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Clinical-pathological and immunohistochemical evaluations of cardiac lesions in cats with chronic kidney disease[1]

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ABSTRACT.- Cid G.C., Jardim M.P.B., Jesus A.C., Costa S.Z.R., Gonçalves I.N., Peixoto T.C., Souza H.J.M. & Nogueira V.A. 2020. **Clinical-pathological and immunohistochemical evaluations of cardiac lesions in cats with chronic renal disease.** *Pesquisa Veterinária Brasileira 40(12):1002-1009.* Universidade Federal Rural do Rio de Janeiro, Rodovia BR-465 Km 7, Zona Rural, Seropédica, RJ 23890-000, Brazil. E-mail: gabrieladecarvalhocid@gmail.com

Chronic kidney disease (CKD) is characterized by irreversible morphostructural lesions that can progressively evolve to chronic renal insufficiency and kidney failure. It is known that the heart and kidneys are closely related, and that communication between these organs occurs through a variety of pathways; subtle physiological changes in one of them are compensated by the other. Histopathological cardiac evaluation through routine staining presents a limitation to identify specific or discreet lesions in the cardiomyocytes. This study aimed to evaluate serum troponin levels in cats with CKD, associated with clinical and pathological findings, as well as to correlate the morphostructural cardiac lesions to determine their distribution through macroscopic and histological assessments and anti-cardiac troponin C (cTnC) immunohistochemistry (IHC). To this end, 20 cats (18 diagnosed with CKD and two controls) were selected. Anti-human cTnC IHC was conducted after necropsy and separation in eight regions of each collected heart. Heart fragments from two cats without CKD were used as controls. The anti-human cTnC antibody is useful in detecting cardiac lesions and has shown decreased expression in cardiomyocytes of cats with CKD. Serum troponin was above the reference values in 11/18 (61.11%) animals and decreased expression for the cTnC antibody was observed in individual cardiomyocytes in 9/18 (50%) animals. It was verified that the number of regions with decreased expression for the cTnC antibody in cardiomyocytes is significantly correlated with serum troponin. The anti-human cTnC antibody has been found effective in detecting cardiac lesions and has shown decreased expression in the cardiomyocytes of cats with CKD. Correlation was observed between increased serum cTnI and loss of immunoreactivity at anti-cTnC antibody IHC in cats with CKD, which proves damage to cardiomyocytes secondary to kidney disease.

INDEX TERMS: Clinics, pathology, immunohistochemistry, cardiac lesions, cats, renal disease, troponin, biomarker, cardiology, nephrology.

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RESUMO.- [**Avaliações clínico-patológica e imuno-histoquímica de lesões cardíacas em gatos com doença renal crônica.**] A doença renal crônica (DRC) é caracterizada por lesões morfoestruturais irreversíveis, que podem evoluir progressivamente para insuficiência renal crônica e falência renal. Sabe-se que o coração e os rins mantêm estreita relação e a comunicação entre esses órgãos ocorre por uma variedade de vias; alterações fisiológicas sutis em um desses órgãos são compensadas pelo outro. A avaliação histopatológica cardíaca mediante a colorações rotineiras são limitadas para identificar lesões específicas ou discretas em cardiomiócitos. O presente trabalho teve como objetivos avaliar os níveis séricos de troponina em gatos com DRC, associados aos achados clínico-patológicos, bem como correlacionar as lesões cardíacas morfoestruturais, a fim de determinar a distribuição destas, por meio da avaliação macroscópica, histológica e imuno-histoquímica com anti-cTnC. Neste estudo foram selecionados 20 gatos (18 diagnosticados com DRC e 2 animais controle). Para a aplicação da técnica de imuno-histoquímica anti-troponina C humana, necropsias foram realizadas e cada coração coletado separadamente em 8 regiões. Fragmentos do coração de 2 gatos sem lesão cardíaca foram utilizados como controle. O anticorpo anti-TnC humano é útil na detecção de lesões cardíacas e apresentou expressão diminuída em cardiomiócitos de gatos com DRC. Em 11/18 animais (61,11%) a troponina sérica encontrava-se acima dos valores de referência e foram observadas diminuição da expressão para anticorpo-cTnC em cardiomiócitos individuais em 9/18 (50%). Notou-se que o número de regiões com diminuição da expressão para anticorpo-cTnC em cardiomiócitos está significativamente correlacionado com a troponina sérica. O anticorpo anti-TnC humano se mostrou eficaz para detectar lesões cardíacas e demonstrou diminuição da expressão nos cardiomiócitos de gatos com DRC. Houve correlação entre o aumento da CTnI sérica e perda da imunorretividade na avaliação imuno-histoquímica com anticorpo anti-TnC em gatos com DRC o que comprova danos em cardiomiócitos secundários a doença renal.

