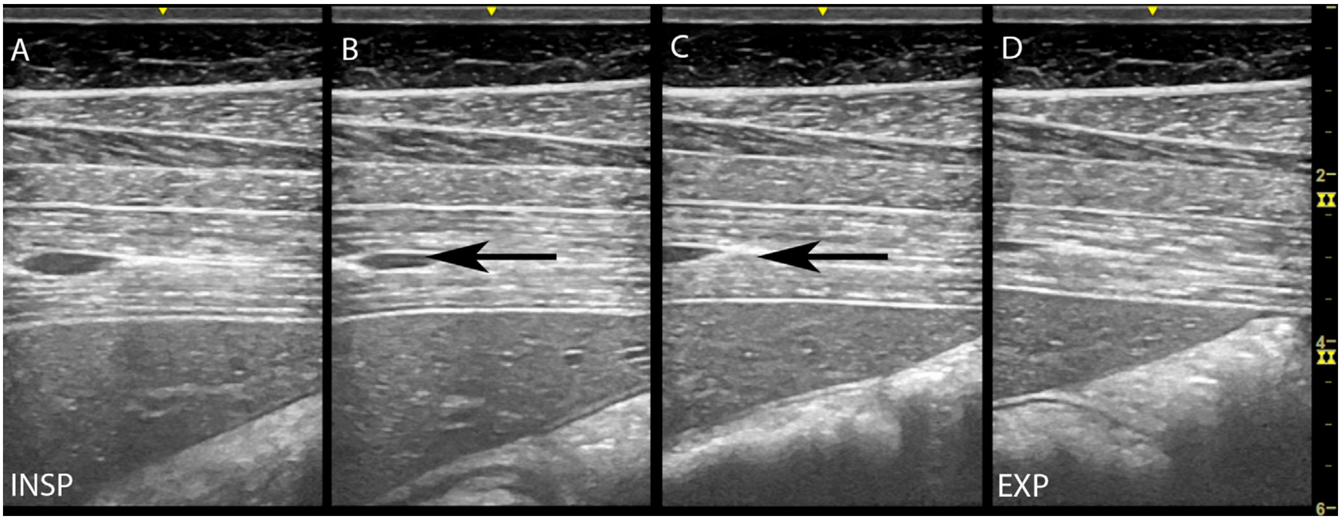

FIGURE 6 Expiration Series of longitudinal ultrasound images, obtained from the 12th intercostal space on the right side, showing the changes in the diaphragm observed during expiration. The muscle fibers of the diaphragm move from ventral to dorsal, from the right to the left side of the screen, in the direction of the arrows and the thickness of the diaphragm usually decreases. When the fiber movement changes direction, this is peak expiration. Panel (A) shows the diaphragm measurement at peak inspiration. Panels (B and C) show the direction of the muscle fiber movement during expiration. Panel (D) shows the diaphragm measurement at peak expiration. The time difference between each image is ~2 s, with the entire duration of expiration lasting 6 s. Dorsal is to the left of the image; images obtained with a variable frequency linear transducer set at 13 MHz [Color figure can be viewed at wileyonlinelibrary.com]

significantly larger than those of the right side during inspiration at ICS 10, 11, and 12, and during expiration at ICS 10 and 11 ($P < .05$).

For the same 22 cine-loops measured off-line twice, on two separate occasions, by observer 1, there was no significant difference between the measurements obtained on each occasion ($P > .05$).

4.4 | Part 4: Interobserver reproducibility

When comparing the diaphragm cine-loops obtained independently by observer 1 and observer 2 on the same occasion (week 3), there was a significant difference in the diaphragm thickness in three of 12 locations. At ICS 12 on the left during inspiration the measurements from the cine-loops obtained by observer 1 (1.26 cm [±0.19]) were greater than those obtained by observer 2 (1.16 cm [±0.13]; $P < .001$), at ICS 10 on the left during inspiration the measurements from the cine-loops obtained by observer 1 (1.27 cm [±0.18]) were greater than those obtained by observer 2 (1.24 cm [±0.18]; $P = .014$), and at ICS 11 on the right during expiration the measurements obtained by observer 1 (1.01 cm [±0.13]) were smaller than those obtained by observer 2 (1.05 cm [±0.15]; $P = .041$).

