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ULTRASONOGRAPHIC RENAL STUDY OF TRYPANOSOMA BRUCEI INFECTED DOGS PRESENTED TO UNIVERSITY OF NIGERIA VETERINARY TEACHING HOSPITAL, NSUKKA

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ABSTRACT

This study was conducted to assess the renal status of *Trypanosoma brucei* infected dogs using ultrasonography and correlate it with kidney function test. A total of eighteen (18) Nigerian local breeds of dogs of both sexes, weighing between 6-10 kg were used for the study. Group I comprised of nine clinically *Trypanosoma brucei* infected dogs selected at the outpatient Department of the University of Nigeria Veterinary Teaching Hospital (UNVTH) and group II was nine the apparently healthy dogs. The uninfected group showed a thin echogenic line representing the capsule. The renal cortex was hypo echoic and finely granular structure. The renal medulla was anechoic and the renal pelvis was seen as an irregular echogenic mass at the hilus of the kidney. The infected group showed more echogenic mass in the renal pelvis and cortical region as well as thickening of capsule and reduction of size. The left and right kidney of the trypanosome infected group showed significant reduction ($p<0.05$) in volume compared to the uninfected group. The mean kidney volume of left and right of the uninfected group were 44.54 ± 3.48 cm^3 and 47.39 ± 47.39 cm^3 respectively, whereas for the trypanosome infected dogs, the mean kidney volume of the left and right kidney was 26.08 ± 17cm^3 and 27.97 ± 3.66cm^3, respectively. The mean blood urea nitrogen (BUN) and Creatinine (CRT) levels differed significantly ($p<0.05$). The mean BUN of infected and uninfected groups was 15.79 ± 9.42 and 7.70 ± 1.95, while CRT was 0.71 ± 0.20 and 0.47 ± 0.15 respectively for the infected and uninfected group. From this study ultrasonography showed the renal morphology, which ultimately represents a better prognosis for sick dogs, particularly on the guidance of therapeutic procedures to be employed and ultrasonograhic images can be correlated with kidney function test as they all point towards the functionality of the kidneys.

KEYWORDS: Ultrasonography; Kidneys; *Trypanosoma* brucei; Dogs.

INTRODUCTION

Diagnostic ultrasound is a non-invasive diagnostic technique based on the pulse-echo principle used to image inside the body (Andualem *et al.*, 2017). Ultrasonography provides information about size, shape, location and the soft issue architecture of the structure or organ being examined (Abd El-Aty and Medan, 2010). Apart from the laboratory findings, application of ultrasonography can be considered as one of the finest diagnostic tools to determine the architectural integrity of the kidney (Ditul and Rajiv, 2014). The most common indication for ordering an ultrasound imaging of the kidney in general veterinary practice is chronically elevated liver enzymes (Tyrrel and Beck, 2006). Other common indication includes urinary tract disease, gastrointestinal disease, endocrine disease, neoplasia, trauma, fever of unknown origin and immune medicated diseases (Kelly, 2015). In small animal practice, Doppler ultrasound has been used to diagnose and monitor canine pregnancy and in precise assessment of maternal and fetal blood flow patterns from vessels as well as fetal and maternal cardiac chambers (Andualem *et al.*, 2017). The vessels routinely examined are the uterine, umbilical, and fetal middle cerebral arteries (Blanco *et al.*, 2008). Another common use of ultrasonography in animals, which is not typically utilized in human medicine, is in the identification of foreign bodies such as plastic, fabric, and wood which cannot be typically visualized by radiographic tests such as plain film x-ray (Tyrrell and Beck, 2006; Ober *et al.*, 2008). Ultrasonography is not primarily used for acute disease processes, as they are often treated symptomatically in veterinary medicine (Tyrrel and Beck, 2006). If the therapeutic procedure employed does not prove successfully resolve symptoms, ultrasonography can be useful in providing differential diagnoses (Kelly, 2015).

