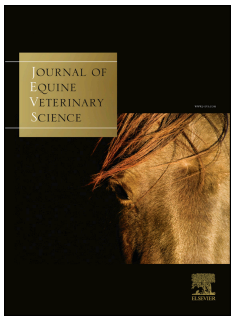
Accepted Manuscript



Renal Measures in Healthy Italian Trotter Foals and Correlation Between Renal and Biometric Measures: Preliminary Study

Ilaria Lippi, Francesca Bonelli, Simonetta Citi, Valentina Meucci, Martina Sartoni, Paola Marmorini, Micaela Sgorbini

PII: S0737-0806(17)30608-1

DOI: 10.1016/j.jevs.2017.09.009

Reference: YJEVS 2388

To appear in: *Journal of Equine Veterinary Science*

Received Date: 29 August 2017

Revised Date: 24 September 2017

Accepted Date: 25 September 2017

Please cite this article as: Lippi I, Bonelli F, Citi S, Meucci V, Sartoni M, Marmorini P, Sgorbini M, Renal Measures in Healthy Italian Trotter Foals and Correlation Between Renal and Biometric Measures: Preliminary Study, *Journal of Equine Veterinary Science* (2017), doi: 10.1016/j.jevs.2017.09.009.

Renal measures in healthy Italian trotter foals and correlation between renal and biometric measures: preliminary study

Ilaria Lippi[1], Francesca Bonelli[1], Simonetta Citi[1], Valentina Meucci[1], Martina Sartoni[2], Paola Marmorini[2], Micaela Sgorbini[1]

[1]Veterinary Teaching Hospital, Department of Veterinary Sciences, via Livornese snc, 56122, San Piero a Grado (PI). Italy.

[2]Private Practitioner, Pisa.

Corresponding author:

Citi Simonetta

Veterinary Teaching Hospital

Department of Veterinary Sciences

Via Livornese snc. 56122, San Piero a Grado (PI), Italy

Phone number +390502210134

Email: simonetta.citi@unipi.it

Abstract

Aim. To evaluate ultrasonographic renal measures in healthy foals aged 1-6 weeks; 2) to verify the correlation between biometric measures to ultrasonographic renal ones.

Materials and methods. A total of 9 Italian trotter foals born in the same stud farm and underwent similar management conditions were enrolled. Inclusion criteria: normal gestation time; unassisted delivery; normal physical examination at all evaluation times.

Length and height of both kidneys were measured by ultrasound weekly from 1 to 6 weeks of life, along with the thoracic and the middle third of the metacarpal area circumferences. Data were expressed as mean and standard deviation and distribution was evaluated. One-way Anova was applied to verify differences related to time. The Pearson correlation test was carried out to evaluate the linearity between time vs all the parameters measured. T student test was used to verify differences in ultrasound measures between right and left kidney at all recorded times. The Pearson test was applied to a mean-variance matrix to verify the correlation between each biometrical vs all renal measures. Significance level was set at $P<0.05$.

Results. One-way Anova showed differences in biometric and renal measures related to time. Correlation test revealed a linear growth. Differences in ultrasound renal measures between right and left kidney were obtained. Correlation was found between biometrical parameters vs kidney measures.

Discussion and conclusions. Renal measures and differences between left and right kidneys were in line with literature. Correlation test revealed a linear growth. Renal growth is correlated with age and biometric measures.

Key words. Foal, ultrasonography, renal ultrasound measures, biometric measures.

Introduction

Ultrasonography of the abdomen represents an important diagnostic tool in equine neonates and it is performed routinely in many equine practices [1,2]. Due to the smaller size and the minimal adipose tissue, abdominal ultrasound is easier to perform in foals than in adult horses [3,4]. Ultrasonographic examinations have been employed for the diagnosis of urinary tract disorders in horses to evaluate size, shape, position and echogenicity of the kidneys [5]. Moreover, ultrasonography has been suggested for guiding percutaneous renal biopsy, and represents the primary noninvasive diagnostic method for evaluating hematuria, renal failure, renal or peri-renal masses and pyelonephritis in the horse [6]. Normal renal parameters and ultrasonography techniques to detect kidneys are well documented in adult horses [7-12]. However, only few studies on the evaluation of ultrasonographic appearance and location of kidneys [1,13], as well as renal dimensions, are available in foals [3,4].

In particular, Hoffman and collegues [3] studied the correlation between the renal anatomic features performed on cadavers of foals and their sonographic appearance in images obtained using different planes. Aleman and collegues [4] reported the ultrasonographic carachteristics, location and variations of thoracic and abdominal organs, included kidneys, with relation to age and some biometrical measures. The authors provided a growth table for comparison with diseased foals.

