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Ultrasonography of the stomach and small intestine in healthy Mangalarga Marchador horses from birth to 5 years of age

[*Ultrassonografia de estômago e intestino delgado de equinos Mangalarga Marchador saudáveis desde o nascimento até cinco anos de idade*]

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ABSTRACT

The aim of the study was to describe location, sonographic characteristics and measures of the stomach and small intestine of equines at different ages. Abdominal ultrasonography was performed on 88 healthy equines of either sex, aged 1, 7 and 15 days, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 months and 1, 3 and 5 years, with 20 animals per group. Location, characteristics and measurements of stomach, duodenum and jejunum were evaluated. Descriptive statistics were performed for each measurement and the percentile for each age was analyzed. The work showed the growth and characteristics of the organs evaluated over time. Adult animals showed a minimum of 4 duodenal movements per minute. It was possible to locate the organs evaluated in all animals. The detailed ultrasound examination of these organs allowed gathering information that could be used to assist in the care of future patients.

Keywords: equine, ultrasound, abdominal, duodenum, jejunum

RESUMO

O objetivo deste estudo foi descrever localização topográfica, características e medidas ultrassonográficas de estômago e intestino delgado de equinos em diferentes idades. Foi realizado um exame ultrassonográfico abdominal em 20 equinos saudáveis de ambos os sexos, com idades de um, sete e 15 dias, um, dois, três, quatro, cinco, seis, sete, oito, nove, 10 e 11 meses e um, três e cinco anos. A localização, as características e as medidas de estômago, duodeno e jejuno foram avaliadas. Foi realizada a estatística descritiva para cada medida e analisado o percentil para cada idade. O trabalho mostrou o crescimento e as características dos órgãos avaliados ao longo do tempo. Animais adultos apresentaram um mínimo de quatro movimentos por minuto de duodeno. Foi possível localizar os órgãos avaliados em todos os animais. O exame ultrassonográfico detalhado desses órgãos permitiu agrupar informações que poderão ser usadas para auxiliar no atendimento a futuros pacientes.

Palavras-chave: equino, ultrassom, abdominal, duodeno, jejuno

INTRODUCTION

Diagnostic ultrasound provides a non-invasive abdominal visualization of gastrointestinal organs and viscera that are otherwise difficult to examine (Reef *et al*., 2004). This test provides additional information on parts of the abdomen that are unavailable to rectal palpation, and it is especially useful in evaluating horses in which the procedure is impossible, unacceptable, or risky (Norman, 2014).

Transcutaneous ultrasound provides instant information about the volume and type of peritoneal effusion, gastric content and its dilatation; small intestine´s content, motility, wall thickness and diameter; and content, motility, and wall thickness of the colon (Jeune and Whitcomb, 2014; Sprayberry,

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2015). A good agreement has already been observed between the measurements of intestinal layers in histological and ultrasound examinations for the duodenum, jejunum, cecum, small colon, and right dorsal large colon (Bevevino *et al*., 2021).

In cases of inflammation and vascular compromise, ultrasound evaluation can provide both diagnostic and prognostic information, which are essential when making surgical decisions with the clients (Norman, 2014). Acute abdomen in horses is considered an emergency, and it is crucial to have an early determination of the need for surgical intervention as a therapeutic measure (Amaral *et al*., 2017). Ultrasound is a fast and non-invasive tool in formulating the accurate diagnosis of horses presenting acute colic, leading to successful treatment and results.

The availability of portable equipment and better protocols for abdominal examination increased the use of transcutaneous ultrasound in the diagnosis of acute colic (Norman, 2014). However, there are few studies that report the ultrasound monitoring of organs in animals of different ages, following their growth. By bringing data detailing the period in which the individual is, it is possible to obtain more accurate information to aid in the early diagnosis of diseases related to the stomach, duodenum and jejunum.

The aim of this study was to evaluate in detail the stomach, duodenum, and jejunum of Mangalarga Marchador horses, a breed native to Brazil, at different ages, to obtain a database that can be useful in future veterinary medicine.

MATERIAL AND METHODS

The work was carried out with 88 Mangalarga Marchador equines of either sex, aged 1 day, 7 days, 15 days, 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 months, 1 year, 3 years and 5 years, with 20 animals of each age group. Full-term foals that showed no clinical changes were added to the experiment, as well as older animals that had not been sick in the last year. Those who had any disease were withdrawn from the study. The work was approved by the Ethics Committee on the Use of Animals of the Federal University of Lavras under protocol number 061/18 and the evaluations took place at *Haras do Henrique*, in Nepomuceno, MG, Brazil.

