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Influence of the Respiratory Cycle on Caudal Vena Cava Diameter Measured by Sonography in Healthy Foals: A Pilot Study

M.C. Tuplin, A.E. Romero, and S.R. Boysen

Background: Intravascular volume assessment in foals is challenging. In humans, intravascular volume status is estimated by the caudal vena cava (CVC) collapsibility index (CVC-CI) defined as (CVC diameter at maximum expiration [CVC_{max}] – CVC diameter at minimal inspiration [CVC_{min}])/CVC_{max} × 100%.

Hypothesis/Objectives: To determine whether the CVC could be sonographically measured in healthy foals, determine differences in CVC_{max} and CVC_{min}, and calculate inter- and intrarater variability between 2 examiners. We hypothesized that the CVC could be measured sonographically at the subxiphoid view and that there would be a difference between CVC_{max} and CVC_{min} values.

Animals: Sixty privately owned foals <1-month-old.

Methods: Prospective study. A longitudinal subxiphoid sonographic window in standing foals was used. The CVC_{max} and CVC_{min} were analyzed by a linear mixed effect model. Inter-rater agreement and intrarater variability were expressed by Bland-Altman and intraclass correlation coefficients, respectively.

Results: Measurements were attained from 58 of 60 foals with mean age of 15 ± 7.9 days and mean weight of 75.7 ± 17.7 kg. The CVC_{max} was significantly different from CVC_{min} ($D = 0.515$, SE = 0.031, $P < 0.001$). Inter-rater agreement of the CVC-CI differed by an average of −0.9% (95% limits of agreement, −12.5 to +10.7%). Intrarater variability of CVC_{max} was 0.540 and 0.545, of CVC_{min} was 0.550 and 0.594, and of CVC-CI was 0.894 and 0.853 for observers 1 and 2, respectively.

Conclusions and Clinical Importance: These results indicate it is possible to reliably measure the CVC sonographically in healthy foals, and the CVC-CI may prove useful in assessing the intravascular volume status in hypovolemic foals.

Key words: Collapsibility index; Fluid estimation; Intravascular volume status; Ultrasound.

Hypovolemia, which is a major cause of morbidity and mortality in sick foals, can occur rapidly as a result of several underlying disease processes.[1,2] Fluid administration is crucial in the treatment plan of many hospitalized foals. However, because of the immaturity of the kidneys, fluid overload can be more of a problem than hypovolemia in foals receiving fluid therapy.[2,3] The methods that are currently used to assess fluid status in foals recently have come under review, as well as the theories related to fluid administration.[3] Currently, there is no clinical indicator or monitoring modality that can accurately determine the presence of hypovolemia, hypervolemia or return to euvolemia in foals.[3]

Abbreviations:

Bpm	beats per minute
Brpm	breathes per minute
°C	degrees Celsius
CI	confidence interval
Cm	centimeter
CVC	caudal vena cava
CVC-CI	caudal vena cava collapsibility index
CVC_{max}	caudal vena cava maximum diameter with expiration
CVC_{min}	caudal vena cava minimum diameter with inspiration
CVP	central venous pressure
DVLC	distributed veterinary learning community
ICC	intraclass correlation coefficient
IQR	interquartile range, 25th percentile to 75th percentile
Kg	kilograms
RA	right atrium
TPR	temperature, pulse, respiration
UCVM	University of Calgary, Faculty of Veterinary Medicine

In human medicine, a similar problem exists in determining intravascular volume status in critical patients.[4–15] Recent studies have identified numerous deficiencies with the current methods used to estimate fluid status, including heart rate, blood pressure, physical examination findings, and laboratory findings.[4,12,14,15] A rapid and noninvasive method recently investigated to assess fluid status in humans involves the use of sonography to measure the change in diameter of the caudal vena cava (CVC) with inspiration and expiration.[4–14,16,17]

The CVC changes diameter during the respiratory cycle.[4–21] With inspiration, the CVC diameter in the cranial abdomen decreases and with expiration it increases. Although a number of variables can affect the

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degree of change in CVC diameter during respiration, there is a strong correlation in people between the degree of change in diameter of the CVC and the fluid status of the patient. Hypovolemic patients have a larger change in CVC diameter during the respiratory cycle, whereas hypervolemic patients have very little or no change in the CVC diameter.

