



VARIATIONS IN RENAL DOPPLER ULTRASOUND IN APPARENTLY HEALTHY AREWA AND TALON HORSES WITHIN ZURU EMIRATE STABLE, ZURU, KEBBI STATE, NIGERIA

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Abstract

Renal Doppler ultrasonography encompasses the measurement of the renal resistive index (RRI) and pulsatility index (PI). The examination is a systematic sonographic evaluation of the kidneys and it is a non-invasive procedure that is used to assess, investigate, monitor and diagnose the renal conditions of animals. These renal indices do not exist for the Arewa and Talon breeds of Nigerian horses which are probably the most common breeds in northern Nigeria. Thus, this study aims to gain a clearer understanding of RRI and PI variations in these breeds and interplay of some physiological parameters such as withers height, body weight (BW), body condition score (BCS), age (young and adult) and to establish equine renal reference data of RRI and PI in these breeds by means of non-invasive pain free imaging modality (Power Doppler Ultrasound). Thirty-two healthy, conscious, non-racing horses were recruited. The overall median RRI and PI values for the right kidney were 0.60 ± 0.006 and 0.75 ± 0.13 , while those for the left kidney were 0.59 ± 0.006 and 0.77 ± 0.18 , respectively. There was no statistically significant difference between the right and the left kidney of either breed, nor among the physiological variables and biochemical analyses studied. Furthermore, no predispositions to RRI or PI variation have been found by us that could be attributed to breed, body weight, body condition score or withers height. There was no difference in renal values between young (≤ 4 years) and adult (≥ 4.5 years) horses. These results recorded can be used as a reference for several prospective studies comparing healthy and diseased individuals and also to study the influence of other conditions (pregnancy, lactation) aimed at assisting

clinicians in making inferential diagnostic and prognostic decisions of renal conditions in horses.

Keywords:

Renal indices, Doppler, Arewa horse, Talon horse, Zuru.

Introduction

Ultrasound (US) examination of the kidneys is a common, non-invasive technique used in both human and veterinary medicine. B-mode US is useful in discriminating upper urinary tract infection (UUTI) and lower urinary tract infection (LUTI); it is also useful for differentiating between focal and diffuse involvement of the kidney, and sometimes between acute kidney disease (AKD) and chronic kidney disease (CKD) (Braum *et al.*, 2008; Floeck, 2009; Imran and Sharma, 2014). Its utility in distinguishing different forms of kidney disease is somewhat restricted, however, in this context specificity is low, particularly in the case of diffuse diseases (toxic, inflammatory, infectious process) (Braum *et al.*, 2008; Floeck, 2009; Tipisca *et al.*, 2016; Bragato *et al.*, 2017). Doppler US, on the other hand, provides more dynamic information related to renal blood flow, which is closely related to renal function (Bragato *et al.*, 2017). It also provides useful information about the effect of different physiological or pathological variables on the flow (Maksuad *et al.*, 2019; Agut *et al.*, 2020); and in clinical diagnosis Doppler US is valuable during renal biopsy as information it provides of microvascular and parenchymal lesions may help minimize the risk of haemorrhage (Floeck, 2009; Mohammed and Oikawa, 2008; Szymanski *et al.*, 2018).

The renal resistive index (RRI) is a parameter helpful for assessing both acute and chronic kidney disease in dogs (Novellas *et al.*, 2010) and cats ((Tipisca *et al.*, 2016; Bragato *et al.*, 2017). Renal lithiasis, end-stage renal disease and hydronephrosis are also potential causes of elevated RRI. Use of RRI may be an early indicator of impaired renal function, especially in the context of chronic kidney disease and may be useful in monitoring kidney function if a significant B-mode US change is not observed (Bragato *et al.*, 2017; Novellas *et al.*, 2010). The renal blood flow resistance can be expressed indirectly in the form of the RRI or pulsatility index (PI). The RRI is altered with intrarenal perfusion and relations to the systemic haemodynamics making it a useful prognostic tool in the human patient (Di Nicolò and Granata, 2017).