The animals were kept in extensive breeding with corn silage supplementation and ration in the trough. The foals were weaned at 4 months of age, in groups with at least 4 individuals. From 5 months onwards, the youngest animals were incorporated into the group of the oldest ones born in the same season.

Before the ultrasound evaluation, the thoracic circumference of the animals was obtained using a tape measure and they underwent a general physical examination, with measurement of heart rate, respiratory rate, capillary refill time and rectal temperature. For restraint of neonates, a second person gently held them by holding the chest and the tail. Larger animals were restrained only in the halter. The foals were examined close to their mothers, a procedure that made the animals feel calmer and safer. Trichotomy using a shearing machine was performed between the 10th intercostal space and the paralumbar fossa on each side. It was used 70% alcohol for the ultrasound contact.

Sonographic location and characteristics of stomach, duodenum and jejunum were evaluated. Furthermore, measurements of wall thickness and diameter of duodenum and jejunum in millimeters (mm) were determined. In animals older than 4 months, it was measured the number of duodenal movements in 1 minute. The abdomen scan started at the 10th intercostal space and ended at the paralumbar fossa, with the transducer moving from dorsal to ventral direction.

The wall thickness of the stomach, duodenum and jejunum was obtained from the straight-line measurement of all layers of the wall of each organ. A line was drawn between the serosa and the mucosa. The mucosal gas interface (hyperechoic) was important in limiting mucosal thickness. Duodenum and jejunum were evaluated when they were in maximum distension.

The ultrasound evaluation was performed using the device Mindray® Z5 equipped with a multifrequency convex transducer, being adjusted to the best frequency, general gain, specific gain, depth and number of focuses

during the examination to optimize image quality. The images were obtained by a single observer. Each measurement was performed three times to obtain reproducibility and the mean was used for statistical analysis (Lippi *et al*., 2017). During the evaluation, the animals remained stationary in order to preserve the topographic location of each organ.

The settings established for the ultrasound examination of the animals varied according to age and size. Frequency of 4.5-5.0 MHz, frame rate of 28-35/s, power of 97% and 2 focuses were used. Depth and gain have been adjusted to improve the quality of image acquisition.

Descriptive statistics were performed for each measurement and the percentile was analyzed in the program Microsoft Excel®.

RESULTS

The ultrasound window for observing the stomach varied between the 10th and 15th left intercostal spaces (ICS), at the level of the shoulder joint or a little more ventral. However, the best observation site was on the 13th or 14th IS. In some animals, it was observed overlapping of the lung, limiting the visualization of this organ from the 12th ICS (Fig. 1).

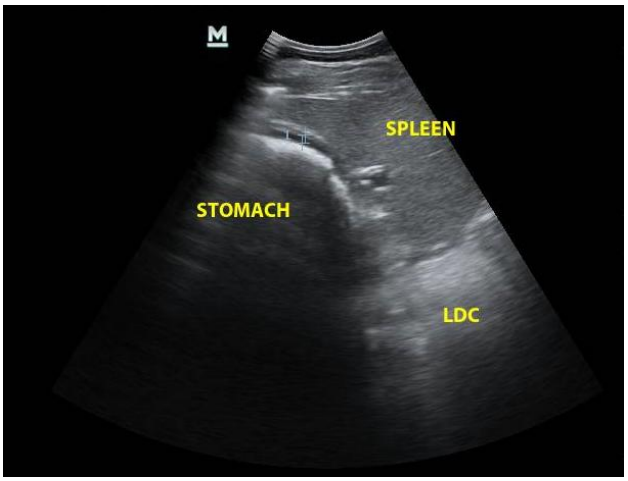

Figure 1. Ultrasonographic image of the stomach in the 12th left intercostal space on shoulder´s level. The image was obtained using a convex transducer at 4.5 MHz at a depth of 14,8 cm LDC: left dorsal colon. Measure: 4,2 mm.