In humans, instead of analyzing CVC diameter alone, the CVC collapsibility index (CVC-CI) is used to estimate intravascular volume status. The difference between the maximal CVC (CVC_{max}) and minimal CVC (CVC_{min}), divided by CVC_{max}, and multiplied by 100%, provides the collapsibility index. The CVC-CI in people is negatively correlated with central venous pressure (CVP) and patient volume status.[4–11,13,16,17,21] The normal CVC-CI is between 20% and 50% in adult humans.[4,5,8] The American Society of Echocardiography and the European Association of Cardiovascular Imaging recommends that CVC diameter and CVC-CI be used together to determine right atrial (RA) pressure.[13,16]

Studies investigating the CVC and the CVC-CI in equine neonates are lacking. The objective of our study was to assess whether the CVC could be identified where it crosses the diaphragm in healthy foals. The hypotheses were that the CVC could be sonographically identified in healthy foals <1 month of age, and that a statistical difference exists between CVC_{max} and CVC_{min} diameters, allowing the CVC-CI to be calculated.

Material and Methods

Ours was a prospective, observational study. Informed client consent, as well as Animal Care and Use Committee approval from the University of Calgary, was obtained.

Animals were privately owned foals, <1 month of age, and assessed as healthy based on a general physical examination. The general physical examination included temperature, pulse and respiration (TPR), mucous membrane color and capillary refill time, <2 second skin tent, absence of ocular and nasal discharge, examination of joints and umbilicus, and appropriate mental status. Weight was determined with a JorVet Walk-on platform scale (J825QM).[a] Height at the withers was recorded with a combination of levels and a measuring tape. Recruitment occurred through veterinary clinics and by word of mouth. Foals were excluded from the study if they did not fit the age criteria, if temperament precluded sonographic examination, if the procedure caused undue stress, or if there were inadequate facilities to perform the study. Data were collected between April 14 and June 27, 2016.

The sonographic machine used was a Mindray M7, with a 3.5–5 MHz convex probe.[b] The depth setting used was 12–20 cm, with the focus position typically set at the level of the CVC or as deep as the limitations of the ultrasound machine would allow. Alcohol was used to provide conduction of the signal.

Duplicate sonographic examinations were performed on standing foals by approaching them from the right side. The probe was placed longitudinally just caudal to xiphoid process, with the marker pointing caudally (see Fig 1). The liver was identified first, and then the diaphragm. If the diaphragm could not be seen, the head of the probe was angled slightly cranial or the probe moved cranial. Once the diaphragm and liver were identified, the probe was moved or fanned laterally toward the right extent of the rib cage of the foal. During this process, the CVC was identified as 2 distinct horizontal and parallel lines interrupting the diaphragm, at a depth of between 12 and 15 cm. If a complete sweep did not identify the CVC, the depth was increased and the process repeated. Once the CVC was identified at the point it crossed the diaphragm, the probe was slowly fanned through all longitudinal planes of the CVC to identify the largest visual width at this location. This procedure helped ensure that the central diameter of the CVC was measured. To ensure M-Mode was perpendicular to the CVC, the probe also was gently rocked until the point where the CVC crossed the diaphragm was located at, or within 5 mm, the center of the ultrasound screen. When centered on the ultrasound screen, M-Mode was used to measure the CVC by placing the M-Mode cursor perpendicular to the CVC. With the ventral and dorsal walls of the CVC clearly visualized in M-Mode, a cineloop was saved that included several inspiratory and expiratory cycles (see Fig 2).