The thickness measurements obtained by observer 1 and observer 2 when measuring the same 22 cine-loops were significantly different during both inspiration ($P = .001$) and expiration ($P = .002$) however, the results were significantly correlated (Spearman Rho = 0.068, $P < .001$). The thickness measurements recorded by observer 1 (inspiration; 1.19 cm [± 0.12]; expiration: 0.98 cm [±0.11]) were larger than the measurements by observer 2 (inspiration: 1.15 cm [±0.14]; expiration: 0.95 cm [±0.10]), with a mean bias of 0.031 cm (Figure 7).

5 | DISCUSSION

This preliminary study was intended to provide a detailed description of a repeatable technique for quantifying effects of training in performance horses using ultrasonographic imaging and measurement of equine diaphragm thickness. Based on our review of the literature, this is the first published report describing this technique. The costal portion of the muscular part of the diaphragm was readily imaged over a wide area on both the left and right sides. The novel technique developed to position the ultrasound transducer in a standardized location allowed examination and measurement of the diaphragm, with good repeatability,[19] in a population of Thoroughbred racehorses.

Although sonographic imaging of the diaphragm is commonly performed in humans, there is no standardized technique. Two main methods are used – a subcostal or a lateral thoracic approach.[1] The subcostal approach is not feasible in horses due to the strong abdominal musculature and patient compliance, so the lateral thoracic approach was utilized. A further imaging consideration is the choice of 2D or M-mode ultrasound modalities.[1] M-mode imaging allows easier measurement of the thickness of the diaphragm and quantification of its excursion in humans,[20] but the image quality in horses was less clear due to a reduced distinction between adjacent structures and the slow respiratory rate of the horse leads to difficulty in measuring this modality. Two-dimensional ultrasonography provided a clearer anatomical image and the use of cine-loop recordings allowed consistent selection of peak inspiration and peak expiration, due to the changes in the direction of diaphragm movement, over consecutive respiratory cycles.

To enable clear tissue interfaces to be observed, facilitating repeatable measurement of the diaphragm, the ultrasound machine settings were selected to provide high contrast images. There are connective

TABLE 1 The locations (intercostal spaces) where the diaphragm was observed and measured in each horse, along with the mean measurements

		Left				Right			
ICS	Insp/Exp	Diaphragm Observed (horses)	Diaphragm Measured (horses)	Mean [±SD] cm	Mean CV %	Diaphragm Observed (horses)	Diaphragm Measured (horses)	Mean [±SD] cm	Mean CV %
6	Insp	0/11	0/11	-	-	4/11	3/11	1.05 [± 0.15]	8.3
	Exp			-	-			0.79 [± 0.17]	6.7
7	Insp	10/11	9/11	0.90 [± 0.13]	7.3	10/11	8/11*	0.87 [± 0.18]	6.7
	Exp			0.75 [± 0.08]	6.6			0.76 [± 0.15]	6.9
8	Insp	11/11	10/11	0.97 [± 0.17]	6.2	11/11	11/11	0.98 [± 0.27]	8.6
	Exp			0.81 [± 0.12]	3.6			0.81 [± 0.19]	7.5
9	Insp	11/11	11/11	1.20 [± 0.27]	6.6	11/11	11/11	1.15 [± 0.23]	7.4
	Exp			0.98 [± 0.20]	4.7			0.96 [± 0.19]	6.0
10	Insp	11/11	11/11	1.40 [± 0.17]	5.1	11/11	11/11	1.26 [± 0.18]	9.0
	Exp			1.12 [± 0.12]	5.1			1.07 [± 0.13]	6.8
11	Insp	11/11	11/11	1.42 [± 0.24]	8.2	11/11	11/11	1.26 [± 0.12]	5.7
	Exp			1.14 [± 0.17]	5.1			1.10 [± 0.09]	5.4
12	Insp	11/11	11/11	1.35 [± 0.17]	7.3	11/11	11/11	1.28 [± 0.14]	7.8
	Exp			1.09 [± 0.15]	5.1			1.13 [± 0.14]	6.7
13	Insp	11/11	11/11	1.23 [± 0.17]	6.8	11/11	11/11	1.19 [± 0.14]	9.2
	Exp			1.03 [± 0.17]	5.4			1.02 [± 0.12]	6.7
14	Insp	11/11	11/11	1.10 [± 0.18]	5.1	11/11	11/11	1.04 [± 0.19]	4.9
	Exp			0.93 [± 0.13]	3.9			0.90 [± 0.15]	4.8
15	Insp	11/11	11/11	0.80 [± 0.21]	5.8	11/11	10/11*	0.79 [± 0.21]	9.5
	Exp			0.71 [± 0.17]	5.3			0.69 [± 0.18]	10.8
16	Insp	8/11	3/11*	0.73 [± 0.11]	6.8	5/11	4/11*	0.67 [± 0.20]	8.5
	Exp			0.66 [± 0.13]	9.8			0.56 [± 0.19]	9.1

*Indicates that in a single horse, the diaphragm could only be consistently measured in one of the two cine-loops obtained. ICS, intercostal space; Insp, inspiration; Exp, expiration; SD, standard deviation; CV, coefficient of variation.