TERMOS DE INDEXAÇÃO: Clínica, patologia, imuno-histoquímica, lesões cardíacas, gatos, doença renal, troponina, biomarcador, cardiologia, nefrologia, felinos.

INTRODUCTION

Chronic kidney disease (CKD) is characterized by irreversible morphostructural lesions that can progressively evolve to chronic renal insufficiency and kidney failure. Kidney failure leads to disruption of metabolic and endocrine functions, hydroelectrolytic and acid-base disorders (Ribeiro et al. 2008), and changes in cardiac function (Martin & Franco 2005). CKD occurs with high frequency in pets, with a prevalence of 30-40% in feline species (Polzin et al. 2005). Consequently, there is a decrease in the ability of this organ to concentrate and eliminate certain toxic substances that, under normal conditions, are eliminated from the body by renal excretion (Polzin et al. 2005).

It is known that the heart and kidneys are closely related, and that communication between these organs occurs through a variety of pathways; subtle physiological changes in one of them are not always compensated by the other (Viswanathan & Gilbert 2011). Cardiac alterations in humans with chronic renal insufficiency were first described in 1947. Macroscopically, there was marked ventricular hypertrophy and fibrosis, and intense interstitial edema was observed on histological examination of the myocardium. That study suggested that the cause of death had been uremia, given that routine clinical dialysis was not available at the time (Langendorf & Pirani 1947). Heart diseases, when associated with nephropathies, can develop more quickly and are difficult to control. CKD can cause anemia, uncontrolled cholesterol and triglycerides levels, and hinders the control of arterial blood pressure. In humans, it accelerates the process of atherosclerosis, with subsequent mineralization and formation of atheromatous plaques in the cerebral and coronary arteries, which can cause myocardial infarction (Ronco et al. 2008).

Routine staining is limited to showing specific or discrete lesions in the cardiomyocytes (Stigger et al. 2001, Carmo et al. 2011). Several immunohistochemical techniques with high specificity and sensitivity have been developed aiming to detect damage to the cardiomyocytes. Among these, cardiac troponin has shown promising results in cattle (Ortmann et al. 2000, Pavarini et al. 2012, Bandinelli et al. 2014, Santos et al. 2016), sheep (Costa et al. 2016), and dogs (D'avila et al. 2016). Necrotic cardiomyocytes, detectable or not by routine optical microscopy exams, show negative immunostaining, while those unaffected are positive for troponin (Jenkins et al. 2010, Bandinelli et al. 2014, Santos et al. 2016 Costa et al. 2016, D'avila et al. 2016).

To date, there have been no studies evaluating immunohistochemistry (IHC) with anti-cTnC to detect cardiac lesions in domestic cats. Thus, the present study aimed to evaluate the serum levels of troponin I in cats with CKD, associated with clinical and pathological findings, as well as to correlate the morphostructural cardiac lesions to determine their distribution through macroscopic and histological assessments and anti-cardiac troponin C (cTnC) IHC.

MATERIALS AND METHODS

This study was approved by the Ethics Committee on Animal Use (EAUC) of "Universidade Federal Rural do Rio de Janeiro" (UFRRJ) under the process no. 7430260817, and was carried out at the Histopathology and Immunohistochemistry Laboratories of the "Setor de Patologia Animal" (PAS), located in Annex I of the "Instituto de Veterinária" of UFRRJ. Laboratory tests (blood count, biochemistry, and troponin I) were performed in a private clinical pathology laboratory.

The study sample was composed of 20 cats (*Felis catus*) - 12 females and eight males: 18 diagnosed with CKD and 2 controls, regardless of breed, sex, and age. After necropsy, fragments from all organs were macroscopically evaluated, collected, and processed for histopathology.