Two sonographic examinations were performed per observer, giving 2 or 4 complete data sets per foal. The duplicate examination occurred after removal of the probe from the foal and saving of the cineloops (approximately 1 minute). Foals were examined by 1 or both observers as availability of the 2 observers performing scans and cooperation of the foals allowed. Whenever possible, examinations were performed by both observers. Foals remained standing and restrained, whereas the person performing the sonographic examination was switched when both observers performed the examination (approximately 2 minutes). The CVC measurements were taken retrospectively on recorded M-Mode cineloops at the largest diameter during expiration and smallest diameter on inspiration. The CVC_{max} and CVC_{min} were recorded within the same respiratory cycle. The CVC-CI was calculated by the following equation: $([CVC_{max} - CVC_{min}]/CVC_{max}) \times 100\%$.

Observer 1 had 12 years of experience with clinical sonography. Observer 2 was a novice sonographer who had completed three 3-hr sonographic laboratory sessions. Both observers were trained for 4 hours by an experienced sonographer familiar with the technique of locating and measuring the CVC. Both observers practiced localizing the CVC at the level of the diaphragm on dogs, scanning at least 5 dogs before applying the skill to foals.

The Bland-Altman method was used to compare inter-rater variability and an R package "MethComp"[c] was used for analysis.[22] A linear mixed effects model was used to detect any difference between CVC_{max} and CVC_{min} by the "nlme" package.[d,23]

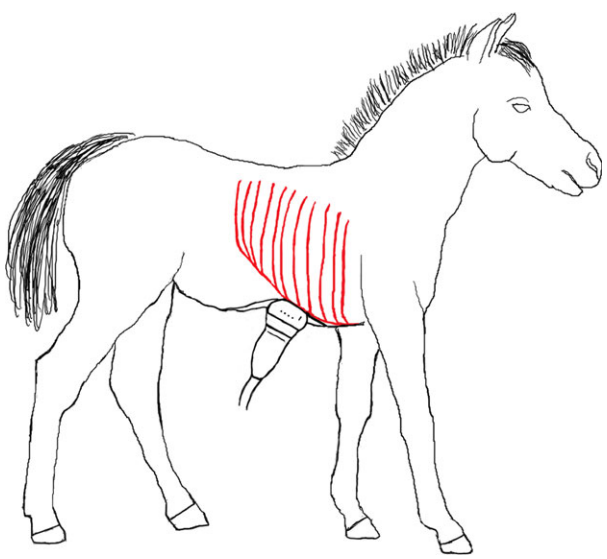

Fig 1. Schematic of the right side of a foal, with approximate location of ribs and sonographic probe shown for the subxiphoid window.

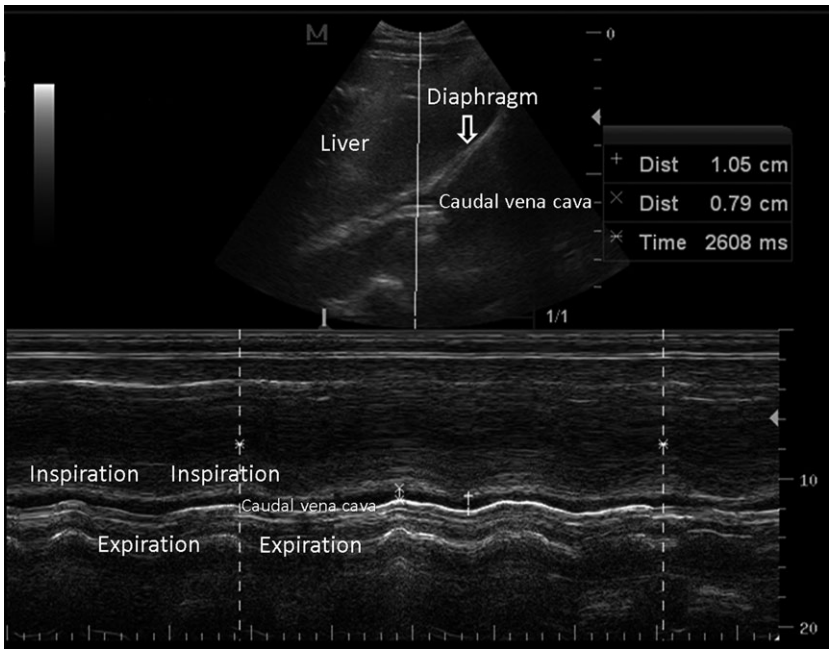

Fig 2. Sonographic image: B-Mode (upper), demonstrating the liver, diaphragm, and caudal vena cava (CVC) with the M-Mode line crossing the CVC perpendicularly at the level of the diaphragm. M-Mode (lower) showing CVC diameter on expiration (trough) and inspiration (peak). Five respiratory cycles are shown in M-Mode. Large white arrows indicate site of measurement for minimum inspiratory CVC diameter (CVC_{min}), and white arrow heads indicate site of measurement for maximum expiratory CVC diameter (CVC_{max}).