Trypanosomosis at present has emerged a significant global health challenge in humans, domesticated animals and wildlife communities (WHO, 1995; Thompson *et al.*, 2013). It is considered as one of the most important diseases affecting livestock, equines, and dogs within the Sub-Saharan region, with high morbidity and mortality (Anderson *et al.*, 2011; Geiger *et al.*, 2018). Trypanosome infection leads to erythrophagocytosis and heme catabolism resulting in iron accumulation in tissues and hyperbilirubinemia, liver dysfunction, and multiple organ failure (Stijleman *et al.*, 2016). Trypansomosis caused by *Trypansoma brucei* is known to sequestrate in various organs including the kidneys from blood stream causing some renal pathology (Aquino *et al.*, 2002; Nwoha *et al.*, 2013). Trypanosomiasis is a chronic progressive disease, and clinical signs may become obvious in advanced stages of the disease (Magona *et al.*, 2003). Trypanosomiasis is characterized by parasitaemia and intermittent fever (Habila *et al.*, 2012). Parasitological examinations are relatively sensitive during the acute phase of the disease. Wet blood film examination is used to detect the presence of motile trypomastigotes but has low sensitivity (Kasozi *et al.*, 2021). Other methods of diagnosis including fluorescence microscopy, dark ground or phase-contrast microscopy. Molecular and serological tests have emerged, which appears to be more sensitive, but not routinely used in parasitic diseases especially trypanosomosis (Biéler *et al.*, 2012). However, there seems to be a knowledge gap on the ultrasonographic features of kidneys of *T. brucei* infected dogs. This work was therefore designed, to highlights the architectural integrity of kidneys of trypanosome infected dogs and compare with established laboratory baselines of dysfunctional kidney.

MATERIALS AND METHODS

Experimental animals

A total of eighteen (18) Nigerian local dogs of both sexes and weighing between 6-10 kg were used for this study. Group I comprised of nine clinically trypanosome infected dogs selected at the outpatient Department of the University of Nigeria Veterinary Teaching Hospital (UNVTH) and group II was nine apparently healthy dogs. The clinical cases and apparently healthy dogs were screened of trypanosomosis using wet mount and buffy coat method (Murray *et al.*, 1977). Transabdominal ultrasonography was carried out on the dogs using a dual frequency (3.5/55.0MHz) curved array transducer (scanner 200VET-pie Medical Equipment B. V;Philipsweg l, 6227 A J Maatricht, Neherlands; Sony up-890 MD/2SYN video graphic printer, Sony corporation, Tokyo Japan). For documentation, a video graphic printer was used to record the images. The right kidney was scanned by placing the probe caudal to the right costo-spinal area where as the left kidney was scanned by placing the probe at the caudal left costo-spinal areas in dorsal recumbence position of the patient at the level of 2nd and 3rd lumbar vertebra.

Real time ultrasound scanning was done to visualize the kidneys. Images of interest were frozen and printed out. In order to find out the volume of each kidney, all recorded parameters were put in a formula of prolate Ellipsoid method and calculated (Felkai *et al.*, 1992)

$V=A_2 \times 0.85/L$ where:

V= Volume

A= Area and

L= Length of the kidney

Kidney function test

3 ml of blood was collected from the cephalic vein of each dog into a vacutainer tube without anticoagulant and the tube was left undisturbed for 30 minutes to allow clotting. The clotted blood was then centrifuged for 2 minutes at 30,000 revolutions per minute using a microhematocrit centrifuge. The serum supernatant was immediately aspirated into labelled sample bottles and refrigerated until use. The standard colorimetric method of Reitman and Frankel (1957) was used for the determination of the levels blood urea nitrogen (BUN) and creatinine (CRT) levels. Manuscript complied with current animal welfare and ethical guidelines, of University of Nigeria Nsukka, Faculty of Veterinary Institutional Animal Care and Use Committee. Reference No. FVM- UNN-IACUC-2019-1131.

Statistical analysis

The group means ± SEM was calculated for each parameter and significant difference between means evaluated using student T-test values and $p<0.05$ were considered significant.

RESULTS

The left and right kidney of the trypanosome infected groups showed significant reduction ($p<0.05$) in volume compared to the uninfected group. The mean kidney volume of left and right kidney of uninfected group was (44.54 ±3.48cm^3) and (47.39 ±4.2cm^3), respectively whereas for the trypanosome infected dogs, the mean kidney volume of left and right kidney was (26.08±17cm^3) and (27.97 ±33.6cm^3) (Table 1). The mean blood urea nitrogen (BUN) of infected and uninfected dogs was 15.79 ± 9.42 and 7.70 ± 1.95 respectively, which differed significantly ($P<0.05$), while the mean creatinine (CRT) of infected and uninfected dogs was 0.71 ± 0.20 and 0.47 ± 0.15 which also showed a significant ($P<0.05$) difference (Table 2). In the uninfected group, all cases showed a thin echogenic line representing the capsule. The renal cortex was hypo echoic and finely granular structure. The renal medulla was anechoic and the renal pelvis was seen as an irregular echogenic mass at the hilus of the kidney (Fig 1a&b). In the infected group the kidneys were more echogenic mass in the renal pelvis and cortical region as well as thickening of capsule and reduction of size respectively (Fig 2a&b).

