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Development and application of a focused ultrasound protocol in neonatal foals

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ABSTRACT

This study aimed to develop a standardized ultrasound (US) protocol using selected thoracic and abdominal acoustic windows, to assess its feasibility and applicability in healthy foals, and to evaluate its usefulness for detecting common thoracic and abdominal conditions in sick foals. The Focused US (FUS) protocol included 12 thoraco-abdominal windows (4 right-side, 3 ventral, 5 left-side) using a curved-array probe. It was applied to cohorts of healthy (n = 17) and sick (n = 23) foals by a veterinary clinician with limited US experience. The FUS showed good feasibility and applicability, with clear visualization of target abdominal structures. Diagnostic usefulness was high for identifying gastrointestinal disease and peritoneal effusions. However, several challenges emerged: the need to change recumbency, the relatively large number of US windows, the need for staff to restrain foals, and the prioritization of other diagnostic or therapeutic procedures in sick foals. A shortened Foal Oriented Compressed US (FOCUS) protocol was developed, including 7 thoraco-abdominal windows from a single right recumbency (4 right-side, 3 ventral), and was tested in healthy (n = 3) and sick (n = 3) foals by three clinicians with varying levels of experience. The FOCUS proved significantly faster than FUS, with a short acquisition time (median 7 min, range 3–10 min). Main limitations include single-operator image acquisition, the small cohort of sick foals assessed using FUS, and limited evaluation of the FOCUS protocol. In conclusion, FUS can be performed by veterinarians without extensive US experience. However, in emergency settings, FOCUS may be preferred due to its greater time efficiency.

1. Introduction

Point-of-care ultrasound (POCUS) exams are time-sensitive and goal-oriented assessments that provide answers to targeted clinical questions based on a patient's clinical signs (Busoni et al. 2011; Vincze et al. 2019; Eberhardt and Schwarzwald, 2022; Bevevino et al. 2023; Corrie et al. 2024; Leduc et al. 2024). The POCUS protocols are increasingly used in human and veterinary medicine due to advances in technology and training (Baston et al. 2019; Norman, 2024). In the adult equine patient, POCUS protocols have been proposed to assess the abdomen in horses with colic (FLASH - fast localized abdominal sonography of horses) (Busoni et al. 2011; Corrie et al. 2024), cardiac and respiratory diseases (CRASH - cardiorespiratory assessment of horses) (Eberhardt and Schwarzwald, 2022; Bevevino et al. 2023), and high-risk pregnancies (REP - rapid examination protocol) (Vincze et al. 2019). Because of differences in ultrasound (US) anatomy and organ systems function between adult horses and foals (Aleman et al. 2002), protocols developed in adults may not be applicable to foals. The US is noninvasive, well tolerated, and can be easily performed on the foal (Porter and Ramirez, 2005; Sprayberry, 2015). Abdominal and thoracic disorders represent a large proportion of diseases encountered in neonatal foals and US is an easily available first-line modality capable of diagnosing most of them (Neal, 2003; McAuliffe, 2004; Porter and Ramirez, 2005; Bain, 2012; le Jeune and Whitcomb, 2014; Sprayberry, 2015; Cribb and Arroyo, 2018). To the best of our knowledge, POCUS protocols have not been described in equine neonatal medicine.

The present study aimed to develop a standardized US protocol based on selected thoracic and abdominal acoustic windows, evaluate its feasibility and applicability for describing the appearance of normal organs in a cohort of healthy foals and for detecting common thoracic

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and abdominal conditions in a cohort of sick foals. We hypothesized that selecting appropriate ultrasonographic windows would allow the development of a practical, focused, and standardized short US protocol capable of detecting common abdominal and thoracic abnormalities in neonatal foals with minimal invasiveness.

2. Materials and methods

2.1. Population and study design

Client-owned foals aged less than 30 days, either born at or admitted for perinatal disease at the Veterinary University Hospital (VUH) of the University of Bologna, Italy, during the 2024 foaling season, were prospectively included on a convenience basis. The study was approved by the Animal Care and Use Committee of the University of Bologna (Approval number, 195113; Approval year, 2024) and informed consent was obtained from the owners prior to foal enrollment. Foals included were healthy or sick. They were considered healthy if born by eutocic parturition from mares with normal pregnancy, had an Apgar score ≥ 8 at birth (Vaala et al. 2002) and a normal clinical condition, including a normal complete blood count and serum biochemistry and an IgG serum concentration >800 mg/dL at 12–24 h of life (Perkins and Wagner, 2015). Sick foals were affected by various pathological conditions diagnosed based on history, clinical evaluation, blood culture, blood count, serum biochemistry, blood gas and electrolytes analysis, and serum IgG determination.

Healthy and sick foals underwent the Focused US (FUS) protocol once during hospitalization. The following data were recorded for each foal at the time of performance of the FUS protocol: breed, sex, age (hours/days), weight (kg), diagnosis (clinical, surgical or post-mortem) and outcome. The FUS protocol feasibility and applicability were tested in healthy and sick foals. In sick foals, diagnostic usefulness was tested by retrospectively comparing FUS protocol findings with the final clinical diagnosis. Based on the location at which US abnormalities were detected and comparisons between FUS results and the final diagnosis, the most informative acoustic windows for detecting abnormalities in diseased foals were identified. A shortened version of the protocol, the Foal Oriented Compressed US (FOCUS) protocol, which includes only the most relevant windows to improve feasibility and applicability, was then developed and applied to a new cohort of foals to evaluate examination times and optimize the protocol for clinical use.