For both models, the assumptions of normality and equal variances required for the model were checked and met. A P value $\leq$ 0.05 was considered statistically significant. Intraclass correlation coefficient (ICC) also was used to express Intra- and inter-rater variability by SPSS[e] software.[24] For inter-rater variability, the mean of the 2 measurements for each observer was used. All other statistical analyses (D'Agostino & Pearson omnibus normality test and column statistics) were performed by Prism software.[f]

Results

Sixty foals were enrolled in the study. The CVC was identified in 58 of 60 foals. One foal was excluded because of excessive gas in the colon, the second was excluded because of synchronous diaphragmatic flutter (singultus), which precluded accurate measurements of the CVC in M-Mode. Breeds included 35 Quarter Horses, 12 Warmbloods, 10 Standardbred, 2 Draft crosses, and 1 Haflinger cross. Age varied from 1 day to 30 days, with a mean of 15 $\pm$ 7.9 days. Foal data are presented in Table 1. Seventeen foals had sonographic examinations performed only by the first observer, 16 by the second observer, and 25 foals were examined by both observers.

The CVC_{min} and CVC-CI data passed the D'Agostino & Pearson omnibus normality test, but the CVC_{max} did not. Among all measurements, CVC_{max} median was 1.99 cm and interquartile range (IQR) was 1.71–2.3 cm, CVC_{min} mean was 1.49 $\pm$ 0.38 cm (median, 1.51 cm; IQR, 1.22–1.72 cm), and the CVC-CI mean was 26 $\pm$ 10% (median, 26%; IQR, 19–32%). Table 2 shows the results from the linear mixed effects model. The intercept was statistically significant which indicates that there was a statistically significant difference between CVC_{max} and CVC_{min}, after adjusting for rater effect ($P < 0.001$). The mean difference between CVC_{max}

Table 1. Foal mean parameters.

Foal Parameters	Mean	Standard Deviation
Weight (kg)	75.7	±17.5
Height (cm)	102.2	±6.4
Temperature (°C)	38.6	±0.4
Pulse (bpm)	113.1	±22.9
Respiration (brpm)	46.9	±21.9

Including the weight in kilograms (kg), the height at the withers in centimeters (cm), the rectal temperature in degrees Celsius (°C), the pulse in beats per minute (bpm) and the respiratory rate in breathes per minute (brpm).

Table 2. Linear mixed effects model of the difference between the maximum and minimum caudal vena cava measurements obtained in healthy foals.

	Parameter Estimates	Std. Error	*P*-value
Fixed effects			
(Intercept)	0.515	0.032	<0.001
Factor (observer 2)	0.025	0.023	0.269
Factor (observer 1)	1	–	–
Random effects			
Animal	0.211	–	–
Rater nested within animal	0.028	–	–

The parameter estimates of the fixed effects from a linear mixed effect model, using animals, and rater nested within animals as random effects.

and CVC_{min} was 0.515 cm, with a standard error of the mean (SEM) of 0.031 cm.

Bland-Altman plots for inter-rater agreement are shown in Figure 3. Inter-rater agreement of the CVC-CI differed by an average of 0.9% (95% limits of agreement, −12.5 to +10.7%). The ICC for intrarater variability of CVC_{max} was 0.540 (95% confidence interval [CI], 0.286–0.724) and 0.545 (95% CI, 0.288–0.728), of CVC_{min} was 0.550 (95% CI, 0.299–0.730) and 0.594 (95% CI, 0.354–0.761), and of CVC-CI was 0.894 (95% CI, 0.812–0.942) and 0.853 (95% CI, 0.741–0.919) for observers 1 and 2, respectively. The ICC for inter-rater variability of CVC_{max} was 0.712 (95% CI, 0.448–0.862), CVC_{min} was 0.686 (95% CI, 0.406–0.848), and CVC-CI was 0.884 (95% CI, 0.755–0.947).