Despite several Doppler ultrasound parameters having found their way in the diagnostics of the kidney in humans and small animals (Tetsuka *et al.*, 2003), so far there is paucity of such reference values in the horses (Barreiro-Vázquez *et al.*, 2021). This thus creates a need to investigate for the factors affecting RRI and PI in horses, particularly in Nigeria where such studies have not been done to the best of the authors knowledge. The present study was designed to obtain baseline data on renal RRI and PI in indigenous Arewa and Talon Nigerian horses, and to give an idea of the effect of physiological variables on these indices in these horses.

Material and Method**Study Location**

The study was designed and undertaken in the Department of Veterinary Surgery and Radiology, College of Veterinary Medicine at the Federal University of Agriculture Zuru,

Kebbi State, in the north-western region of Nigeria. The geographical coordinates of Zuru is 11°24'24.260" N and 5°14'26.38" E.

The examination of Renal Doppler was performed using the Edan colour ultrasound scanner (Brand YSENMED, model YSB-L5PRO), with 3.5 MHz convex probe. Before the study was started, ethical clearance, approval and consent of the clients were obtained from the Federal University of Agriculture Zuru's Committee on Animal Use and Care (FUAZ-CAUC) and the management of His Royal Highness stables and Madaki farm in Zuru emirate council, respectively. The care and handling of animals was done in accord with the guidelines and principles of the care and use of experimental animals in Nigeria. Informed consent was then obtained from the owners for the clinical research, and for publication of the results.

Animals and Study Design

The horse population involved in this study are from a stable which is privately owned and was situated in Madaki farm stable (16 horses) and late Sani Sami Gomo stable (16 horses) Zuru Emirate, Zuru, Kebbi State. Thus, a total of thirty-two (32) healthy non-racing horses were studied herein including 16 young (2 fillies and 2 colts) and 16 adult (2 stallions and 2 mares) horses of Arewa and Talon breeds each in the two stables (see table 1 below).

Table 1: Study Animal Distribution per stable

Stable	Late Sani Sami Stable				Madaki Farm stable			
Breeds	Talon breeds		Arewa breeds		Talon breeds		Arewa breeds	
Young (≤ 4 Yrs)	2 Fillies	2 Colts	2 Fillies	2 Colts	2 Fillies	2 Colts	2 Fillies	2 Colts
Adult (≥ 4.5 Yrs)	2 stallions	2 Mares	2 stallions	2 Mares	2 stallions	2 Mares	2 stallions	2 Mares
Total	4	4	4	4	4	4	4	4

While working horse were trained at least three times weekly, the adult horses were used for ceremonies, the Durba riding, for pleasure and few of them were occasionally retired. Each horse was kept in a separate stall and the smaller ones were put into large stalls that were subdivided and kept with their mothers. 100% of horses had access to a pasture with green grass every day. The horses are been fed with roughages such as hay, green pastures (grass), wheat bran ad libitum and commercial concentrate feed three times daily based on the feeding schedules and grain supplements (millet). All horses are housed alone and cared for by trained men (grooms) to tend to horses and all have access to clean water.

Inclusive Factors

Horses selected were those that were apparently healthy as determined by a detailed medical history taken from the owner/carer, a thorough clinical examination, a complete blood count and blood biochemistry (serum creatinine, urea, aspartate aminotransferase, e.t.c), and urinary analysis. Therefore, among the horses that entered the study none had past or present abnormalities of the urinary system or cardiovascular system, no ultrasonographic changes in the kidneys, and no drugs for the last 3 months before the study.

Preclinical Examination

All the horses were subjected to clinical evaluation according to the findings of physical examination, body weight (mass, Kg) and wither height of each horse (cm) measured with a girth tape. Body condition score and complete blood cell count, serum biochemical profiles, urine analyses and urine protein/creatinine ratios were performed. Heart, pulse and respiratory rates were all taken in the traditional way before the procedure and these were found to be within normal ranges for horses. Haematological, biochemical and urinalysis results obtained for the horses on enrolment were all normal for the horse.

Animal Preparation and Restraint

No sedative/chemicals were used to restrain the horses at any point of the procedure. All the horses chosen were given basic veterinary care such as vital parameter checks and transabdominal ultrasound. The horses were checked in their own stalls, stable corridor, in front of their stalls or in the chute section in the stable as conditions were deemed appropriate, to help minimise stress and simulate 'home' environmental conditions.