2.2. Ultrasound protocol

The FUS protocol was designed to target abdominal regions and organs where US abnormalities are most commonly reported in foals (Porter and Ramirez, 2005; Sprayberry, 2015; Magri, 2018; Schwarzwald, 2019; McCoy et al. 2020). Foals were positioned on a soft mattress in front of the box, so that they were always accessible to the mare, and were not sedated for the US examination. Twelve topographic locations were assessed using 90° denatured ethyl alcohol, without clipping and using a portable US machine (Philips CX50) equipped with a curvilinear 5–1 MHz transducer: 4 on the right side, 3 in the ventral abdomen, and 5 on the left side (Table 1; Fig. 1). All FUS exams were performed by a veterinary clinician without extensive experience in equine US who had been trained for 6 h by a European specialist in equine internal medicine with particular focus on imaging. One video clip (10 s in duration) and one still image were recorded per window and stored in DICOM format for subsequent analysis.

The shortened FOCUS protocol (Foal Oriented Compressed US - FOCUS), developed based on the comparison between FUS findings and final diagnosis in sick foals, was then tested on 3 healthy and 3 sick foals by 3 clinicians with varying levels of experience to record the average time required to complete the examination.

Table 1
Scanning protocol with ultrasound probe placement sites for the 12 windows of the FUS (focused ultrasound) protocol, divided into the right side (4 windows), ventral abdomen (3 windows), and left side (5 windows). US = Ultrasound; ICS = Intercostal space.

Side	Number	US window	Scanning procedure
Right	1	Thoracic	Place the probe at the level of the 5th-6th ICS
	2	Hepato-duodeno-colic	Place the probe transversely at the level of the 15th-17th ICS
	3	Ventro-lateral abdomen	Place the probe transversely, retro-costally, in the right ventral abdomen
	4	Nephro-cecum-duodenal	Place the probe transversely at the level of the 14th-15th ICS
Ventral	5	Retro-sternal	Place the probe longitudinally, caudal to the sternum
	6	Meso-gastric	Place the probe transversely, immediately caudal to the umbilical stump
	7	Hypo-gastric	Place the probe transversely, 3–4 cm caudal to the umbilical stump
Left	8	Cardiac	Place the probe on the cardiac projection area, at the level of 4th ICS, parallel to the major cardiac axis
	9	Thoracic	Place the probe transversely at the level of the 5th-6th
	10	Spleno-gastric	Place the probe transversely between 10th and 12th ICS
	11	Ventro-lateral abdomen	Place the probe transversely, retro-costally, in the left ventral abdomen
	12	Nephro-splenic	Place the probe transversely at the level of the left paralumbar fossa or the last ICS (16th-17th)

2.3. Image analysis

The US images were reviewed using a DICOM viewer to evaluate organ visualization in each imaging window and to perform a series of measurements in healthy foals. These included the two-dimensional organ diameters, gastrointestinal wall thickness, and assessment of intestinal motility. Duodenal motility was quantified as the number of contractile acts per 10 s, while jejunal motility was qualitatively classified as normal (continuous and rhythmic contractions), reduced (intermittent motility: rhythmic contractions interspersed with periods of inactivity), or absent (no visible intestinal contractions) (Abraham et al. 2014; Haugaard et al. 2023). Evaluations were conducted at the ventro-lateral abdomen, meso-gastric, and hypo-gastric windows.

The presence or absence of the following abnormalities (Table 2: definitions of abnormalities) was assessed in sick foals: dilated stomach (Porter and Ramirez, 2005; Sprayberry, 2015); dilated and turgid small intestinal loops (Porter and Ramirez, 2005; Busoni et al. 2011); thickened intestinal wall (Magri, 2018); “target-like” lesions (Porter and Ramirez, 2005; Sprayberry, 2015); presence of meconium in foals older than 36 h or with associated clinical signs (Sprayberry, 2015; Magri, 2018; Wong and Wilkins, 2024); increased peritoneal free fluid (Busoni et al. 2011); “flask-like” bladder (Bain, 2012; Bernick et al. 2024); abnormalities of the pleuropulmonary surface (Porter and Ramirez, 2005; Bevevino et al. 2023); pleural effusion (Porter and Ramirez, 2005; Sprayberry, 2015); increased pericardial free fluid (Schwarzwald, 2019; Bevevino et al. 2023). The FUS results were compared retrospectively with the findings from serial clinical examinations, surgical and non-surgical outcomes, or post-mortem reports.

2.4. Statistical analysis

Data from the image analysis and interpretation was collected in a Microsoft Excel worksheet (Microsoft Excel®, Microsoft 365 MSO, Version 2401). Statistical analysis was performed in R environment (X) using functions in *readxl* and *knitr* libraries (R Core Team, 2024). The