Discussion

We demonstrated that it is possible to identify and measure the diameter of the CVC at the subxiphoid site in standing healthy foals <1 month of age. The CVC_{max} and CVC_{min} showed a significant difference in size during the respiratory cycle (mean difference, 0.52 cm) making it possible to calculate the CVC-CI. Human medical literature suggests the CVC-CI is a good indicator of intravascular volume status because it is less affected by hypovolemic compensatory mechanisms than are other clinical parameters (e.g, blood pressure, capillary refill time) used to indirectly assess volume status.[12] Experienced practitioners have identified the challenge of assessing intravascular volume status in foals.[1–3,25–27] Our study determined that it is possible to calculate the CVC-CI in healthy foals < 1 month of age, which is the first step in determining if this technique may be useful in assessing intravascular volume status in foals. Further studies would be required to establish the clinical validity of the CVC-CI in sick foals, including whether there is a correlation with right atrial pressures and other methodologies used to assess intravascular volume status.

The statistical difference of CVC_{max} and CVC_{min} in the current study is similar to what has been reported in human medicine; the CVC diameter is larger during expiration than inspiration.[4–21] This occurs for several reasons including a change in pressures within the thorax during the respiratory cycle, the compliance of the CVC, and the motion of the diaphragm.[18,19] Respiration results in a change in positive and negative pressures within the thorax.[9,18] These pressure changes influence the vascular volume within the thorax and abdomen. Negative pressure draws blood into the thoracic CVC from the abdominal CVC, causing the CVC to decrease in size within the abdomen whereas the positive pressure of expiration pushes blood from the thoracic CVC into the abdominal CVC. Currently, most studies of CVC-CI have been performed in humans and it is unknown how species variation impacts pressure changes within the thorax and abdomen and CVC diameter during the respiratory cycle. Veins are compliant and not subject to the same compensatory vasoconstriction as arteries are, allowing intravascular volume