The area of the fur where the transabdominal kidney ultrasound examination was performed (intercostal space 16-17 left and 15-17 right according to Hoffmann *et al.*, 1997) was clipped with an electric clipper. The skin was meticulously washed with chlorhexidine, soap and water and followed by alcohol washing with chlorhexidine solution, allowed to dry and overlaid with aquasonic coupling gel.

B-mode and Doppler Sonographic Examination

Trans-abdominal curvilinear probes were used for B-mode and Doppler scans. Ultrasound B-mode, Pulsed Wave Doppler and color Doppler examinations were performed on both the kidneys to measure the RRI and PI of the intrarenal arteries. The structure, echogenicity and size of the kidneys was evaluated by gray scale ultrasound before Doppler ultrasound. Probe placement, frequency and other parameters of images were adjusted based on depth of the kidneys and fat tissue content in the body to optimize the quality of image. The kidneys were scanned in sagittal/ parasagittal and dorsal plane (as described by Hoffmann *et al.*, 1995).

Color flow Doppler ultrasonography (CFDU) was subsequently performed with scanning of the kidney in longitudinal plane in B-mode, after which the CFDU was turned on to allow the visualization of the intrarenal vasculatures. The border of the medullary pyramids and the corticomedullary junctions were sites of the localization of the interlobar arteries and the arcuate arteries, respectively (Rivers *et al.*, 1997). Afterwards, Doppler ultrasonography changes using the pulse-wave modality were obtained for each one of the intrarenal arteries with Doppler sample width of 1–2 mm. The smallest scale that could be used without alias was chosen and the slowest filter was used. Minimum of one 8 second PW Doppler tracing per kidney was stored. Three similar sequential Doppler waveforms were obtained each time. Using manual delimitation with the internal calipers of the ultrasonographic machine, the software of this machine automatically calculated the value of the RRI and the PI. Three RRI values were taken from intrarenal arteries (interlobular or arcuate) and a mean RRI for each kidney was calculated. Images were captured on hard media, two-dimensional (2D) colour doppler and pulsed wave (PW) Doppler images were saved and DICOM stored.

Data Analysis

Data was edited and analysed statistically by IBM SPSS v24.0 (SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to test the normality of distribution of variables. Differences between the right and left kidneys and interlobar to arcuate arteries RRI values were determined by using Mann–Whitney test. Age: (Kruskal–Wallis test), RRI and PI values in different age cohorts were assessed for the effect of age. The Wilcoxon matched pairs signed rank test was used to compare differences in RRI and PI values between the different ages of the horses. Fisher's test was used to calculate the independence of each of the qualitative variables between the age groups and one-way linear regression (right kidney/left kidney) was used to assess the relationship between the RRI value and the other parameters. Mean and standard deviation (SD) were used to present data. Statistically significant results were based on p-values of ≤ 0.05 .

Results

The study enrolled 32 horses: 16/32 Arewa and 16/32 Talon, comprising 8/32 fillies, 8/32 colts, 8/32 stallions, and 8/32 mares. The age range was 3-15 years with a median of 9. There were 16/32 (50%) horses in each group (young and adult). The body weight (BW) ranged from 232–483 kg (median: 335 kg). The body condition score (BCS) based on the 1-9 Henneke scale was 3-5/9 (median: 4/9). The average height of the young Arewa would be 10.1–hands (103 cm) wither height, but the young Talon would measure on average 9.7–hands (100 cm) wither height. The average height for the mature Arewa horses was 14.3 hands (145cm) while for the mature Talon horses 14 hands (142cm).

A significant difference ($p < 0.002$) in mean age was present in the groups studied. Body mass and height of the young horses were significantly lower ($p < 0.001$) while there was no significant difference between the mean BCS. Young horses had significantly higher ($p < 0.05$) mean heart rate, mean pulse rate and mean respiratory rate than adult.

Table 2. Mean values with standard deviation of body weight, wither height, body condition score, and other physical parameters in the enrolled young and adult Arewa and Talon horses.