The aims of the present study were; 1) to evaluate ultrasonographic renal measures in healthy foals aged between 1 to 6 weeks; 2) to verify the correlation between some biometric measures to ultrasonographic renal ones.

Materials and Methods

Animals and inclusion criteria

The present observational study was approved by the Institutional Animal Care and Use Committee of the University of Pisa (D.R. 23506/2015). The owner's written consent was obtained for all the foals

included in this study.

A total of 9 Italian trotter foals were enrolled in the study. All the animals were born in the same stud farm and underwent similar management conditions. Five out of 9 were fillies and 4 out of 9 colts. The following inclusion criteria were set: (1) mares' physiological gestation time (>320 days) [14]; (2) controlled delivery by an operator plus no need of aids during birth; (3) normal clinical examination at all times of ultrasound evaluation (hear rate, respiratory rate, body temperature within physiological ranges, moisty mucous membrane and capillary refill time ≤2 seconds, no alterations of mental status, absence of particular behaviors, physiological defecation, urination and milk ingestion, no abnormalities at lymphonodes palpation) [15].

Animals handling and timing

All the foals were submitted to kidney ultrasound and biometric evaluations starting from day 7 (T7), and every seven days (T14, T21, T28, T35), till 6 weeks (T42) of life. All the procedures were supported by 3 operators: one operator held the mare, one operator manually restrained the foal and a third operator performed the biometric measurements and the ultrasound examination. All the procedures were performed in the same handling box and no pharmacological support was used. During the procedures foals were always in the visual field of mares.

Biometric measures

The biometric measures, obtained in the standing foal, on were: 1) Thoracic (TH) circumference measured from the end of the withers around the thorax immediately caudal to the axillary region; 2) Metacarpal Area (MA) circumference measured at the middle third level. All measures were carried out using a flexible measuring tape for livestock and recorded in cm. Each measure was performed 3 times for reproducibility and the average was used for statistical analysis. Biometric evaluations were always carried out before the ultrasonographic evaluation.

88

89 Kidney Ultrasound

90 Alcohol and ultrasound gel were applied to the unclipped hair coat to provide appropriate contact. The
91 ultrasound examination was performed with each foal standing to avoid any change in position or
92 overlapping from other organs, as proposed by others [3,4]. One of the authors performed all the
93 ultrasound examinations using the scanning windows reported in literature [3,4] and a portable
94 ultrasound system (Mylab30™, Esaote, Florence. Italy) equipped with a multi-frequency convex probe
95 (5-7.5 MHz). The ultrasound settings were changed as needed to optimize image quality.
96 The following measures were obtained: 1) Renal Length (RL) defined as the longest cranio-caudal axis
97 of the kidney (Fig. 1); Renal Height (RH) defined as the longest dorso-ventral axis of the kidney. Both
98 measures were obtained using the sagittal plane until the image size was maximal and the renal pelvis
99 could be seen clearly.
100 All the images were recorded as cineloops of 10s each by the ultrasound machine software. Renal
101 dimensions were measured on a still image by the same observer using electronic calipers associated
102 with the ultrasound software (Mylab software, Esaote, Florence, Italy). Each measurement was
103 performed 3 times for reproducibility and the average was used for statistical analysis.
104

105 Statistical analysis

106 Data concerning biometric and ultrasonographic measures were expressed as mean±standard deviation
107 and Kolmogorov-Smirnov test was applied to verify data distribution. Data showed a Gaussian
108 distribution, thus a one-way Anova test for paired data and Tukey's multiple comparisons test as *post*
109 *hoc* were carried out to verify differences both for renal and biometric measures related to time. The
110 Pearson correlation test was carried out to evaluate the linearity between time vs all the parameters
111 measured. A T-student test was performed to verify differences between the ultrasonographic measures

of right and left kidney at different time points. The Pearson correlation test was applied to a mean-variance matrix to verify the correlation between MA or TH circumferences vs right and left RL, right and left RH. Significance level was set at $P < 0.05$. Commercial statistical software was used (GraphPad Prism 6. USA).

Results

Data concerning biometric and ultrasonographic measures relating to time are reported in Tables 1 and 2, respectively. Regarding biometric measures, both the TH and MA circumferences statistically increased since the third week of life (Table 1) and the growth is continuous over time till 6 weeks of life. The RL and the RH of the right kidney statistically increased since the second week of life, along with the RL of left kidney, while the left kidney RH increases since the third week of life (Table 2).