The following inclusion criteria were adopted: cats with biochemical profile of azotemia, at stage II or higher of the International Renal Interest Society (IRIS), with serum creatinine concentrations >1.6mg/dL (IRIS 2019), and record of clinical signs and complementary exams consistent with the diagnosis of CKD. Animals with primary heart disease or other conditions that could elevate serum troponin levels, such as hypertrophic cardiomyopathy, hyperthyroidism, primary or metastatic cardiac neoplasia, were excluded from the study. This exclusion was based on a thorough clinical evaluation with the aid of imaging and laboratory tests to diagnose CKD. Histological analysis of the thyroid gland was performed in the 20 animals, and usual morphology was observed, without hyperplasia or any other changes. Arterial blood pressure was measured on 12 of the 18 cats with CKD.

Laboratory tests including biochemistry, blood count, and serum troponin were performed in all cats in this study. Blood samples were collected from each cat to perform blood count and biochemistry tests in all consultations within the sample collection period in this study until death. Collection for serum troponin measurement was performed in animals at CKD stages II, III, or IV.

During this study, 18 cats with CKD and two control animals were necropsied. On macroscopic examination, several organs were evaluated in detail, and the data were recorded in forms prepared for this research. At necropsy, the hearts were collected whole for gross evaluation according to the technique described by Bandinelli et al. (2014). Then they were cross-sectioned in a total of four complete sections equidistant from each other. Eight fragments from different regions of each heart were collected separately and placed in previously identified flasks containing 10% buffered formalin solution. Fragments were collected from the apex, interventricular septum, left and right ventricles, left and right papillary muscles, and left and right atria. Heart fragments from two cats without renal and cardiac injuries were used as control. For histopathological examination, each sample was fixed and routinely processed for histology, the sections were stained with hematoxylin and eosin (HE), and the slides were examined under an optical microscope.

Anti-human troponin C IHC was performed on serial slides obtained from sections of the eight heart regions. The histological sections were dewaxed in two xylene baths (20 min each), hydrated in four alcohol baths (5 min each), and incubated for 15 min in 3% hydrogen peroxide diluted on the spot. They remained for another 15 min in the same solution and were then diluted to block endogenous peroxidases. After washing the slides with distilled water, they were kept in a water bath with citrate buffer (pH 6.0) at approximately 98 °C for 15 min for antigen recovery. Blocking of nonspecific reactions was performed using 5% skimmed milk (Molico® - Brazilian industry) for 30 min. The sections were incubated overnight with anti-human troponin C antibody (Novocastra® clone 1A2) in a 1:100 dilution (1µl antibody to 100µl PBS or ADS-125 antibody diluent). The REVEAL biotin-free polymer-HRP detection system (Spring) was used with diaminobenzidine (DAB) as chromogen. All sections were stained with Harris' hematoxylin and evaluated under an optical microscope.

The Spearman's correlation coefficients were calculated to measure the relationship between the laboratory variables and the number of heart regions with decreased expression for cTnC antibody in individual cardiomyocytes. The Fisher's exact test was applied to assess whether plasma troponin was independent of the IRIS stages. The data were processed using the *R* 3.6.12 software. A significance level of 5% ($p<0.05$) was adopted for all statistical analyses.

RESULTS

A total of 18 cats diagnosed with CKD and 2 control animals were assessed between March 2016 and November 2018. Of these 18 cats, 11 (61.15%) were female and 7 (38.9%) male, with ages varying from 4 to 23 years (mean of 10.5 years). Eleven (11, 61.1%) cats were weighed using the body score with a scale ranging from 1 to 5 (1 = cachectic, 2 = underweight, 3 = ideal, 4 = overweight, and 5 = obese). With regard to breed, varied patterns were observed: most cats were crossbred (14, 77.8%), two were Persian (11.1%), one was Birman (5.55%), and one Siamese (5.55%).

In the analysis of creatinine and urea, parameters above the reference values were found in the 18 animals with CKD. In the serum troponin test, data above the reference values were found in 10/18 (55.6%) animals. The highest values for creatinine and urea, as well as for serum troponin, the classification of animals according to IRIS, the presence or absence of cardiac injury at HE, and the loss of cardiac troponin at IHC are described in Table 1.