The stomach was visualized as a convex line, which corresponds to the greatest curvature. It lies medial to the spleen and left dorsal to the large colon. In foals up to 15 days old, it was possible to see a hypoechoic content, while in older animals, only the gas interface was observed after the gastric wall. The layers of the stomach wall (serosa, muscularis, submucosa and mucosa) were distinguishable. The serosa is the last hyperechoic line and mucosa is the first hypoechoic line of gastric wall. The gas-mucosal interface is a good reference point for limiting the mucosal layer. Percentile values are shown in Table 1. From 3 months of age onwards, at least 50% of the individuals presented a value for gastric wall thickness greater than or equal to 2.1 mm. One-day-old neonates had a maximum value of 2.0 mm and animals from 7 months of age had values greater than 3.0 mm. Five-year-old animals showed values greater than 4.0 mm.

The ascending duodenum was observed between the 11th and 16th right ICS at the height of the middle third of the humerus and the descending duodenum was seen in the 17th right ICS below the coxal tubercle. In most animals, it was only possible to observe the ascending duodenum between the 13th and 15th ICS. The best viewing window to it was in the first caudal ICS to the first organ viewing ICS. This organ appears as a round or oval structure with a hyperechoic wall and may have anechoic or hyperechoic content (Fig. 2).

Table 1. Stomach wall thickness percentiles (mm) of Mangalarga Marchador horses between 1 day and 5 years of age

Values (mm)	Age (days)																
	1	7	15	30	60	90	120	150	180	210	240	270	300	330	360	1095	1825
Minimum	1.0	1.3	1.2	1.3	1.4	1.6	1.8	1.7	1.7	1.6	1.6	1.8	2.0	1.5	1.6	2.2	2.1
P10	1.1	1.5	1.3	1.4	1.5	1.6	1.8	1.9	1.8	2.0	1.7	1.9	2.3	1.7	1.9	2.2	2.2
P25	1.4	1.6	1.5	1.6	1.6	1.8	2.0	2.0	2.0	2.1	2.0	2.0	2.6	1.8	2.0	2.6	2.5
P50	1.7	2.0	1.9	1.8	1.8	2.2	2.2	2.1	2.1	2.5	2.3	2.5	2.8	2.1	2.4	2.7	2.9
P75	1.8	2.3	2.5	2.0	1.9	2.3	2.4	2.4	2.2	2.7	2.7	3.0	2.9	2.5	3.0	3.0	3.6
P90	2.1	2.6	3.1	2.1	2.1	2.4	2.4	2.7	2.6	2.9	2.9	3.2	3.2	3.2	3.5	3.3	4.0
Maximum	2.3	3.2	3.5	3.0	2.6	2.8	2.7	2.8	2.9	3.1	3.4	3.8	3.5	3.8	3.9	3.7	4.5

A value greater than 20 mm for duodenum's diameter was observed in at least 50% of neonates from 7 days of age, 75% of foals from 3 months of age and 90% of animals from 6 months of age (Table 2). Only 1 individual aged 9 months had a maximum value of 54.0 mm. Animals aged 3 and 5 years had diameter values greater than 5 cm. 1-day-old neonates had a maximum duodenal wall thickness of 2.0 mm. Two 5-year-old animals had a value of 4.6 mm. The median value of the thickness was above 2.2 mm in animals from 3 months of age. Animals from 4 months of age showed between 2 and 9 peristaltic movements per minute. Only 5-year-old animals showed a minimum of 4 movements per minute (Table 3).

Table 2. Percentiles of wall thickness and duodenal diameter (mm) of Mangalarga Marchador horses between 1 day and 5 years of age