Breed	Arewa (n=16)		Talon (n=16)	
	Young (n=8)	Adult (n=8)	Young (n=8)	Adult (n=8)
Body mass (kg)	212.1 \pm 32.3	488.4 \pm 79.5*	219.9 \pm 52.6	465.4 \pm 75.4
Wither height (cm)	122.8 \pm 24.0	150.9 \pm 31.2*	102.1 \pm 38.8	131.3 \pm 22.9
Body condition score	3.5 \pm 2.1	3.0 \pm 2.9	4.0 \pm 1.5	3.5 \pm 2.0
Heart rate (bpm)	49.0 \pm 12.2	35.0 \pm 3.44	43.9 \pm 9.2	33.5 \pm 13.7
Pulse rate (Pulse/min)	63.9 \pm 6.5*	37.9 \pm 2.2	68.6 \pm 4.4	39.3 \pm 2.5
Respiratory rate (Cycle/min)	35.2 \pm 9.1*	20.9 \pm 2.5	33.4 \pm 6.1	19.2 \pm 5.5

Note: n–number of animals in the group, bpm–beats per minute, kg–kilogram, cm–centimetre,

* statistically significance $p < 0.05$ between Young and adult horses;

The ultrasound procedure

The completion of the examination mean time to satisfaction was typically under 50 minutes per animal. The greater challenge was seen in Doppler measurements where individual factors like the cooperation of the animal and manipulations to get quality Doppler numbers were incurred.

B-Mode US

No structural B-mode US abnormality was detected in the kidneys and neighbouring structures in all the 32 horses enrolled and no horses exhibited significant discomfort during the examination. The B-mode US sonogram in one of the horses is shown in Figure 1 which depicts some the renal dimensions taken and clearly demonstrating the medulla and cortex; the renal cortex is more echoic than the medulla. The first two averages of the B-mode dimension of both kidneys are given in Table 3.

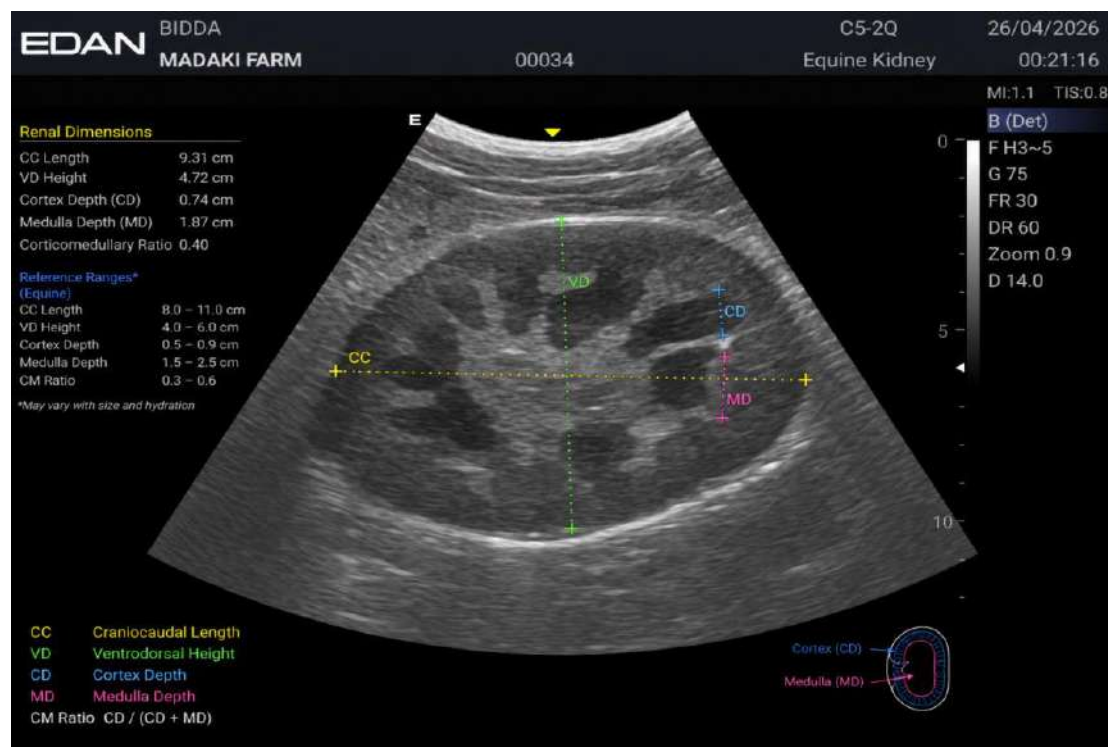


Figure 1: sonogram of the B-mode US in horses depicting the cortex been more echoic than the medulla