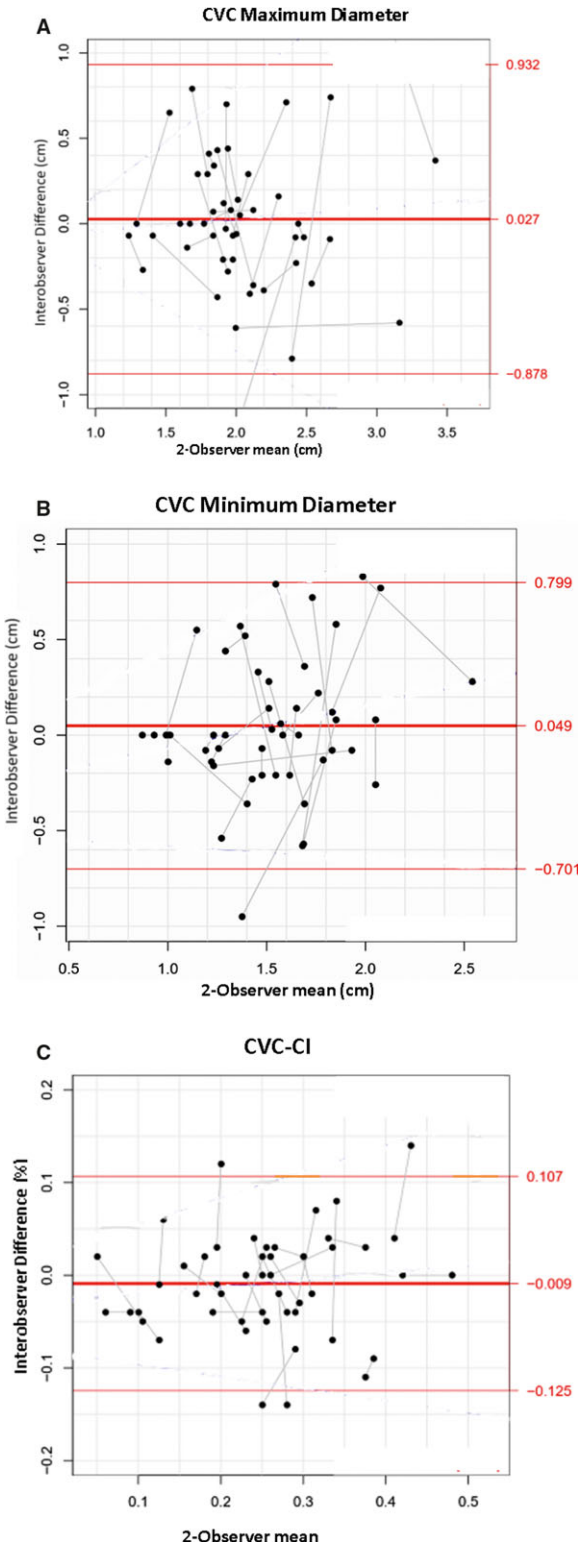

Fig 3. (**A,B,C**) Bland-Altman plots for the caudal vena cava (CVC) maximum, CVC minimum diameter, and the caudal vena cave collapsibility index (CVC-CI) inter-rater variability (n = 25). The lines connecting the dots indicate the first and second examination performed by each observer for an individual foal. The bold horizontal line is the mean difference between observers 1 and 2. The finer horizontal lines represent the 95% limit of agreements for mean difference.

status to be better assessed with the changes in size of the CVC compared with larger arterial vessels such as the aorta.[12,14,21] Movement of the diaphragm also affects the collapsibility of the CVC,[19,28] although the importance of this finding is uncertain.

The Bland-Altman inter-rater agreements did not show a statistical difference, indicating good repeatability for the measurements assessed. The CVC-CI 95% limits of agreement for inter-rater variability showed variation of up to 23%, which may influence clinical decision making. This variation is not surprising given a 68% Bland-Altman variation in the 95% limits of agreement for CVC-CI has been reported in healthy humans.[29] However, the greatest variation of CVC-CI occurs in healthy populations, with less variation occurring in patients with values outside of established reference intervals.[4] Therefore, although variation is substantial in healthy human patients, calculation of the CVC-CI still has clinical utility and prognostic relevance when values are at either extreme. Further studies are needed to determine whether this trend also is present in foals, whereby hypovolemic foals or foals with increased right atrial pressures will have less variation in CVC-CI values. The large variation in the 95% limits of agreement has several potential causes. It may be a result of compression of the CVC by the ultrasound probe or not measuring the central diameter of the CVC, both of which would falsely decrease the CVC diameter. Increased respiratory effort has been shown to increase the degree of CVC collapse in humans, and a change in respiratory effort by the foal (because of the stress of separation from the mare or handling) may have occurred in the time it took observers to measure and record the CVC.[19,28] Finally, the angle of M-Mode relative to the CVC may have varied between observers, which would falsely increase the size of the CVC. Good inter-rater agreement on all measurements suggests that the skill to find the CVC in foals is not difficult because 1 of the observers had minimal sonographic experience, whereas the other had considerably more experience. These findings are similar to those of a study reported previously that also failed to find a statistical difference between an experienced operator and a less experienced operator in determining the CVC/aorta diameter in humans.[14]

The intrarater variability determined by ICC for CVC_{max} and CVC_{min} was fair for both observers.[30] Causes of variation for CVC_{max} and CVC_{min} intrarater variability are likely similar to those discussed for inter-rater variability. However, intrarater variability of CVC-CI was excellent for both observers. This finding suggests that the percentage change in the CVC during the respiratory cycle is consistent despite individual variation in the measurement of CVC_{max} and CVC_{min}. For example, if the ultrasound beam is not at the exact center of the CVC in the longitudinal plane, as long as the CVC_{max} and CVC_{min} are measured within the same longitudinal plane the impact on the CVC-CI will be less variable than for either measurement alone. Therefore, the CVC-CI may be a better measure than absolute CVC values. Similarly, the ICC inter-rater variability also suggests the CVC-CI may be a better measure than absolute CVC values.