Clinically, 14/18 (77.7%) animals presented reduced appetite, 16/18 (88.8%) showed weight loss, and 6/18

Table 1. Classification of IRIS and laboratory tests

Cat	IRIS stage	Creatinine 0.51.6mg/dL (reference)	Urea 42.854.2mg/dL (reference)	Troponin serum 0.030.16ng/L (reference)	Cardiac lesion (HE)	Areas with troponin loss (IHC)
1	II	2.0	53.0	0.03	No	No
2	III	2.91	72.7	0.09	Yes	(+)
3	IV	13.0	130.0	0.673	No	No
4	IV	6.81	308.2	39,497	Yes	+++
5	IV	6.6	152	0.11	No	No
6	IV	11.3	430.6	0.120	No	No
7	II	2.2	188.5	1,558	Yes	(+)
8	IV	6.33	206.1	0.448	Yes	(+)
9	IV	6.8	226	0.990	Yes	(+)
10	IV	8.8	241	0.085	No	No
11	IV	8.2	325	0.023	No	No
12	IV	17.0	276.0	0.166	No	No
13	II	2.8	-	9,332	Yes	(+)
14	IV	8.40	226	1,237	Yes	(+)
15	IV	9.4	276	51,019	Yes	+++
16	IV	8.0	200.0	0.046	No	No
17	IV	10.9	309	0.206	Yes	(+)
18	II	2.8	47	0.131	No	No
19*	-	-	-	-	No	No
20*	-	1.6	-	0.11	No	No

* Control animals; (+) Discrete, + mild, ++ moderate, +++ marked.

(33.33%) had vomiting episodes. All cats that had arterial blood pressure measured (12/18) showed values within normality (120-140 mmHg).

In the macroscopic analysis of the cats, the main findings were pale mucous membrane (8/18, 44.44%), kidneys reduced in size (13/18, 72.2%) and with irregular capsular surface (15/18, 83.3%). The main histological lesions in the kidneys included predominantly diffuse interstitial lymphoplasmacytic infiltrate in the corticomedullary region (18/18, 100%), Bowman's capsule thickening (15/18, 83.3%), atrophy and glomerular sclerosis (16/18, 88.8%), mineralization (9/18, 50%), vacuolar degeneration of contorted tubular epithelial cells (6/18, 33.3%), and interstitial fibrosis (5/18, 27.7%).

Histopathological analysis of the heart revealed hypereosinophilic cardiomyocytes in 9/18 (50%) cats, presented singly in seven (38.8%) (Fig.1A) and in large areas in two (11.11%) (Fig.2A) animals; foci of fibrosis in 3/18 (16.6%) felines; mineralization of myocytes and tunica intima of heart vessels also in 3/18 (16.6%) animals; deposition of amorphous eosinophilic substance with reduction of the vascular lumen, characterizing arteriosclerosis, in 1/18 (5.5%) cat. Control animals did not show any important changes (Fig.3A,B).

Decreased expression for cTnC antibody was observed in cardiomyocytes (affected areas) in 9/18 cats (50%), singly in seven (38.8%) (Fig.1B) and in extensive areas in two (11.11%) cats (Fig.2B). Of these 18 animals, 11 showed increased serum troponin I levels: six (66.7%) with only one affected region and three (33.3%) with two affected regions. Of the eight heart areas observed, the most frequently affected region was the apex (27.8%), followed by the left ventricle (16.7%), right papillary muscle (11.1%), left papillary muscle (5.6 %), and right ventricle (5.6%).

It was observed that the number of regions with decreased expression for cTnC antibody in cardiomyocytes is strongly and significantly correlated only with serum troponin I. There were no significant correlations with the other variables of the laboratory tests (Table 2).

Of the 18 cats analyzed, five (27.77%) were in stage II, one (5.55%) in stage III, and 13 (72.2%) in stage IV of the IRIS. A *p*-value of 0.6998 was obtained in the Fisher's exact test, suggesting independence between IRIS stage and the number