A positive linear relationship was found between time vs MA (Pearson r 0.98; P=0.0004) and TH (Pearson r 0.99; P=0.0002) circumferences, right and left RL (both Pearson r 0.99; P<0.0001), right (Pearson r 0.99; P=0.0002) and left RH (Pearson r 0.99; P<0.0001).

Moreover, a positive linear relationship was also found between MA vs right (Pearson r 0.98; P=0.0004) and left RL (Pearson r 0.98; P=0.00009), and between TH vs right (Pearson r 0.99; P=0.0004) and left (Pearson r 0.99; P=0.0003) RH.

The T-student test revealed differences in RL measure between the right and left kidney at T28 (P=0.008), T35 (P<0.0001) and T42 (P=0.009), while no differences were obtained for RH between right and left kidney at all recorded times.

Discussion

Renal ultrasound in foal is an important diagnostic tool for the early assessment of kidney diseases [1-4]. To the best of the authors' knowledge there was only one study [4] evaluating renal

ultrasonographic measures in foals and their correlation with the growing rate. In particular, Aleman and collegues [4] evaluated ultrasonographically the thoracic and abdominal organs of 10 foals of different breed from birth till 6 months of age and compared the measures to age, body weight and height. In the present study we aimed to evaluate weekly the renal ultrasonographic measures during the first 6 weeks of life in a cohort of 9 Italian trotter foals and correlate these measures to age and biometric measures, such as thoracic and middle-third of the metacarpal area circumferences.

The measures obtained for the length and height of both kidneys were similar to those reported in other studies at different times [4].

Regarding the age, our results showed a continual growth of both kidneys starting from the second-third week of life, while the growth started since the first week in a previous study [4]. This difference might be due to the different ultrasound machines used and/or to the different bred enrolled. In the previous study [4] the authors included foals of different breed, while in this work we enrolled only Italian trotter foals, in order to study breed-specific and age-specific ultrasonographic parameters in light of potential differences in normal growth rate between horse breeds. Moreover, the growing has a linear tendency with time.

The left kidney was statistically longer than the right one starting from T28, while no differences were observed for the height. Our results are in line with those reported by others, both for renal height and length [4].

Biometrical measures obtained in the present study were in line with what reported for Standardbred foals [16] and the growth showed a positive linear relationship with time. In the paper by Aleman and colleagues [4], body weight and withers height were the biometrical measures compared with kidneys ultrasonographic measures and they found no correlations between them. In the present study we opted to consider the middle-third of the metacarpal area and the thoracic circumferences and we found a positive linear relationship between each biometric measures vs renal ones. This difference might be

due to the different bred enrolled because in our study we included only Italian trotter foals, while in the previous study [4] mixed breed were used.

This study has some limits. The authors included a low number of foals, thus larger sample would be assessed to establish reference ranges and relative growth for ultrasonographic renal measures. Moreover, no blood work or urine analysis have been performed, according to owner's wishis.

Conclusion

In conclusions, we found a continuous growth starting from 14-21 days of life for both kidneys and a difference between the length of left and right kidney starting from 1 month of age. Correlations have been found between time vs all the parameters measured and between each biometrical measures vs kidney length and height.

Funding

This research was supported by the University of Pisa.

References

[1] Reef VB. Equine pediatric ultrasonography. Comp Cont Educ Pract Vet 1991;13:1277-95.

[2] Reef VB. Pediatric abdominal ultrasonography. In: Reef VB, editor. Equine Diagnostic Ultrasound, WB Saunders, Philadelphia,USA; 1998, p. 364-403.

[3] Hoffmann KL, Wood AK, McCarthy PH. Ultrasonography of the equine neonatal kidney. Equine Vet J 2000;32:109-13.

[4] Aleman M, Gillis CL, Nieto JE, Renaudin CD, Bea J. Ultrasonographic anatomy and biometric analysis of the thoracic and abdominal organs in healthy foals from birth to age 6 months. Equine Vet Journal 2002;34: 649-55.

[5] Divers TJ. Diseases of the urinary system. In: Colahan PT, Mayhew IG, Merritt AM, Moore JN, editors. Equine Medicine und Surgery, 4th edn. American Veterinary Publications Inc, Goleta, California; 1991, p. 1542-4.

[6] Divers TJ, Yeager AE. The value of ultrasonographic examination in the diagnosis and management of renal diseases in horses. Equine Vet Educ 1995;7(6):334-41.