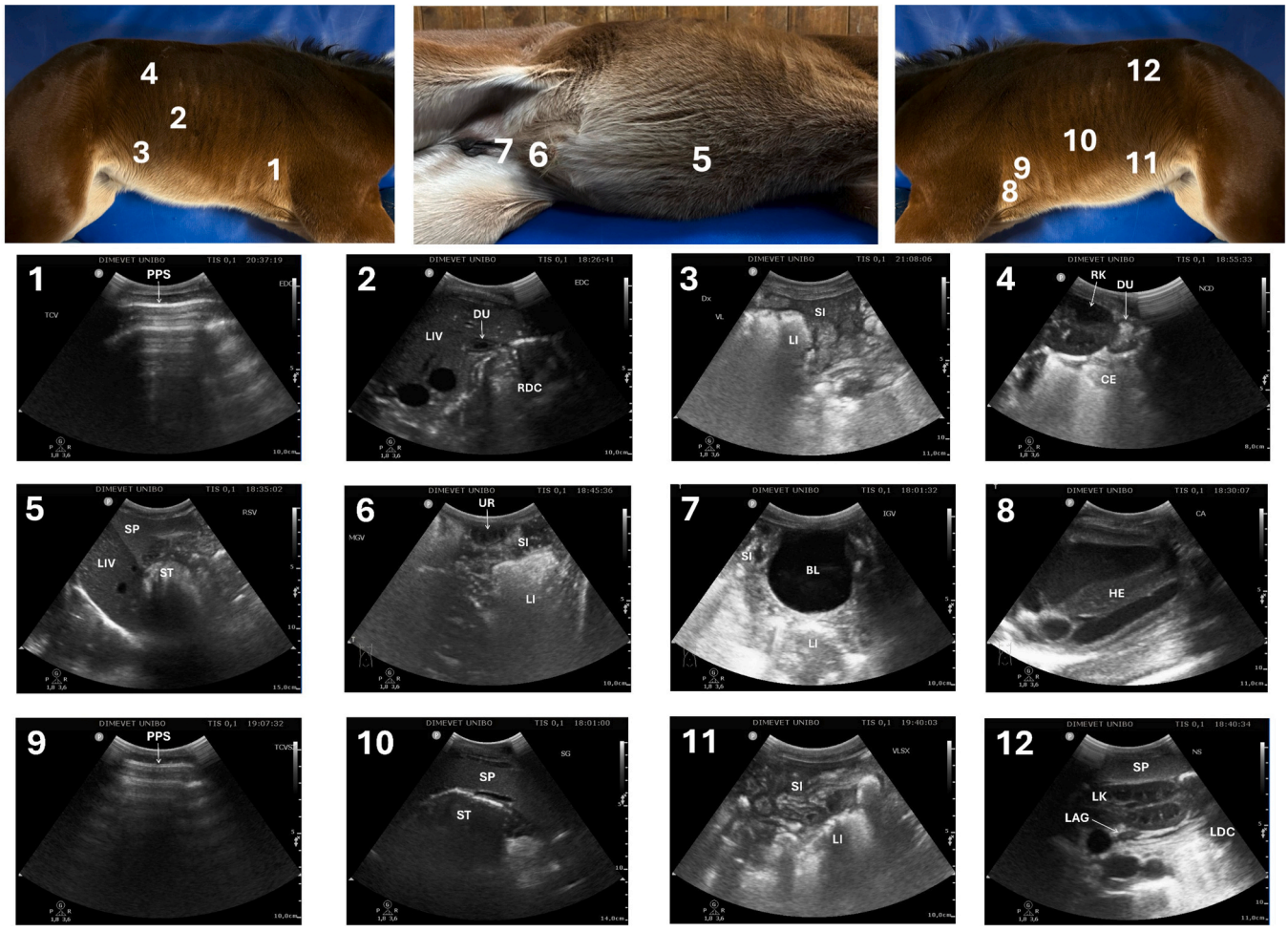

Fig. 1. The 12-window FUS protocol. Probe placement site (top) and corresponding ultrasound image in a healthy foal (bottom) for the 12 acoustic windows of the FUS protocol. Four windows on the right side: 1. Right cranio-ventral thoracic window - Pleuropulmonary surface (PPS); 2. Hepato-duodeno-colic window - Liver (LI), right dorsal colon (RDC) and duodenum (DU); 3. Ventro-lateral abdomen window - Small intestine (SI) and large intestine (LI); 4. Nephro-cecum-duodenal window - Cranial pole of the right kidney (RK), cecum (CE) and duodenum (DU). Three windows on the ventral abdomen: 5. Retro-sternal window - Liver (LI), spleen (SP) and stomach (ST); 6. Meso-gastric window - Umbilical remnants (UR), small intestine (SI) and large intestine (LI). 7. Hypo-gastric window - Bladder (BL), small intestine (SI) and large intestine (LI). Five windows on the left side: 8. Cardiac window - Heart (HE); 9. Left cranio-ventral thoracic window - Pleuropulmonary surface (PPS); 10. Spleno-gastric window - Spleen (SP) and stomach (ST); 11. Ventro-lateral abdomen window - Small intestine (SI) and large intestine (LI); 12. Nephro-splenic window - Spleen (SP), left kidney (LK), left dorsal colon (LDC) and left adrenal gland (LAG).

data distribution was tested with Shapiro-Wilk test. Most of the data did not follow a normal distribution. The ordinal and numerical variables were presented as median, interquartile range, minimum and maximum.

3. Results

3.1. Population

Forty foals of various breeds (15 Standardbreds, 11 Warmbloods, 6 Quarter Horses, 5 Arabians, 3 Others) were included: 27 colts and 13 fillies, aged 9 h to 18 d (median age 43 h). Foals' weight ranged from 13 to 90 kg with a median weight of 45 kg. Seventeen/40 foals were healthy (aged 12h-11 d, median 24 h) and 23/40 were sick (aged 9h-18 d, median 53 h). Sick foals were affected by systemic or localized infections (n = 10), neonatal encephalopathy (n = 4), meconium retention/impaction (n = 3), uroperitoneum (n = 3), gastric ulceration (n = 2), and prematurity (n = 1). Seventeen/23 sick foals survived and 6/23 were euthanized.

To test the shortened FOCUS protocol, six additional foals of various breeds (3 Standardbreds, 1 Warmblood, 1 Arabian, 1 Other) were included: 5 fillies and 1 colt, aged 1–7 d (median age 72 h). Foals' weight ranged from 40 to 55 kg with a median value of 50 kg. Three/6 foals were healthy (aged 48–108 h, median 96 h) and 3/6 were sick (aged 24 h-7 d, median 34 h). Sick foals were affected by uroperitoneum (n = 1) and meconium impaction (n = 2). Two/3 sick foals survived and 1/3 was euthanized.