TABLE 2 Mean thickness measurements obtained from each intercostal space on each occasion, the mean value from all three occasions with the *P*-value comparison between the different occasions

			Occasion 1		Occasion 2		Occasion 3		Overall – all three occasions		*P*-value
Side	ICS	Insp/Exp	Thickness Mean [±SD] cm	CV %	Thickness Mean [±SD] cm	CV %	Thickness Mean [±SD] cm	CV %	Thickness Mean ± SD cm	CV %	RM ANOVA Sig.
Left	10	Insp	1.40 [± 0.17]	5.1	1.39 [± 0.16]	6.0	1.35 [± 0.07]	6.1	1.38 [± 0.11]	8.3	.096
		Exp	1.12 [± 0.12]	5.1	1.09 [± 0.12]	5.4	1.07 [± 0.09]	3.7	1.09 [± 0.10]	6.2	.119
	11	Insp	1.42 [± 0.24]	8.2	1.35 [± 0.17]	5.4	1.27 [± 0.15]	7.2	1.35 [± 0.14]	11.1	.229
		Exp	1.14 [± 0.17]	5.1	1.07 [± 0.10]	4.7	1.02 [± 0.11]	5.5	1.08 [± 0.11]	8.4	.162
	12	Insp	1.35 [± 0.17]	7.3	1.32 [± 0.18]	8.4	1.24 [± 0.19]	9.9	1.30 [± 0.14]	12.4	.359
		Exp	1.09 [± 0.15]	5.1	1.05 [± 0.12]	7.2	0.99 [± 0.14]	5.4	1.04 [± 0.11]	9.7	.293
Right	10	Insp	1.26 [± 0.18]	9.0	1.30 [± 0.19]	8.5	1.22 [± 0.17]	7.8	1.25 [± 0.15]	10.7	.419
		Exp	1.07 [± 0.13]	6.8	1.05 [± 0.18]	4.9	1.00 [± 0.14]	4.8	1.04 [± 0.13]	8.8	.457
	11	Insp	1.26 [± 0.12]	5.7	1.29 [± 0.15]	6.8	1.23 [± 0.13]	6.9	1.26 [± 0.10]	10.2	.438
		Exp	1.10 [± 0.09]	5.4	1.05 [± 0.12]	5.9	1.01 [± 0.13]	6.0	1.06 [± 0.09]	9.2	.175
	12	Insp	1.28 [± 0.14]	7.8	1.28 [± 0.19]	10.9	1.25 [± 0.10]	8.1	1.26 [± 0.11]	11.7	.538
		Exp	1.13 [± 0.14]	6.7	1.06 [± 0.13]	7.7	1.05 [± 0.07]	9.2	1.07 [± 0.09]	10.2	.189

Abbreviations: ICS, intercostal space; Insp, inspiration; Exp, expiration; SD, standard deviation; CV, coefficient of variation; RM ANOVA sig, repeated measures analysis of variance significance.

TABLE 3 Mean thickness measurements obtained from the left and right sides, and two-tailed significance, for each intercostal space during inspiration and expiration

ICS	Insp/Exp	Mean thickness [±SD] Left (cm)	Mean thickness [±SD] Right (cm)	Significance (two-tailed)
10	Insp	1.37 [±0.51]	1.26 [±0.20]	.000*
	Exp	1.09 [±0.12]	1.04 [±0.16]	.000*
11	Insp	1.34 [±0.21]	1.26 [±0.15]	.000*
	Exp	1.07 [±0.14]	1.05 [±0.13]	.023*
12	Insp	1.30 [±0.21]	1.27 [±0.19]	.004*
	Exp	1.04 [±0.15]	1.07 [±0.14]	.002*

Abbreviations: ICS, intercostal space; Insp, inspiration; Exp, expiration; SD, standard deviation.
*Significance at $P < .05$.