Doppler US

Both the transverse (APT) and transverse-oblique (APTO) anatomical planes were used bilaterally to examine the arcuate arteries for convenience of the Doppler procedure. In all, adequate well defined pulsed wave Doppler spectra were successfully obtained in 30 of 32 horses (93%) from the right and left kidneys, respectively.

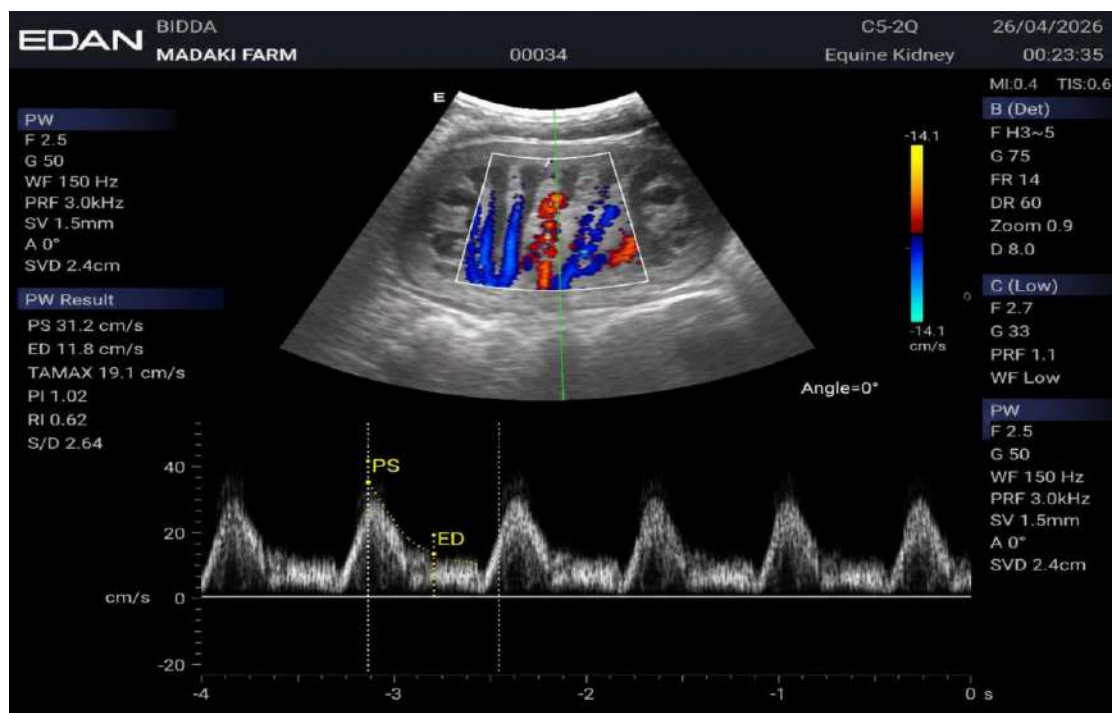


Figure 2. Sonogram of the intrarenal arterial Doppler pulsed wave velocity in a healthy, conscious Arewa horse for the determination of RRI and PI.

Table 3. Sizes of right and left kidneys (in cm) measured by B-mode ultrasonography in the non-racing Arewa and Talon Nigerian horses.

	Arewa Breed				Talon Breed			
	Young horses		Adult horses		Young horses		Adult horses	
Kidney	CCD (mean ± SD)	DVD (mean ± SD)	CCD (mean ± SD)	VDD (mean ± SD)	CCD (mean ± SD)	DVD (mean ± SD)	CCD (mean ± SD)	VDD (mean ± SD)
Right	7.9±1.1	7.3±1.4	10.6 ±	9.0±1.4	8.3±4.1	11.3±1.0	10.1±0.7	10.0±6.4
Left	10.1±3.2	9.9 ±1.0	11.2±1.8	10.3±1.7	11.5±0.9	6.90±2.3	11.9±1.2	7.8±6.1

Note: CCD- craniocaudal diameter, VDD-ventrodorsal diameter; SD Standard deviation

A statistical summary of the correlations between the RRI values in the right and left kidney, between the 2 breeds, male and female and young and adult horses and between the other parameters and variables measured is shown in Table 4.