3.2. FUS protocol feasibility

The full FUS protocol (12 windows) was not completed in 3 out of 17 healthy and 6 out of 23 sick foals. The cranio-ventral left thoracic, cranio-ventral right thoracic, cardiac, and ventro-lateral right abdominal windows were not acquired in 6, 3, 3, and 3 sick foals, respectively.

3.3. FUS protocol applicability and findings in healthy foals

Ultrasound images for the 12 acoustic windows of the FUS protocol in a healthy foal are shown in Fig. 1. Main FUS protocol findings in healthy foals are summarized in Table 3.

According to their anatomical topography, the US windows of the FUS protocol consistently allowed visualization of all major intra-abdominal organs (stomach, small and large intestine, liver, spleen, left and right kidneys, urinary bladder, and umbilical remnants) and intra-thoracic organs (heart and pleuropulmonary surface), in nearly all

Table 2
Ultrasonographic abnormalities assessed with FUS (focused ultrasound) and their defining ultrasonographic criteria.

Abnormality	Definition	Reference
1. Dilated stomach	Combination of a fluid-filled stomach with gas interfaces, a subjectively increased size and more ventral and caudal extension of stomach than typically observed.	Porter and Ramirez, 2005 Sprayberry, 2015
2. Dilated and turgid small intestinal loops	Diameter > 2.5–3.0 cm, turgid appearance (round transverse section), fluid content, and hypomotility or amotility.	Busoni et al. 2011 Porter and Ramirez, 2005
3. Thickened intestinal wall	Wall thickness > 3 mm.	Magri, 2018
4. "Target-like" lesion	Intussusceptum segment surrounded by the intussuscipiens segment giving alternating layers of different echogenicity.	Porter and Ramirez, 2005 Sprayberry, 2015
5. Meconium in foals older than 36 h or with associated clinical signs	Intraluminal hypoechoic mass or hyper-/hypoechoic or mixed echogenicity ball surrounded by hyperechoic content.	Sprayberry, 2015 Magri, 2018
6. Increased peritoneal free fluid	Amount of peritoneal fluid that is > 1–1.5 cm in depth.	Busoni et al. 2011
7. "Flask-like" bladder	Low filled, collapsed and folded bladder.	Bain, 2012 Bernick et al. 2024
8. Abnormalities of the pleuropulmonary surface	Irregularity of the pleural line, presence of ring-down/comet-tail artefacts and/or hypoechoic subpleural pulmonary consolidations.	Bevevino et al. 2023 Porter and Ramirez, 2005
9. Pleural effusion	Amount of pleural fluid that is > 0.5–1 cm in depth.	Porter and Ramirez, 2005 Sprayberry, 2015
10. Pericardial free fluid	Amount of pericardial fluid that is more than a few millimeters in depth.	Bevevino et al. 2023 Schwarzwald, 2019

foals.

Abdominal organs, especially the gastrointestinal tract, were visualized in more than one window. Values for the two-dimensional organ diameters, gastrointestinal wall thickness, and assessment of duodenal motility for each individual US window are reported in Table 3. Jejunal motility was classified as normal in 13/15 foals and reduced in 2/15 foals at the right ventro-lateral abdomen window. At the meso-gastric, hypo-gastric and left ventro-lateral abdomen windows, motility was classified as normal in 16/17 foals and reduced in 1/17 foals.

3.4. FUS protocol applicability and findings in sick foals

Main FUS protocol findings in sick foals are summarized in Table 4.

Of the 23 sick foals, 13 had abdominal involvement, 4 had thoracic involvement, 2 had both abdominal and thoracic involvement, and 4 had neither (e.g., neonatal encephalopathy). Of the 13 foals with abdominal involvement, 4had a surgical disorder (2 bladder rupture, 1 urachus rupture, 1 jejunal intussusception), and 9 had a medical disorder (4 meconium impaction, 2 enteritis, 2 septic peritonitis, 1 gastric ulceration).

Gastric dilation (Fig. 2 A), reduced small or large intestinal motility and fluid-filled small intestinal loops (Fig. 2B) were seen in foals with enteritis. Turgid small intestinal loops with no motility were seen in foals with meconium impaction, enteritis and jejunal intussusception (Fig. 2 C). The presence of meconium in the large intestine was documented at multiple sites in a total of 10/23 foals: 8 in the sternal flexure, 6 in the right ventral colon/cecum, 4 in the right dorsal colon, 5 in the left ventral colon, and 4 in the small colon. Of the 10 foals, 3 had reduced intestinal motility and 3 were treated for meconium impaction (Fig. 2D). Peritoneal effusion (Fig. 2E) was documented in 5/23 foals, 2 with septic peritonitis and 3 with uroperitoneum. Bladder rupture was identified in 2 foals with uroperitoneum due to thick "flask like" bladder (Fig. 2 F) or discontinuity of the bladder wall, while a rupture of the urachus was not identified in the remaining foal. Gastric dilation was most frequently identified in the retro-sternal window (4/23 foals); turgid loops of small intestine in the meso-gastric (3/23 foals) and right ventro-lateral abdomen (2/23 foals) windows; presence of meconium in the retro-sternal (7/23 foals) and ventro-lateral abdomen (6/23) windows; abnormalities of intestinal wall echostructure (marked wall thickening, "target-like" lesions) in the ventro-lateral abdomen (1/23 foals) and meso-gastric (1/23 foals) windows (Fig. 2 C); peritoneal effusion in the ventral abdomen, especially in the retro-sternal window (6/23 foals). Thoracic abnormalities (B-lines, subpleural consolidations, irregularity of the pleuropulmonary surface) were seen in foals with the final diagnosis of bronchopneumonia (2/23 foals).