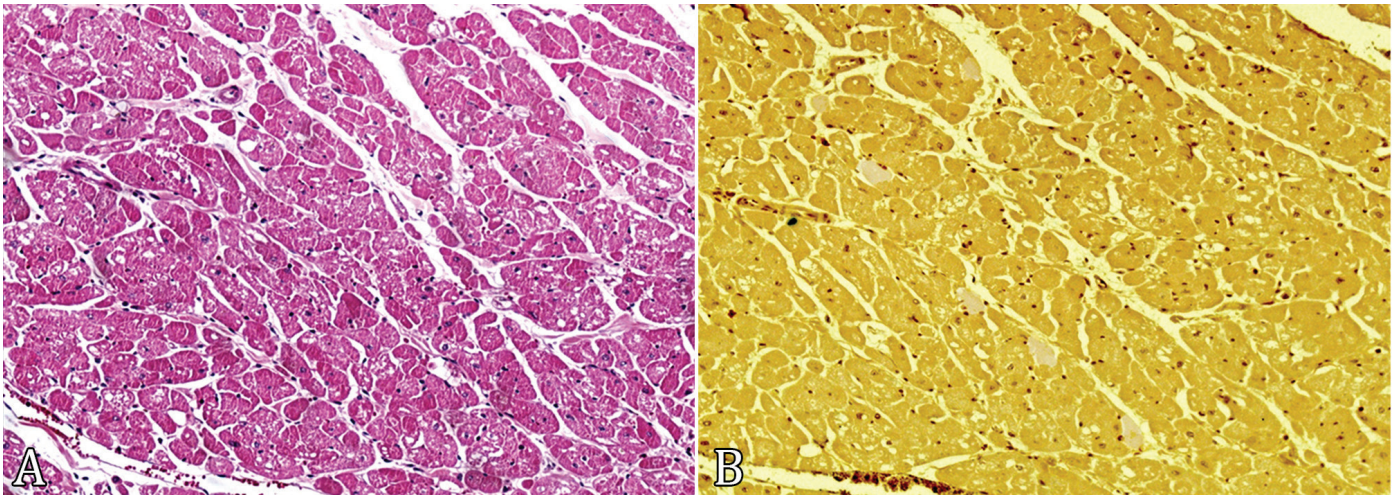

Fig.1. (**A**) Few individual hypereosinophilic cardiomyocytes. Cat 2. HE, obj.20x. (**B**) Decrease in expression for anti-human cTnC antibody corresponding to the area with hypereosinophilic cardiomyocytes. Cat 2. IHC, obj.20x.

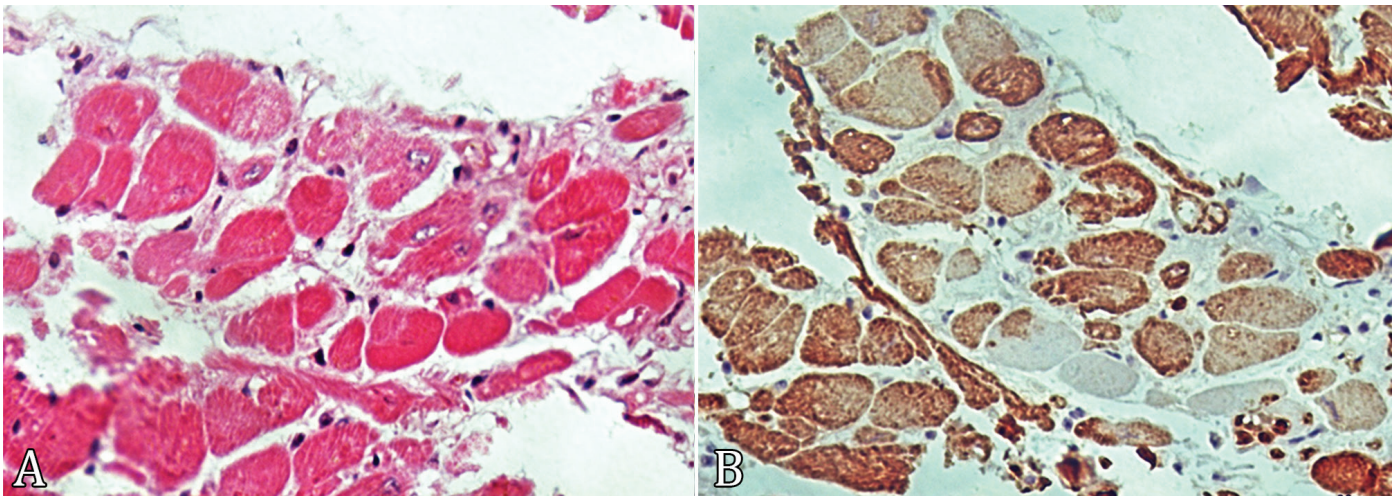

Fig.2. (**A**) Area delimited with individual hypereosinophilic cardiomyocytes . Cat 2. HE, obj.40x. (**B**) Moderate decrease in expression for anti-human cTnC antibody corresponding to the area with hypereosinophilic cardiomyocytes. Cat 2. IHC, obj.40x.

of regions with decreased expression for cTnC antibody in cardiomyocytes, that is, the number of affected regions was not associated with the IRIS stage.