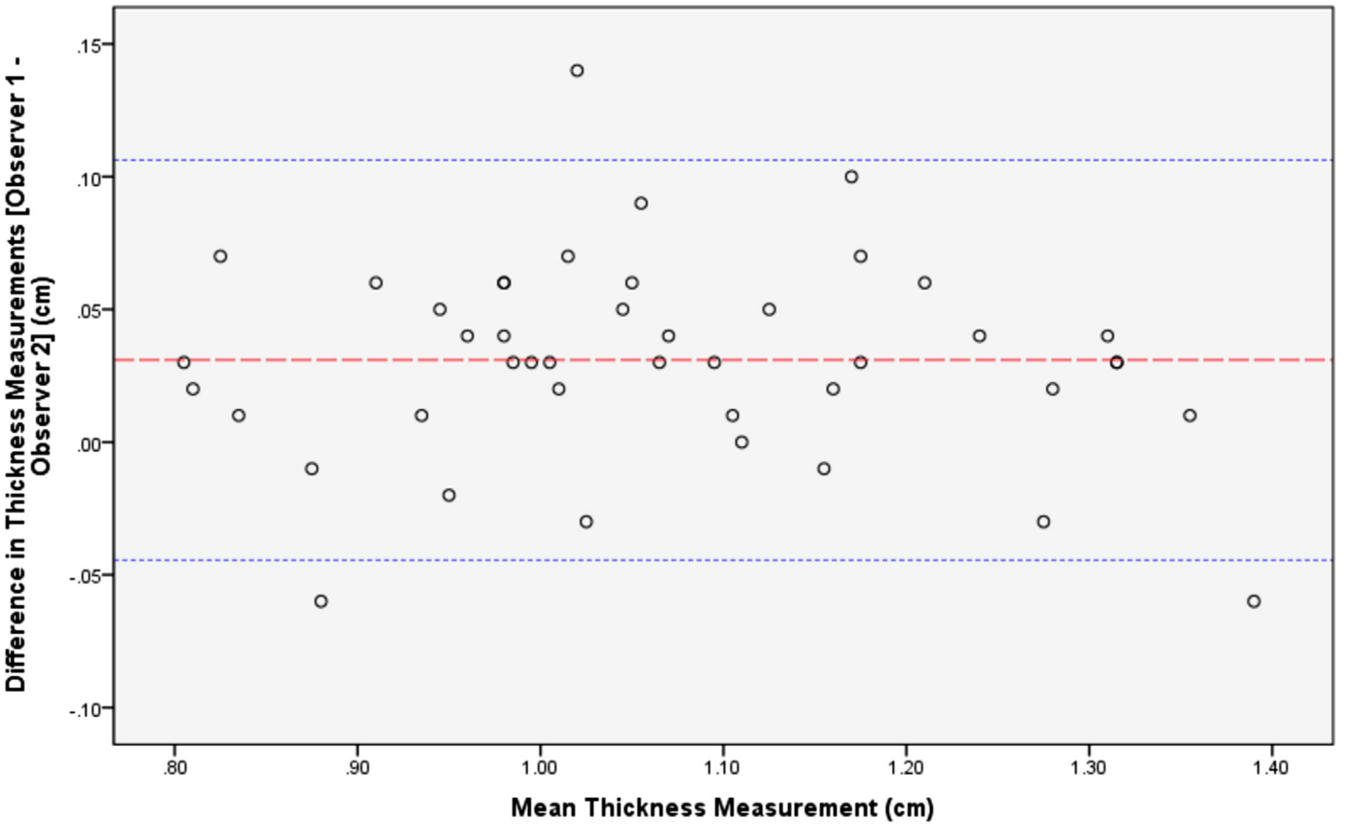

FIGURE 7 Bland-Altman plot illustrating the agreement between the mean diaphragm thickness measurements from observer 1 and observer 2, when measuring the same 22 randomly selected cine-loops. The mean bias (red dashed line) indicates that the measurements by observer 1 were on average 0.031 cm greater than the measurements recorded by observer 2. The 95% limits of agreements (blue dotted lines) between the two observers' measurements are displayed as ±1.96 SDs of the mean difference (0.106 cm and −0.044 cm, respectively) [Color figure can be viewed at wileyonlinelibrary.com]

tissue sheets over the superficial thoracic and deep abdominal surfaces of the diaphragm. These tissue interfaces, along with the thoracic pleura and peritoneum adjacent to the superficial and deep surfaces respectively,[13] generate a clearly delineated border to the diaphragm on ultrasonographic images, between which the muscular tissue of the diaphragm can be measured. The measurements were performed from inside edge to inside edge to account for edge enhancement with such a high contrast image and the authors were interested in the muscle thickness not the connective tissue portion of the diaphragm.