Table 4. Comparison of Renal resistivity index vs other variable of Arewa and Talon Nigerian horses obtained at the level of the arcuate arteries via a PW Doppler for the right and left kidneys

BREED	Arewa		Talon	
	RKD (n=8)	LKD (n=8)	RKD (n=6)	LKD (n=8)
Median ±SE	0.55±0.01	0.53±0.01	0.52±0.01	0.51±0.001
Max	0.61	0.63	0.55	0.53
Min	0.49	0.47	0.49	0.50
AGE	Young (≤4 years)		Adult (≥4.5 years)	
	RKD	LKD	RKD	LKD
Median±SE	0.56±0.01	0.53±0.01	0.55±0.02	0.54±0.03

Max	0.61	0.63	0.66	0.62
Min	0.51	0.55	0.55	0.52
SEX	Female (n Filly=8, n Mare=8)		Male (n Colt=8, n Stallion=8)	
	Filly RKD/Mare RKD	Filly LKD/Mare LKD	Colt RKD/ Stallion RKD	Colt RKD/ Stallion RKD
Median±SE	0.56±0.04/0.50 ± 0.02	0.51±0.05/0.53 ± 0.06	0.49±0.07/0.53 ± 0.04	0.60 ± 0.05/0.52 ± 0.05
Max. value	0.64/0.66	0.61/0.62	0.67/0.59	0.62/0.60
Min. value	0.33/0.40	0.46/0.41	0.35/0.44	0.39/0.42
OVERALL	Right Kidney		Left Kidney	
Median±SE	0.60±0.006		0.59±0.006	
Max	0.69		0.66	
Min	0.33		0.39	

Note: SE- Standard Error; Max- maximum value; min: minimum value; RKD- Right kidney; LKD- Left kidney; n- number of horses included

For the entire cohort, the overall mean RRI of the right kidney was 0.60 ± 0.06 (range: 0.33–0.69) and that of the left kidney was 0.59 ± 0.06 (range: 0.39–0.66). The mean PI of the right kidney was 0.75 ± 0.13 (range: 0.33–0.89), whereas the left kidney yielded a mean PI of 0.77 ± 0.18 (range: 0.53–0.81). In young horses, the mean right kidney RRI stood at 0.56 ± 0.01 and the left kidney value at 0.53 ± 0.01 ; in adults, the corresponding means were 0.55 ± 0.02 (right) and 0.54 ± 0.03 (left).

There was no statistical difference between RRI values for young vs adult horses in either the left kidney ($p = 0.3017$) and/or right kidney ($p = 0.4454$). Likewise, no significant correlation was found between the RRI and breed (Arewa vs Talon), age category (young vs adult), sex (male vs female), body weight, body condition score or withers height of the horses.

Discussion

Using the sagittal plane and transverse plane for the right kidney and using the dorsal plane in the left kidney, like Hoffmann *et al.*, 1997 did, it was assessed that this was an effective and optimal window for Doppler evaluation. On the other hand, Fraccero *et al.*, 2020 used a transverse/transverse-oblique plane for bilateral renal Doppler examination; and they reiterated that Doppler examination technique might differ according to the anatomy of the individual animal. Therefore, is no standard preferred technique for Doppler examination in the horse.

RRI and PI values measured here fall within the normal range reported for healthy (unstressed) adult dogs and cats (Bragato *et al.*, 2017; Novellas *et al.*, 2007), horses and donkeys (Fraccero *et al.*, 2020; Macri *et al.*, 2015) and humans (Scoutt *et al.*, 1999) alike. However, interestingly, a fraction of our own PI values was lower than the ones reported for cats (1.29) and dogs (1.52) by Novellas *et al.*, 2007.