[7] Hoffmann KL, Wood AK, McCarthy PH. Sonographic-anatomic correlation and imaging protocol for the kidney of horses. Am J Vet Res 1995;56(11):1403-12.

[8] Reef VB, Whittier M, Griswold E. Sonographic evaluation of the adult abdomen. Clin Tech Equine Pract 2004;3:294-307.

[9] Draper ACE, Bowen M, Hallowell GD. Reference ranges and reliability of transabdominal ultrasonographic renal dimensions in thoroughbred horses. Vet Radiol Ultrasound 2012;53(3):336-41.

194 [10] Slovis N. Ultrasonography of the liver, spleen, kidney, bladder and peritoneal cavity. In: Kidd
195 JA, Lu KG, Frazer ML, editors. Atlas of equine ultrasonography. Wiley-Blackwell, USA; 2014,
196 p. 409-25.

197 [11] Habershon-Butcher J, Bowen M, Hallowell G. Validation of a novel translumbar ultrasound
198 technique for measuring renal dimensions in horses. Vet Radiol Ultrasound 2014;55(3):323-30.

199 [12] Williams S, Cooper JD, Freeman SL. Evaluation of normal findings using a detailed and
200 focused technique for transcutaneous abdominal ultrasonography in the horse. BMC Vet Res
201 2014;10(Suppl 1)S5.

202 [13] Behn C, Bostedt H. Technique of abdominal ultrasonography in newborns and normal findings.
203 Berl Munch Tierarztl Wschr 2000;113: 335-43.

204 [14] Lester GD. Prematurity, dismaturity and assessment of maturity. In: McKinnon AO, Squires
205 EL, Vaala WE, Varner DD, editors. Equine reproduction, 2nd ed. Wiley-Blackwell, USA; 2011,
206 p. 121-7.

207 [15] Stoneham SJ. Assessing the newborn foal. In: Paradis MR, editor. Equine Neonatal Medicine: a
208 case-based approach, 1st ed. Elsevier Inc., Philadelphia, USA;2006, p. 1-11.

209 [16] Vaccari Simonini F, Catalano AL, Martuzzi F, Beretti V, Filippini S. Biometric measurement of
210 Thoroughbred and Italian Trotter breed foals. Annali Facoltà Medicina Veterinaria, Università di
211 Parma 2006;26:335-40.

212

213

	MA circumference (cm)							TH circumference (cm)						
	T7	**T14**	**T21**	**T28**	**T35**	**T42**	**P**	**T7**	**T14**	**T21**	**T28**	**T35**	**T42**	**P**
1	12	13	13	14	14	14.5		89	91	97	103	105	107	
2	12	12.5	12.5	13	13	13		87	90	96	97.5	97.5	100	
3	12	12	12,5	13	14	14		87	89	93	94	102	108	
4	11	11.5	12	12	12	13		88	90	92	94	102.5	106	
5	12	13	13	14.5	14.5	14.5		96	100	101	101	102	104	
6	12	12	13.5	14	14	14		89.5	90.5	97	100	100	100	
7	12	12	12.5	12.5	13	13.5		94	95	96	96	98	100	
8	13	14	14	14	14	14		73	93	97	100	100	101	
9	12	13	13	13	13	13.5		89	93	97	101	101	101	
X	**12^{a}**	**12.6^{ab}**	**12.9^{b}**	**13.3^{bc}**	**13.5^{bc}**	**13.8^{c}**	**0.0001**	**88.1^{a}**	**92.4^{ab}**	**96.2^{b}**	**98.5^{bc}**	**100.9^{bc}**	**103.0^{c}**	**0.0001**
SD	**0.5**	**0.8**	**0.6**	**0.8**	**0.8**	**0.6**		**6.4**	**3.4**	**2.6**	**3.3**	**2.3**	**3.3**	