The thickest part of the diaphragm within each ICS was at the lung border. However, there was often a variation in the location of the lung border at peak inspiration and peak expiration due to changes in lung volume. The authors felt that measurement of the thickness at the lung border was therefore not reliable as the location varied breath by breath (*unpublished data*), so an alternative method was devised to measure the thickness of the diaphragm in the zone of apposition, mirroring one of the techniques reported in humans.[18] The thickness of the diaphragm within each ICS was roughly uniform from just below the lung border until the taper before the region of insertion on the thoracic wall. The external landmarks used to guide ultrasound transducer placement were selected to enable observation of the diaphragm where the borders were parallel in the greatest number of ICSs. The method of transducer placement worked well for all horses in the majority of ICS, occasionally the ventral lung border was observed during inspiration but only impeded measurement infrequently (<1% of the time).

The measurements obtained from the left and right sides were significantly different, during both inspiration and expiration, on all three examination occasions. The reason for this difference is unknown. Although the dorsal and ventral borders of where the diaphragm could be observed were symmetrical on the left and right sides, the abdominal viscera adjacent to the diaphragm on the left and right sides is different. The liver situated on the right side is relatively "immobile" whereas the stomach on the left side is more mobile and could apply different pressure leading to differential contraction, and therefore thickness, of the diaphragm in situ. The study by Poole et al,[5] measuring the thickness of the equine diaphragm post-mortally, did not report a significant difference between the measurements obtained on the left and right side. As such, it is speculated that the difference in thickness on the left and right sides is related to in vivo apposition of abdominal structures with the diaphragm.

The process of obtaining the cine-loops showed a good intraobserver repeatability and a moderate interobserver reproducibility. The measurement of the same 22 cine-loops showed a good intraobserver repeatability, but the interobserver reproducibility was poor. Further analysis showed a systematic bias whereby measurements obtained by observer 1 were consistently greater than those recorded by observer 2. These results indicate that the same observer should perform the measurement of diaphragm cine-loops, particularly when there are repeated examinations, to enable consistent comparison of the measurements.

The diaphragm is an important respiratory muscle, with a correlation between diaphragm strength and thickness reported in humans.[13,14] Specific inspiratory muscle training results in an increase in the measurement of diaphragm thickness[16] and a delay in the onset of respiratory muscle metaboreflex.[10] This study shows that it is possible to measure the thickness of the equine diaphragm with good repeatability.[19] Further investigation is required to determine whether there is a measurable change in diaphragm thickness in response to training in horses, and to explore the relationship between diaphragm thickness and other parameters such as inspiratory muscle strength and performance.

The main limitation of this study was the variation in the lung volume at peak inspiration and peak expiration that could have caused variation in the diaphragm thickness measurements. A larger inspired volume is associated with greater contraction of the diaphragm and a greater thickness measurement.[14] An attempt was made to ensure the horses were breathing at an even respiratory rate and cine-loops were rejected at the time if the horse took a spontaneous deep breath, had a respiratory rate outside of the 6-14 brpm range or moved during the examination. A further limitation was the lack of comparison between the novel technique developed and a gold standard method of diaphragm measurement. In humans and small animals, CT examination could be used however, this is not possible in horses due to patient size so there is no gold standard for in vivo measurement.

In conclusion, the findings of the present study indicated that ultrasonographic imaging and measurement of the equine diaphragm is a feasible method for use in Thoroughbred racehorses. The novel technique developed to position the ultrasound transducer in a standardized location allowed examination and measurement of the diaphragm in a number of different horses, with good repeatability.

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Category 1

(a) Conception and Design: Fitzharris, Meehan, Allen
(b) Acquisition of Data: Fitzharris, Meehan
(c) Analysis and Interpretation of Data: Fitzharris, Hezzell, Allen

Category 2

(a) Drafting the Article: Fitzharris
(b) Revising Article for Intellectual Content: Fitzharris, Meehan, Hezzell, Allen

Category 3

(a) Final Approval of the Completed Article: Fitzharris, Meehan, Hezzell, Allen

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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