The CVC-CI values found in our study (mean, $26 \pm 10\%$) are lower than what has been reported in healthy, adult humans (mean, $47.3 \pm 8.9\%$).[31] The difference between studies may be due species differences, the age of the foals examined or both. A study in humans found that healthy neonates had lower CVC-CI values (mean, $28 \pm 13\%$) compared to adults, and very similar values to those found in the foals in our study.[11] Some of the difference in CVC-CI between human neonates and adults may be the result of neonates having a larger percentage of total body water, because 60% of an adult's body mass is water, whereas neonates at term have 75% of body weight as water.[32] The relative proportion of intracellular compared to extracellular fluid also may account for differences in humans, because the extracellular compartment is 53% in neonates compared to 33% in adults.[11,32] Data in horses are similar, with mean total body water for foals within 24 hours of birth being 0.744 L/kg ($\pm$0.024 L/kg).[33] This value is larger than what is reported for human neonates at birth (0.696 L/kg) and adult horses (0.67 ± 0.06 L/kg). The extracellular fluid volume in foals also is increased relative to adult horses and is similar to the intracellular fluid volume (1:1). This ratio is expected to reach 2:1 intracellular to extracellular fluid in an adult horse.[33] Further research is needed to establish validated CVC-CI reference values for healthy and unhealthy foals of different ages.

Our study had several limitations that should be considered. All M-Mode cineloops were evaluated by 1 nonblinded observer, which may have caused bias in the CVC_{max} and CVC_{min} measurements obtained. Also, we evaluated healthy foals, and it is unknown what changes or clinical relevance measuring the CVC in sick foals might produce. Foals in our study were assessed in the standing position. The position of the patient is known to influence CVC diameter in people, with left lateral recumbency creating the smallest diameter, right lateral recumbency creating the largest diameter and dorsal recumbency providing intermediate values.[10] No studies currently describe the change of CVC diameter in the prone position. Sick foals will tend to be scanned in recumbency, whereas in our study foals were scanned in the standing position. Thus, the effect of body position on CVC-CI in foals needs further investigation. In other species, changes in the abdominal CVC diameter have been assessed by many sonographic windows, whereas our study only assessed data from a single sonographic window. Although this window is most commonly described in human medicine, it is possible that there are other windows that would allow better measurement of the CVC. Small sample size is also a concern, and a larger sample size may have detected differences that truly existed between observers (type II error). Finally, data from 2 foals were not collected. One foal had gas in the colon that made an image of the CVC as it crossed the diaphragm impossible to obtain. Difficulty in obtaining images because of intra-abdominal bowel gas has been reported in humans.[8] The second foal had

synchronous diaphragmatic flutter, so although we could identify the CVC, we were unable to attain a cineloop with complete respiratory cycles.

Conclusions

The CVC can be measured in healthy standing foals and a significant difference was seen between CVC_{max} and CVC_{min}. This finding allowed us to calculate the CVC-CI, which may have clinical relevance in assessment of volume status. The lack of significance of inter-rater variability between 2 observers suggests that the CVC-CI can be easily and consistently calculated during sonographic measurement by both novice and more experienced sonographers. Finally, it is a noninvasive and rapid procedure that healthy foals tolerate well and has the potential to provide a technique to assess the intravascular volume status of foals by calculation of the CVC-CI. Further research into validation of reference values for normal and abnormal CVC-CI values is required before this concept can be applied in clinical settings.

Footnotes

[a] Jorgenson Labs, Loveland, Colorado
[b] Shenzhen Mindray Bio-Medical Electronics Co., Nanshan, Shenzhen, China
[c] "MethComp" package version 1.22.2 for the repeated measured inter-rater agreement
[d] "nlme" package version 3.1-129 for the linear mixed effects models
[e] IBM SPSS Statistics, IBM Corporation, Armonk, New York
[f] GraphPad Prism version 7.0d, San Diego, California

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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