3.5. FOCUS protocol

The FUS protocol in healthy foals showed that some anatomical structures can be visualized from more than one window. Left-side views did not provide additional information compared with right-side views. Furthermore, more consistent sites of abnormalities were detected in sick foals. On this basis, the 12-window FUS protocol was reduced to a 7-window FOCUS protocol, including the 4 windows on the right side and the 3 windows on the ventral abdomen (Fig. 3). The left cranio-ventral thoracic, cardiac, spleno-gastric, nephro-splenic and left ventro-lateral abdomen windows were excluded. This allowed operators to avoid a change of recumbency during the examination. The time required to perform the FOCUS protocol was less than 10 min for all three operators, ranging from 3 to 10 min, with a median duration of 7 min. In healthy foals, the duration ranged from 3 to 9 min (median 6 min), whereas in sick foals it ranged from 7 to 10 min (median 8 min).

4. Discussion

In this study, a FUS protocol with standardized windows for goal-oriented assessment of the abdomen and thorax was developed in neonatal foals. The FUS acoustic windows were selected based on US protocols established for adult horses (Busoni et al. 2011; Vincze et al. 2019; Eberhardt and Schwarzwald, 2022; Bevevino et al. 2023; Corrie et al. 2024; Leduc et al. 2024), the unique anatomy and physiology of foal organ systems (Aleman et al. 2002; Nieth, Wehrend, 2019), and the common locations and organs where lesions are frequently detected in foals (Neal, 2003; McAuliffe, 2004; Porter and Ramirez, 2005; Bain, 2012; le Jeune and Whitcomb, 2014; Sprayberry, 2015; Cribb and Arroyo, 2018).

In healthy foals, despite the large number of views, the FUS protocol was feasible, with almost all US windows successfully acquired, enabling visualization of the target abdominal structures. The retro-sternal and hypo-gastric windows were particularly noteworthy, as the organ topography observed in these windows is specific to foals and not shared with adult horses. The retro-sternal window provides clear images of the liver, spleen and greater curvature of the stomach (Magri, 2018), in contrast to adult horses, where only the large colon is visible (Slack, 2012). The hypo-gastric window facilitates easy examination of the bladder (McAuliffe, 2004), unlike in adult horses, where the bladder cannot be visualized transabdominally.

In foals, several anatomical structures could be visualized and assessed from multiple windows: the stomach from both the retro-sternal and spleno-gastric windows; the small and large intestines from both the ventro-lateral abdomen and three ventral windows; the duodenum from both the nephro-cecum-duodenal and hepato-duodeno-colic windows; the spleen from the retro-sternal, nephro-splenic, and spleno-gastric windows; and the liver from both the hepato-duodeno-

Table 3
Frequency of organ visualization (N) and measurements of different organs for each ultrasonographic window in 17 healthy foals. US = Ultrasound; IQR = Interquartile range.

Side	US window	Organ visualization		Organ measurements			
		Organ	N	Measurement	Median	Range	IQR
Right	Cranio-ventral thoracic	Pleuropulmonary surface	17				
	Hepato-duodeno-colic	Liver (right lateral and caudate lobes)	17				
		Right dorsal colon	17	Wall thickness (cm)	0.34	0.25–0.37	0.33–0.35
		Duodenum	16	Wall thickness (cm)	0.24	0.21–0.28	0.23–0.25
				Contractile acts/10 s	1	0–2	1–1
	Ventro-lateral abdomen	Small intestine	15	Wall thickness (cm)	0.23	0.22–0.25	0.23–0.24
		Large intestine	17	Wall thickness (cm)	0.32	0.28–0.37	0.3–0.33
	Nephro-cecum-duodenal	Right kidney, cranial pole	17	Diameter, latero-medial (cm)	3.8	3.27–5.07	3.47–4.08
		Base of cecum	17	Wall thickness (cm)	0.29	0.25–0.31	0.27–0.29
		Duodenum	15	Wall thickness (cm)	0.22	0.21–0.27	0.21–0.23
				Contractile acts/10 s (n)	1	0–2	0–1
Ventral	Retro-sternal	Liver (left lateral lobe)	17				
		Spleen	16				
		Stomach (large curvature)	16	Diameter, cranio-caudal (cm)	5.73	4.48–7.85	5.12–5.90
				Wall thickness (cm)	0.39	0.37–0.42	0.37–0.41
		Small intestine	17				
		Large intestine	12				
	Meso-gastric	Umbilical remnants (2 umbilical arteries and urachus in the center)	12	Diameter, major (cm)	2.08	1.77–2.9	2.03–2.33
				Diameter, minor (cm)	1.06	0.8–1.33	0.99–1.20
		Small intestine	17	Wall thickness (cm)	0.23	0.2–0.27	0.21–0.25
		Large intestine	17				
	Hypo-gastric	Urinary bladder	17	Diameter, height (cm)	4.22	1.25–7.95	3.23–5.69
				Diameter, width (cm)	4.99	1.86–7.95	3.28–7.43
		Umbilical arteries	6				
		Small intestine	17				
		Large intestine	17				
Left	Cardiac	Heart, 4-chamber-long-axis scan	14				
	Cranio-ventral thoracic	Pleuropulmonary surface	17				
	Spleno-gastric	Spleen	17				
		Splenic hilum	11				
		Stomach	15	Diameter, dorso-ventral (cm)	7.48	7.06–7.9	7.27–7.69
				Diameter, medio-lateral (cm)	7.9	6.23–10.4	7.43–10
				Wall thickness (cm)	0.38	0.32–0.42	0.35–0.42
		Small intestine	10				
	Ventro-lateral abdomen	Small intestine	17	Wall thickness (cm)	0.23	0.2–0.24	0.21–0.23
		Large intestine	17	Wall thickness (cm)	0.32	0.29–0.34	0.31–0.33
	Nephro-splenic	Spleen	12				
		Left kidney	16	Diameter, latero-medial (cm)	5.96	5–7.13	5.74–6.55
				Diameter, dorso-ventral (cm)	3.53	3–4.4	3.34–4.18
		Left dorsal colon	16				
		Left adrenal gland	9				