214 Table 1. Biometric measures (cm) at different time points. Results are expressed as mean (X)±standard

215 Deviation (SD). In the same row: a≠ab≠b≠bc≠c.

216 Legend - MA: Metacarpal Area; TH: thoracic.

217

	RIGHT KIDNEY														LEFT KIDNEY													
	RL (cm)							RH (cm)							RL (cm)							RH (cm)						
	T7	T14	T21	T28	T35	T42	P	T7	T14	T21	T28	T35	T42	P	T7	T14	T21	T28	T35	T42	P	T7	T14	T21	T28	T35	T42	P
1	8.4	8.2	8.9	9.0	9.0	9.3		3.9	3.9	4.9	4.9	4.9	4.8		8.7	9.9	9.6	9.8	9.8	10.6		4.6	4.5	4.5	4.6	4.5	4.6	
2	8.1	8.5	8.6	8.8	9.0	10.7		3.9	4.3	4.6	4.5	4.6	4.9		7.1	7.3	7.4	9.7	10.0	10.3		3.8	4.0	4.0	4.4	4.5	4.7	
3	7.0	7.6	8.9	9.0	9.0	9.2		4.2	4.3	4.5	5.6	5.7	6.0		9.0	9.2	10.4	10.1	10.1	11.1		4.0	4.3	4.5	4.6	4.8	5.2	
4	7.0	7.5	7.8	7.8	8.0	8.2		4.1	4.2	4.5	4.1	4.1	4.3		6.1	7.0	7.5	8.0	10.2	10.8		3.5	3.5	3.5	3.9	4.0	4.5	
5	8.8	9.1	9.5	9.5	9.5	9.5		4.3	4.4	5.0	5.0	5.0	5.2		7.7	8.2	9.8	9.8	10.0	10.2		4.3	4.4	4.6	4.6	4.7	4.7	
6	6.3	6.3	6.7	6.9	8.7	8.8		3.1	3.1	3.8	4.1	4.6	4.6		7.5	7.8	8.2	9.8	9.9	10.0		4.9	4.9	5.1	5.0	5.0	5.1	
7	8.0	8.4	8.5	8.4	9.0	9.0		3.8	3.9	4.0	4.1	4.1	4.2		7.9	8.0	8.1	10.0	11.2	11.2		3.8	3.9	3.9	4.2	4.1	4.2	
8	7.8	8.2	8.3	9.0	9.0	9.1		3.8	3.7	3.7	4.5	5.1	5.1		8.8	10.8	10.9	9.9	10.0	10.0		4.1	4.1	4.4	4.6	4.6	4.7	
9	7.1	7.3	8.2	8.8	8.8	9.0		3.6	4.1	4.0	4.1	4.2	4.3		7.7	8.4	8.5	9.3	9.3	9.4		3.3	3.8	3.9	4.4	4.4	4.5	
X	**7.6^{a}**	**7.9^{b}**	**8.4^{bc}**	**8.6^{c}**	**8.9^{c}**	**9.2^{c}**	**0.0001**	**3.9^{a}**	**4.0^{ab}**	**4.3^{b}**	**4.5^{bc}**	**4.7^{c}**	**4.8^{c}**	**0.001**	**7.8^{a}**	**8.5^{b}**	**8.9^{b}**	**9.6^{b}**	**10.1^{b}**	**10.4^{c}**	**0.0005**	**4.0^{a}**	**4.1^{a}**	**4.3^{ab}**	**4.5^{b}**	**4.5^{b}**	**4.7^{b}**	**0.0004**
SD	**0.8**	**0.8**	**0.8**	**0.8**	**0.4**	**0.7**		**0.4**	**0.4**	**0.5**	**0.5**	**0.5**	**0.6**		**0.9**	**1.2**	**1.3**	**0.6**	**0.5**	**0.6**		**0.5**	**0.4**	**0.5**	**0.3**	**0.3**	**0.3**	

218

219 Table 2. Renal Length (RL) and Renal Height (RH) of both left and right kidneys (cm) at different time points. Results are expressed as mean

220 (X)±Standard Deviation (SD). In the same row: In the same row: a≠b≠ab≠b≠bc≠c.

Fig. 1. Sagittal image of the left kidney (7-day-old filly foal). Cr = cranial; V = ventral. Scale (cm) on the right side and a grey scale bar located on the left side of the image. For details of probe placement see text. D1: Renal Lenght; D2: Renal height.

Highlights

1) We evaluated ultrasonographic renal measures in 9 Italian trotter healthy foals aged 1-6 weeks and correlated them to some biometric measures.
2) We found a continuous growth starting from 14-21 days of life for both kidneys and a difference between the length of left and right kidney starting from 1 month of age.
3) Correlations have been found between time vs all the parameters measured and each biometrical measures vs kidney length and height.

Dear Editor,

the present *in vivo* experimental trial in a clinical setting was approved by the Institutional Animal Care and Use Committee of the University of Pisa (D.R. 23506/2015). The owner's written consent was obtained for all the foals included in this study.

Yours sincerely,

Prof. Micaela Sgorbini

Dear Editor,

The Authors' contribution to the manuscript is equally distributed and no conflict of interest exists.

Yours sincerely,

Prof. Micaela Sgorbini