Values (mm)	Age (days)																
	1	7	15	30	60	90	120	150	180	210	240	270	300	330	360	1095	1825
Wall thickness (mm)																	
Minimum	1.1	1.4	1.2	1.4	1.2	1.9	2.0	2.1	1.8	1.7	1.8	1.2	1.9	2.0	1.4	2.1	1.2
P10	1.3	1.4	1.5	1.4	1.5	1.9	2.2	2.2	1.9	2.1	2.0	1.9	2.0	2.2	2.0	2.2	2.2
P25	1.5	1.6	1.5	1.7	1.7	2.0	2.3	2.2	2.3	2.3	2.3	2.1	2.1	2.3	2.3	2.4	2.5
P50	1.7	1.8	1.8	1.8	1.8	2.3	2.4	2.4	2.5	2.4	2.5	2.3	2.5	2.4	2.6	2.7	2.7
P75	1.9	2.4	2.3	2.3	2.2	2.4	2.6	2.7	2.6	2.6	2.8	2.5	2.8	2.9	3.0	2.9	3.0
P90	1.9	2.9	2.5	2.8	2.6	2.9	2.7	3.0	2.6	2.7	2.9	3.0	3.2	3.0	3.3	3.3	3.5
Maximum	2.0	3.0	2.7	2.9	3.3	3.2	2.9	3.4	3.1	2.8	3.6	3.1	3.6	3.8	3.4	3.7	4.6
Diameter (mm)																	
Minimum	11.7	13.0	12.3	13.6	13.3	15.6	21.3	15.6	21.5	22.8	22.2	22.3	20.7	20.5	19.6	25.5	12.8
P10	12.9	16.6	15.8	15.6	1.5.5	20.1	23.8	19.8	24.8	26.8	29.1	27.2	25.6	23.3	22.4	26.7	24.8
P25	14.9	18.0	18.3	19.4	17.0	22.9	26.2	23.5	26.4	27.9	30.6	29.8	28.9	28.9	28.0	30.9	27.3
P50	18.3	22.7	21.1	22.3	21.0	24.9	27.9	25.5	29.8	31.0	34.2	31.3	31.9	35.0	31.9	36.6	34.1
P75	20.2	27.1	22.4	26.2	25.1	26.9	29.0	29.2	35.9	34.3	36.3	36.2	34.8	38.7	35.1	40.5	41.5
P90	23.7	28.0	25.5	29.7	34.1	31.3	31.6	30.4	39.0	37.1	40.7	43.4	38.1	43.6	38.4	45.9	51.0
Maximum	36.3	31.1	29.0	30.6	41.1	40.8	42.8	32.7	39.6	39.9	44.9	54.0	43.4	48.4	48.6	53.6	56.3

Table 3. Duodenal movements per minute of Mangalarga Marchador horses between 4 months and 5 years of age

Values	Age(days)																
	1	7	15	30	60	90	120	150	180	210	240	270	300	330	360	1095	1825
Minimum							2	2	3	3	2	3	2	2	2	2	4
P10							3	3	3	3	3	4	4	3	4	3	4
P25							3	3	4	3	4	4	4	4	4	4	4
P50							4	4	4	4	5	5	5	5	4	5	5
P75							5	4	5	5	6	6	6	5	5	6	6
P90							6	6	5	6	6	7	6	6	6	6	6
Maximum							7	6	6	7	8	9	6	7	9	7	7

Jejunum was observed in most of the left abdomen in foals up to 9 months old, becoming visible since the 13th ICS. However, its best viewing window is located on the flank at or just below the hip joint in all horses. In some foals, it was possible to observe it in the ventral abdomen (Fig. 3).

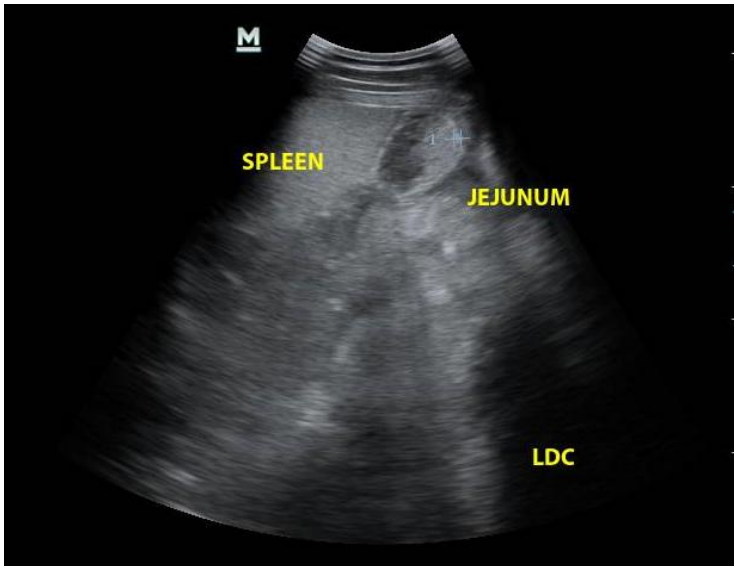

Figure 3. Ultrasonographic image of the jejunum in the middle third of the left paralumbar fossa. The image was obtained with convex transducer at 4.5 MHz at a depth of 18.5 cm. LDC: left dorsal colon. Measure: 2.7 mm.