colic and retro-sternal windows. The wall thickness of the stomach, small and large intestine, bladder diameter, kidney diameter and smaller diameter of the internal umbilical remnants were consistently within previously reported ranges (McAuliffe, 2004; Magri, 2018; McCoy et al. 2020; Nieth, Krohn, 2020).

One limitation of the present study is the lack of age stratification among the foals and the absence of individuals with umbilical disease. Certain anatomical structures, such as the umbilicus, can undergo rapid changes during the early postnatal period, and umbilical disorders are clinically relevant in neonates (McCoy et al. 2020). However, the majority of foals (36/40) were examined within a relatively narrow age range (9–96 h), which likely reduced the impact of age-related variability. Umbilical remnants were consistently visualized in healthy foals. The maximum diameter of the internal umbilical remnants exceeded previously reported reference values (< 2.5 cm) (McCoy et al. 2020). In healthy foals, this discrepancy may be attributed to measurement overestimation due to the limited lateral resolution of the low-frequency probe used for imaging superficial structures. In clinical settings, umbilical remnants are typically assessed with a linear probe (McCoy et al. 2020); however, in the context of FUS, the use of a convex probe was required to achieve a complete abdominal evaluation. Therefore, precise measurement of umbilical remnants was beyond the scope of this study, and further investigation is warranted to evaluate the protocol's effectiveness in detecting umbilical disease.

No reference values for gut motility in foals are available in the literature; however, the number of duodenal contractile events was comparable to the reference values reported for adult horses (<1 contractile event per 10 sec) (Gomaa et al. 2011; Patton et al. 2022). Jejunal motility was assessed using criteria described in the literature for both foals and adult horses, classifying motility as normal, reduced, or absent, and identifying the presence of dilated or turgid loops (Abraham et al. 2014; Haugaard et al. 2023).

The diagnostic usefulness of the FUS protocol was assessed by retrospectively comparing the findings with the final clinical diagnosis,

Table 4
Frequency (N) of diagnosis (left column) and ultrasound findings (right column) in 14 out of 23 sick foals presenting with FUS (*focused ultrasound*) abnormalities.

Diagnosis		N	US findings	N
Uroperitoneum	Bladder rupture	2	Peritoneal effusion	2
			Thick “flask like bladder”	2
			Discontinuity of bladder wall	1
	Urachus rupture	1	Peritoneal effusion	1
Jejunal intussusception		1	“Target-like” lesions	1
			Turgid small intestine loops	1
Meconium impaction		4	Meconium in large intestine	4
			Reduced intestinal motility	3
			Turgid small intestine loops	1
Enteritis		2	Gastric dilation	2
			Reduced intestinal motility	2
			Turgid small intestine loops	1
Septic peritonitis		2	Peritoneal effusion	2
Bronchopneumonia		2	Irregularity of pleuropulmonary surface	2
			B-lines	2
			Subpleural consolidation	2

which was made independently of the FUS results. The diagnostic performance was high, particularly for gastrointestinal diseases, with FUS findings aligning closely with the final clinical diagnosis. The ventro-lateral windows, especially the right, as well as the retro-sternal and meso-gastric windows, were particularly useful for identifying gastro-intestinal abnormalities. The ventral abdominal windows, notably the retro-sternal window, were effective in detecting increased free abdominal fluid.

Abnormalities in gastrointestinal US patterns, motility, and diameters were identified and associated with a final diagnosis of gastrointestinal disease in most cases. Meconium was detected in multiple locations, including the jejunum and large colon, in a sick foal. Notably, in healthy foals, the presence of meconium in the large intestine beyond the physiological period (>36 h) (Wong and Wilkins, 2024) can be considered an incidental finding if not associated with colic signs. Thus, the significance of meconium should be interpreted in the context of the foal’s age and the presence of colic symptoms.

Diagnostic performance was similarly high in identifying peritoneal effusions of various origins. Uroperitoneum due to bladder rupture was highly suspected in two cases based on the identification of a characteristic “flask-like” bladder or discontinuity of the bladder wall, which was later confirmed by peritoneal fluid analysis. The failure to detect urachal rupture may be attributed to the limited resolution of the probe used to examine this superficial structure. However, in many clinical cases, the presumptive diagnosis relies more on accompanying clinical and ultrasonographic signs than on direct visualization of the structural anomaly, even with higher-resolution probes.

Although the FUS protocol was feasible and applicable in both healthy and sick foals, several factors prolonged the examination duration: the need for positional changes, the large number of US windows (12), and the requirement for staff to restrain the foals during the procedure.

The FUS protocol was not entirely performed more frequently in sick foals than in healthy foals. Similar to healthy foals, certain US windows were occasionally omitted, but in sick foals, this was more influenced by their clinical status and the prioritization of other diagnostic and therapeutic procedures. In sick foals, the left cranio-ventral thoracic and cardiac windows were among the least performed, likely due to their acquisition being delayed until after a change in recumbency.