Table 1: The mean volume (size)-cm^3 of the Right and Left kidneys of *Trypanosoma brucei* naturally infected and uninfected dogs.

	Right Kidney	Left Kidney
Group I	28.00±8.22[a]	26.07±8.90[a]
Group II	47.40±2.70[b]	44.53±3.46[b]

[ab] Different superscripts in a row indicate significant differences between the group means ($p<0.05$).

Table 2: The mean blood urea nitrogen (BUN) and creatinine (CRT) of *Trypanosoma brucei* naturally infected and uninfected dogs.

	Blood urea nitrogen (BUN)	Creatinine (CRT)
Group I	15.79±9.42[a]	0.71±0.20[a]
Group II	7.70±1.95[b]	0.47±0.15[b]

[ab] Different superscripts in a row indicate significant differences between the group means ($p<0.05$).

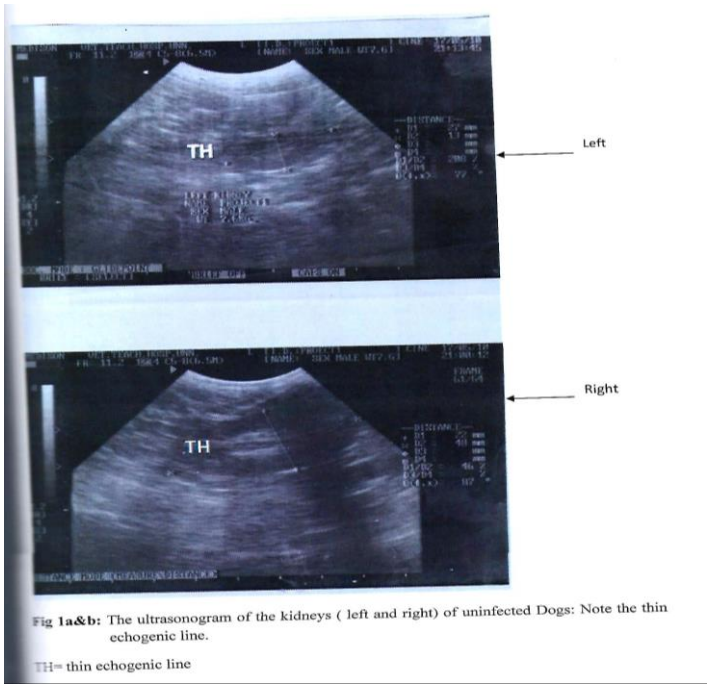

Fig 1a&b: The ultrasonogram of the kidneys (left and right) of uninfected Dogs: Note the thin echogenic line.

TH= thin echogenic line

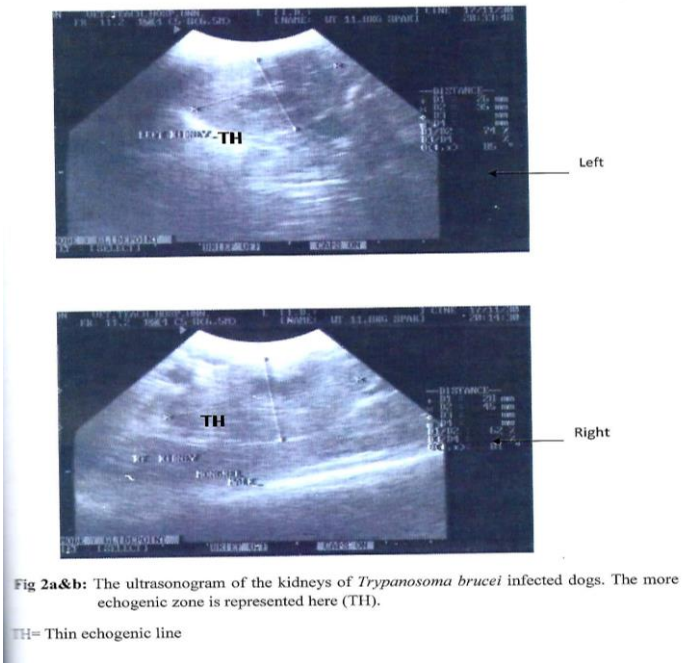

Fig 2a&b: The ultrasonogram of the kidneys of *Trypanosoma brucei* infected dogs. The more echogenic zone is represented here (TH).