Animals with 15 days of life had already reached a jejunal diameter greater than 30.0 mm, from 7 months of age there is a diameter greater than 40.0 mm and at 1 year of life, values above 50.0 mm were seen (Tab. 4). The maximum jejunal wall thickness was 2.4 mm for 1-day-old neonates. From that period on, the animals showed maximum values above 3.0 mm, except for the ages of 4 and 6 months. At least 75% of the animals from 3 months of age had wall thickness greater than 2.0 mm.

Table 4: Percentiles of wall thickness and jejunal diameter (cm) of Mangalarga Marchador horses between 1 day and 5 years of age

Values (mm)	Age (days)																
	1	7	15	30	60	90	120	150	180	210	240	270	300	330	360	1095	1825
Wall thickness (mm)																	
Minimum	01.0	1.1	1.1	1.4	1.0	1.7	2.0	2.0	1.7	1.9	1.8	1.4	2.1	1.2	1.9	2.0	1.7
P10	01.2	1.4	1.2	1.5	1.4	2.1	2.0	2.2	2.0	2.0	1.9	1.8	2.2	1.6	2.0	2.2	2.0
P25	1.3	1.6	1.4	1.6	1.5	2.2	2.1	2.3	2.2	2.1	2.1	2.2	2.3	2.1	2.1	2.3	2.2
P50	1.5	1.9	1.9	2.0	1.8	2.3	2.3	2.5	2.4	2.3	2.2	2.4	2.4	2.4	2.3	2.5	2.4
P75	1.8	2.2	2.4	2.4	2.0	2.4	2.6	2.8	2.6	2.5	2.5	2.6	2.5	2.7	2.5	2.7	2.9
P90	2.1	2.6	2.8	2.9	2.7	2.6	2.8	3.1	2.7	2.6	2.6	2.7	2.8	2.9	2.6	3.0	3.0
Maximum	2.4	3.1	3.3	3.4	3.3	3.4	2.9	3.4	2.9	3.2	3.3	3.3	3.1	3.5	3.2	3.3	3.6
Diameter (mm)																	
Minimum	11.3	12.1	13.7	10.7	16.6	20.6	17.9	17.2	20.3	12.4	18.1	21.3	17.9	19.0	17.1	21.6	18.5
P10	13.0	14.5	13.9	15.7	17.3	21.3	21.1	19.9	22.9	22.4	18.8	21.6	19.6	23.7	21.8	24.2	21.9
P25	13.5	15.4	16.7	18.1	21.1	22.7	22.9	21.3	23.8	24.6	23.1	23.9	24.8	25.6	24.3	27.3	25.0
P50	15.7	18.2	18.9	21.7	23.0	26.1	25.2	23.7	26.0	28.2	28.2	30.7	30.9	30.8	26.0	32.3	30.5
P75	18.3	19.2	22.2	28.5	27.1	29.2	27.5	27.5	29.2	31.2	33.9	38.8	34.1	33.6	29.7	40.2	34.9
P90	21.8	20.3	23.8	32.1	30.9	31.7	30.5	28.5	31.3	38.5	37.9	40.6	37.9	37.0	42.2	47.0	39.1
Maximum	26.5	28.5	30.2	34.4	33.5	33.9	31.0	33.1	37.0	43.2	45.4	47.5	46.1	45.0	51.1	51.8	48.9

DISCUSSION

Although reference intervals for equine organs have been observed (Aleman *et al*., 2002; Jones *et al*., 2003; Farooq *et al*., 2018), long-term monitoring of the growth of these organs in the first year of life had not been carried out yet. These results show a growth curve of the stomach, duodenum, and jejunum according to the animal´s life span.

The stomach´s image was obtained within the limits observed by previous studies in other breeds and different ages (Freeman, 2003; Reef, 2003; Nasr *et al*., 2014; Farooq *et al*., 2018). However, the viewing window of this study was smaller, possibly due to the overlapping of the left lung on the organ. The gastric wall ranges from hypoechoic to echogenic with a hyperechoic echo of gas from the mucosal surface and can be up to 7.5 mm thick (Reef, 2003). In foals, it was possible to observe stomach contents up to 15 days of age, which is longer than in other studies (Aleman *et al*., 2002). In this study, the maximum value of stomach wall thickness found was 4.5 mm in adult animals. Younger animals showed lower values for wall thickness, corroborating other studies (Aleman *et al*., 2002). The differences found may be due to variations in breed, age, body weight or even diet. Another possibility is the volume ingested in the stomach during the examination since the animals did not undergo any type of fasting.