Right and left kidneys did not differ significantly in RRI nor did age (young or adult) or sex, body weight, body condition score or withers height. This is in agreement with the results obtained in human (Keogan *et al.*, 2011; Ansarin *et al.*, 2011), healthy dogs and cats (Novellas *et al.*, 2007; Chang *et al.*, 2010) and donkeys (Fraccero *et al.*, 2020). On the other hand, Macri *et al.*, 2015 and Fraccero *et al.*, 2020 were able to signal differences in RRI between the right and left kidney in horses, and Tipisca *et al.*, 2016 had higher RI in the left kidney in cats. Such differences could be a reflection of species or breed anatomic and/or technical differences as Barreiro-Vázquez *et al.*, 2021 suggested. The inter-kidney RRI difference is used as a

diagnostic tool to help identify upper urinary tract obstructions in the human patient (Sayani *et al.*, 2012). With regard to age, some differences in RRI were reported between elderly horses (over 18 years old) and foals and adults, although, again, this RRI was never higher than 0.70 (Siwinska *et al.*, 2019); we were not able to verify any differences, as none of our horses enrolled exceeded the age of 15 years. Ageing is known to have a positive effect on RRI (Kaiser *et al.*, 2007; Macrì *et al.*, 2015) mainly in human subjects aged > 50 years and with mixed results in small animals (Tipisca *et al.*, 2016; Ostrowska *et al.*, 2016).

We did not note any significant differences in RRI between geldings and mares, agreeing with Macrì *et al.*, 2015 (5 geldings, 8 mares) as well as with the human study by Tetsuka *et al.*, 2003. However, Ponte *et al.*, 2014 did obtain a sex-related difference in a larger human study, which may have been due to the increased number of elderly women enrolled in their study.

A weak correlation between RRI and body weight was found in cats by Park *et al.*, 2008 and they also suggested that this posed in the cat may be true for dogs, although, like in the present study, there was no correlation between body weight and RRI in a similar study in dogs and cats reported by Ostrowska *et al.*, 2016. Few studies attempted to investigate the relationship between body fat distribution and intrarenal haemodynamics in equines however this study did not do so. Fat content in the body of horses is very variable and positively related to body weight and BCS (Dugdale *et al.*, 2012). In humans, central fat distribution and excess weight are linked with an unfavourable renal haemodynamic profile which is potentially involved in chronic renal damage (Kwakernaak *et al.*, 2013) and will be associated with an increase in RRI (Buscemi *et al.*, 2009).

Conclusion

In Nigerian Arewa and Talon horses, RRI and PI can be assessed on the pulsed-wave Doppler mode but this is time-consuming and requires ultrasonographic skill, technical prowess and experience. The median values of both the RRI and the PI for both kidneys are in the range reported previously; there was no significant difference between the right and left kidney. There were no differences among breed, body weight, body condition score or withers height and there were no differences between young or adult horses with respect to either RRI or PI. These results can be used as a reference for several animal studies to be performed comparing healthy and diseased individuals and also to study the influence of other conditions (pregnancy, lactation). It is thus important to combine Doppler assessment with hematological, biochemical, and physiological evaluations in the interpretation of renal function vis a vis the established reference values for clinical practice.

Recommendation

Extensive research is necessary to complement our findings and aim to investigate the relationship between RRI and morpho-functional characteristics of the kidneys and distribution of body fat. It is required to study if renal indices are related to different stages of pregnancy, acute and chronic kidney failure and if they have diagnostic and prognostic significance. To support appropriate correlations with age, renal indices should be analyzed on a greater number of horses of a larger age range. Furthermore, RRI has been shown to be influenced by arterial blood pressure (Andrikou *et al.*, 2018), so further research is needed to elucidate this in horses in Nigeria.

Technical Challenges encountered

There were some delays in the procedure time as handlers were sometimes reluctant to help which added to research time. Depending on the animal cooperation, pulsed-wave Doppler image acquisition was sometimes difficult, time-consuming and tedious, particularly because of incessant body movement and non-cooperative nature of the horses.

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Informed Consent Statement: Informed consent was obtained from the owners for the clinical research, and for publication of the results from the management of His Royal Highness stables and Madaki farm in Zuru emirate council.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

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