To address this, a shortened 7-window protocol, called FOCUS, was successfully implemented and tested on 6 foals by 3 operators with varying levels of US experience. In this shortened protocol, the left thoracic and cardiac windows are replaced by a single right-side combined window. Positioned more cranio-ventrally than the left thoracic window, just caudal to the triceps muscle, this window allows simultaneous visualization of the pleural and pericardial spaces, aiding in the identification of relevant effusions and changes. In equine patients,

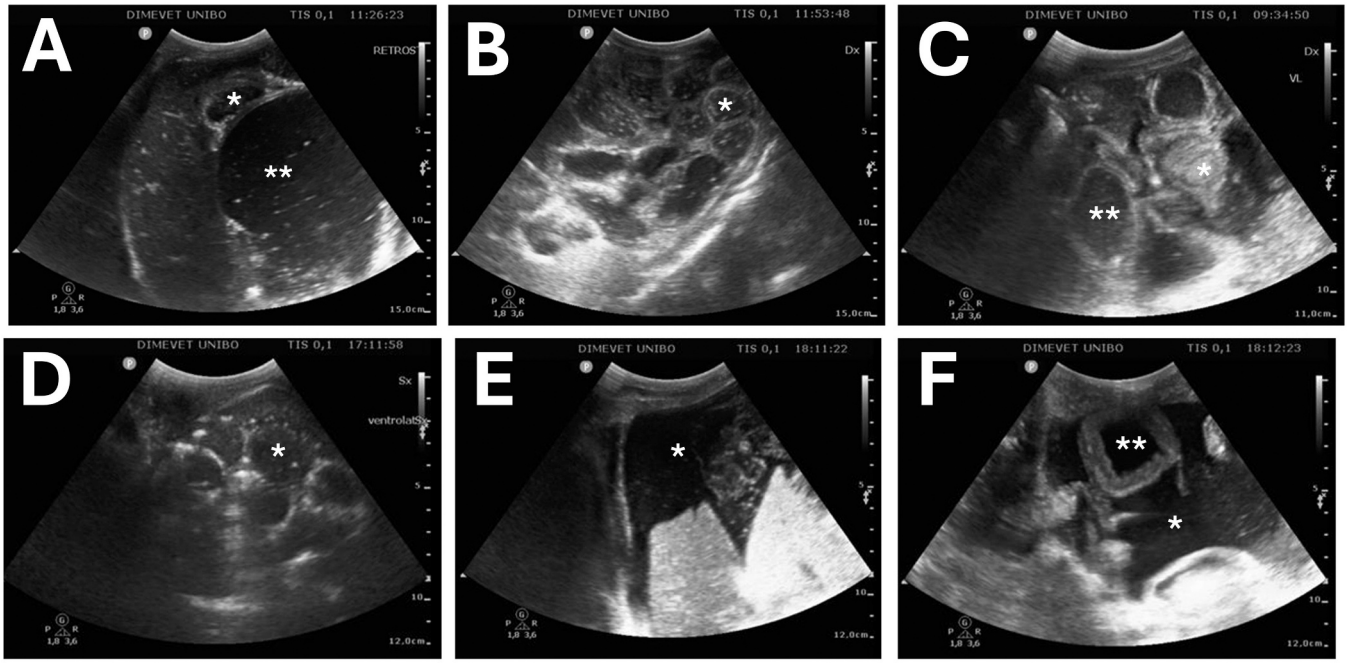

Fig. 2. FUS findings in sick foals. A. Retro-sternal window. The diaphragm, liver, small intestine loop with fluid pattern (*), and dilated, fluid-filled stomach (**) are shown from left to right in a foal affected by enteritis. **B.** Ventro-lateral right abdominal window. Dilated small intestine loops with fluid content (*) in a foal affected by enteritis. **C.** Ventro-lateral right abdominal window. "Target-like" lesion (*) and turgid small intestine loops (**) in a foal affected by jejunal intussusception. **D.** Ventro-lateral left abdominal window. Hypoechoic masses in the large intestine (*) of a foal with meconium impaction. **E.** Retro-sternal window. Severe peritoneal effusion (*) in a foal affected by uroperitoneum. **F.** Hypogastric window. Peritoneal effusion (*) and the presence of a thick "flask-like" bladder (**) in a foal affected by uroperitoneum.