TH= Thin echogenic line

DISCUSSION

Ultrasonographic images of the kidneys of *Trypanosoma brucei* infected dogs obtained from this study revealed variation in architectural integrity compared with the kidney images of apparently healthy dogs. This finding suggests some level of pathologic changes in the kidneys of trypanosome infected dogs. The renal ultrasonographic alterations, as showed by the infected dogs in our study, such as reduction in size and loss of cortico-medullary definition, can be defined as signs of chronic renal inflammatory disease (Vac, 2004). These kidney pathologies and architectural alternations may be due to the propensity of *T. brucei* to invade connective tissues and cause inflammation, cellular degeneration and necrosis (Taylor and Authie, 2004). There were reductions in size of both the left and right kidneys in the trypanosome infected dogs, which is attributable to renal impairment, age factor, renal fibrosis or calcification (Ettinger and Feldman, 1995). A similar result was obtained in the ultrasonographic kidney feature of dogs infected with visceral leishmaniasis, with marked loss of natural characteristics of the renal architecture and increased echogenicity of the renal cortical parenchyma when compared to the spleen. These findings are evidences of advanced kidney disease (Burk 1996; Vac 2004; Gama *et al.,* 2009). Extravascular invasion by trypanosomes has been suggested as a likely cause of the kidney architectural alterations and size reduction in this study, though diseases with acute course are likely not to result in reduction in kidney size. Acute kidney diseases are usually associated with normal or enlarged kidney size (Ettinger and Feldman, 1995). However, the endemicity trypanosomosis and uncontrolled use of trypanocides in Nsukka area which may have resulted in the reduction in the size of the kidneys of infected dogs (Umeakuana *et al.,* 2016)

Furthermore, being naturally infected dogs, one cannot rule out effects of underlying infections which may contribute to kidney architectural alterations coupled with the profound immunosuppression that occurs in trypanosomosis (Anene *et al.,* 1989; Hoare, 1992). The marked immunosuppression lowers the host's resistance to opportunistic infection and may thus contribute to the kidney architectural changes. In one of the infected dogs, the left kidney showed more echogenic mass in renal pelvis as well as in the cortical region and this might be due to high fat and fibrous tissues content. Barr (1990) recorded similar findings in his work on assessing the renal size in the dogs using ultrasonography.

In this study, the ultrasonography can be correlated with the kidney function test conducted, as the all point towards renal pathology. This is in consistent with the laboratory findings of (Gama *et al.,* 2009), who discovered abnormally high values for blood urea nitrogen and creatinine levels, as well as severe proteinuria in kidneys of dogs with compromised architectural integrity. The elevated serum levels of blood urea nitrogen and creatinine, associated with proteinuria, can be considered clinical evidence of renal injury caused by deposition of immunocomplexes in the basal membranes of the renal parenchyma as described by various authors (Ciaramella *et al*. 1997; Ferrer 1992; Moura *et al*. 2002).

CONCLUSION

It can be concluded from this study, that laboratory results for kidney disease should be correlated with ultrasonographic images, as ultrasonography will show the renal morphology, which ultimately could represent a better prognosis for sick dogs, particularly on the guidance of therapeutic procedures to be employed.

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Animal right

The study complied with animal welfare and ethical guidelines, of University of Nigeria Nsukka, Faculty of Veterinary Medicine Animal Care and Use Committee.

Conflict of interest

The authors hereby declare no conflict of interest in this study.

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No fund was received for this research work.

Data availability

Data used in this study was obtained from this work and other materials were gotten from the Google and Google scholar search engines.

Author's contribution

PO Akpa conceptualized the work, the ultrasonography and laboratory work were carried out by PO Akpa and CS Ukwueze, while CS Ukwueze prepared the manuscript.

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