Small intestine echoes are recognized by their small tubular and circular appearance, small diameter, fluid content, and frequent peristaltic movements (Freeman, 2002; Reef, 2003). When there are optimal imaging conditions, it is possible to identify five layers in the intestinal wall. The ultrasound layers are serous, muscular, submucosal, mucosa and mucosal interface (Bevevino *et al*., 2021). The latter is the result of ingestion or gas on the mucosal surface (Freeman, 2002). The small intestine was visualized covering a larger area in younger foals, presenting a hypoechoic wall and continuous motility and difficult to quantify (Aleman *et al*., 2002).

The form and content variation of the duodenum is expected since this organ is surrounded by other structures and the animals did not undergo any type of fasting. The duodenum is flattened by adjacent organs, being observed a more oval to round shape only in distension during peristaltic movement (Kirberger *et al*., 1995). The contents of the duodenum can vary from a hyperechoic gas, hypoechoic or hyperechoic fluid, mucus or ingesta, and sometimes a fluid pattern with hyperechoic patches (Reef, 2003; Farooq *et al*., 2018). 24-hour fasting significantly improves high-quality imaging of the small intestine (Norman *et al*., 2010). It results in better visibility, definition, circumferential visibility, and dilation of the small intestine.

When observing the diameter of the duodenum, it is important to note whether there is full duodenal contraction, as its absence may be an indication of dilatation. Duodenal distension and contraction are indicators of peristaltic activity (Kirberger *et al*., 1995). Small intestine distension can be seen on transabdominal ultrasound before being noticed on rectal palpation (Cavalleri *et al*., 2013). Therefore, any changes should be investigated.

The variation in the frequency of movements per minute found was wide, unlike previous studies (Norman *et al*., 2010; Farooq *et al*., 2018). Intestinal movement shows the transit of ingesta and may be related to the feeding time of the animals. In this study, there were individuals on pasture and in pens. Horses kept in stables feed in a shorter period when compared to those kept in the free environment (McGreevy, 2004). Generally, fasted horses show less strain and contractions of the duodenum, while more active contractions appear to be present when this structure is filled with fluid (Kirberger *et al*., 1995; Farooq *et al*., 2018). Therefore, the observed peristalsis may be related to the animal's diet.

The diameter measurements of the jejunum tend to vary. This is because when observing the same loop, it does not always stretch in the same way and to the same extent. The mean of the largest diameter of the jejunal loops, in cross-section, was 44.8±7.3 mm, with a wall thickness of 2.5±0.3 mm in another abdominal imaging study (Amaral and Froes, 2014), being similar to the measurements found in adult animals in this study. Peristaltic waves, anechoic fluid, and hyperechoic gas are often seen in its lumen (Reef, 2003). The jejunum cannot be distinguished with certainty from the ileum by ultrasound imaging. The ileum is located cranial and medial to the cecum but can be difficult to obtain its image in larger horses (Freeman, 2002).

Regarding the measurements of duodenal and jejunal wall thickness, it can be observed that they present an average variation from birth to the first year of age. The thickness of the stomach wall remains more constant until 7 months of age and then increases. Duodenum and jejunum thickness measurements are greater than those reported in some previous studies in horses of other breeds (Reef, 2003; Farooq *et al*., 2018; Bevevino *et al*., 2021), but similar to other studies (Aleman *et al*., 2002; Bithell *et al*., 2010; Kirberger *et al*., 1995). These differences can be explained by the methodology used, age of the animals or even intestinal distension. Although this work aimed to evaluate the loops at maximum distension, sometimes this action was made impossible by the compression of adjacent organs.

The measurements evaluated ranged between the animals from 1 to 12 months. Perhaps this happened because of the growth and development of foals. Some grew faster and gained more weight while others developed more slowly. In this time, it was also possible to notice more easily how birth weight associated with the type of handling influences the growth of the animal.

In clinical practice, high resolution images are not always available. The thickness of the abdominal wall, retroperitoneal fat and other individual characteristics determine the need to use transducers of different frequencies (Bevevino *et al*., 2021). Thus, obtaining a good quality image depends on the individual patient’s situation and device settings. Sonography is a very important practical option, and very enlightening for the owners, being of great value for obtaining information for the elaboration of a diagnosis of equine patients.

CONCLUSION

Abdominal ultrasound not only offers structural information, but also functional information, such as gastrointestinal tract motility. It was possible to define values to help clinicians in diagnosis of some affections on Mangalarga Marchador horses.

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