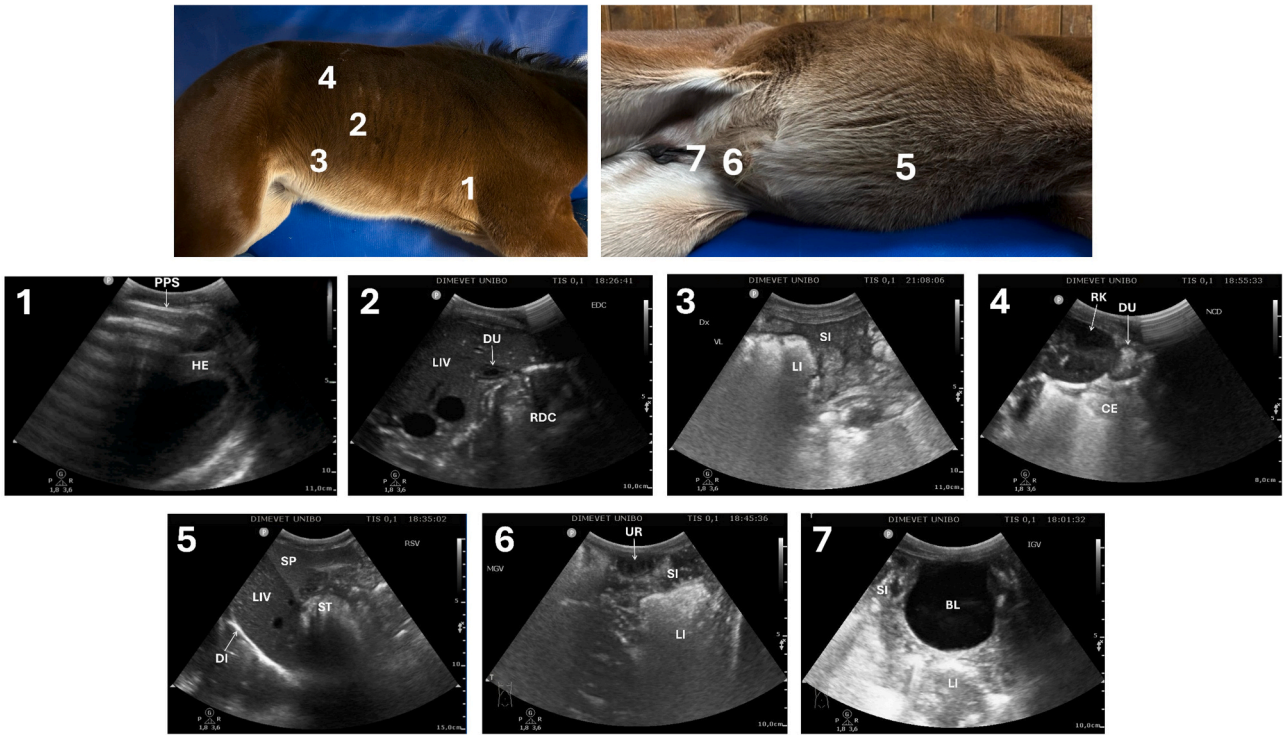

Fig. 3. The 7-window FOCUS protocol. Probe placement site (top) and corresponding ultrasound image in a healthy foal (bottom) for the 7 acoustic windows of the FOCUS protocol. Four windows on the right side: 1. Right cardiac-thoracic window - Pleuropulmonary surface (PPS) and heart (HE); 2. Hepato-duodeno-colic window - Liver (LI), right dorsal colon (RDC) and duodenum (DU); 3. Ventro-lateral abdomen window - Small intestine (SI) and large intestine (LI); 4. Nephro-cecum-duodenal window - Cranial pole of the right kidney (RK), cecum (CE) and duodenum (DU). Three on the ventral abdomen: 5. Retro-sternal window - Liver (LI), spleen (SP) and stomach (ST); 6. Meso-gastric window - Umbilical remnants (UR), small intestine (SI) and large intestine (LI); 7. Hypo-gastric window - Bladder (BL), small intestine (SI) and large intestine (LI).

pleural effusion is typically bilateral due to the presence of an "incomplete mediastinum"; however, unilateral effusion may occur, with clinical evidence suggesting a predilection for the right hemithorax (Busoni et al. 2011; Johns, 2023). The spleno-gastric window is replaced by the retro-sternal window, which not only visualizes the spleen and stomach but also the liver and potential free peritoneal fluid. The nephro-splenic window, primarily useful for diagnosing congenital renal abnormalities and nephrosplenic hernias (both rare in foals) (Bartmann et al. 2002; Chaney, 2007), is more relevant in cases with signs of renal dysfunction, warranting a more thorough kidney examination. The findings from the left ventro-lateral abdomen window can generally be substituted by the contralateral window. While the variable topography of the jejunum and the increased sensitivity of US when examining multiple loops should be considered, the reduced development of the large intestine in neonates allows the jejunum to be more easily visualized from several windows compared to adult horses (Porter and Ramirez, 2005).

The FOCUS protocol was quicker than the extended FUS protocol in a preliminary evaluation on a small sample of healthy and sick foals, with a short acquisition time (median 7 min, range 3–10 min). It is expected to have greater clinical applicability in emergency cases.

The main limitations of this study include the involvement of a single operator for acquiring FUS images, which precluded the assessment of inter-operator variability, the relatively small cohort of sick foals on which FUS was applied, and the limited number of foals on which FOCUS was tested. However, for the purpose of developing a feasible, practical, and efficient standardized short, focused protocol in neonatal foals, such as FOCUS, the number of foals included in this study was considered sufficient to yield reliable preliminary results. A larger, prospective, multicenter study will be necessary to validate the protocol's applicability across different settings. Additionally, the inclusion of a greater number of sick foals with well-defined conditions will enable the assessment of FOCUS diagnostic accuracy in emergency contexts.

5. Conclusions

In conclusion, this study suggests that FUS is a viable technique for detecting common intra-abdominal and intra-thoracic abnormalities in neonatal foals, even when performed by veterinarians with limited US experience. Given its greater acquisition efficiency and clinical applicability, the FOCUS protocol could be strategically used in sick foals during emergency situations.

CRediT authorship contribution statement

Carolina Castagnetti: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation. **Aliai Lanci:** Writing – review & editing, Writing – original draft, Visualization. **Jole Mariella:** Writing – review & editing, Writing – original draft, Visualization. **Francesca Freccero:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Valeria Busoni:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Ilaria Imposimato:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Nicola Ellero:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alessandra Maggi:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Generative AI and AI-assisted technologies in the writing process

No Generative AI and AI-assisted technologies were used in the

writing process.

Ethical statement

The authors confirm that the ethical policies of the Veterinary Journal, as noted on the Journal's author guidelines page, have been adhered to. This study was approved by the Animal Care and Use Committee of the University of Bologna (Approval number, 195113; Approval year, 2024) and informed consent was obtained from the owners prior to the inclusion of the foals.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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Data availability

We would like to inform you that the dataset associated with our manuscript is publicly available in the University of Bologna repository.

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