DISCUSSION

Cardiorenal syndrome (CRS) is widely studied in medicine (Martin & Franco 2005). In veterinary medicine, CRS has gained notoriety because of its high prevalence and wide variety among dogs and cats (Pouchelon et al. 2015). According to the classification by Pouchelon et al. (2015), animals developed cardiovascular disease/dysfunction secondary to primary CKD and concomitant with impairment of both systems. Kidney-mediated systemic hypertension can result in increased afterload, left ventricular hypertrophy, worsening of mitral or aortic insufficiency, arrhythmia, vasculopathy, or retinopathy. In addition, volume overload generates systemic arterial hypertension, hypokalemia, or hyperkalemia and, consequently, cardiac arrhythmia (Pouchelon et al. 2015). In this study, the animals showed signs, clinical exams, laboratory findings, and images compatible with CKD. However, they did not present impairment of the cardiovascular system, which confirms the classification as primary kidney disease.

The use of serum cTnI as a biomarker of cardiac injury in this study assisted with identifying cardiomyocyte injury in the feline species. Langhorn et al. (2019) observed an increase in serum cTnI concentration in cats with compromised renal function without evidence of structural cardiac injury, which confirms its possible prognostic indicator in cats with CKD. These data corroborate the findings of the present study, since cats with CKD showed a significant increase in serum cTnI. The results reported by Langhorn et al. (2019) reinforce the hypothesis that increased impairment of renal function reflects myocardial injury or, at least partially, damage to the myocytes induced by a non-cardiac disease. This damage is correlated with codependency of the cardiorenal system, where its interaction involves shared mechanisms (Ronco et al. 2014, Keller et al. 2016), as well as with the causes of cardiac remodeling by CKD that would include activation of the renin-angiotensin-aldosterone system (RAAS) of the sympathetic nervous system (SNS) after a decline in glomerular filtration rate (GFR), which induce heart disease and fibrosis, in addition to uremic toxins, hyperphosphatemia, and non-anemia. Regenerative hypotheses have been recently raised (Langhorn et al. 2019).

In the animals of this study, only one serum cTnI measurement was performed because of the high costs of the exam and the lack of availability of the owners to collect the material. However, according to the literature, increased serum cTnI concentration occurs 4-6 h after an injury or acute necrosis of the human myocardium (Nelson & Thompson 2006, Jenkins et al. 2010), and can remain elevated for an average of 7-10 days after an acute episode (Sleeper et al. 2001), which demonstrates that its prolonged release time allows confirmation of the diagnosis up to three weeks (Godoy et al. 1998). A retrospective study conducted by Porciello et al. (2008) showed that dogs and cats with acute or chronic renal failure generally have high levels of cTnI, with an average of 72% of

Table 2. Spearman's correlation coefficient between the number of regions with decreased expression for cTnC antibody in cardiomyocytes of cats with chronic kidney disease (CKD) and laboratory test variables

Laboratory tests	Spearman's correlation	*p*-value	N
Maximum creatinine	-0.1203	0.6345	18
Maximum urea	0.2015	0.4381	17
Hematocrit	0.1797	0.4900	17
Leukogram	0.1966	0.4656	16
Troponin	0.7754	0.0002	18
Creatinine 1	0.1101	0.6636	18
Creatinine 2	-0.2848	0.3457	13
Creatinine 3	-0.2062	0.6242	8
Creatinine 4	-0.2236	0.7177	5
Creatinine 5	-1,0000	1,0000	2
Urea 1	0.3243	0.2041	17
Urea 2	-0.1927	0.5938	10
Urea 3	-0.4743	0.4195	5
Urea 4	0	1,0000	3
Urea 5	*	*	1

N = Number of cats subjected to the examination; * It was not possible to calculate because there was only one observation.

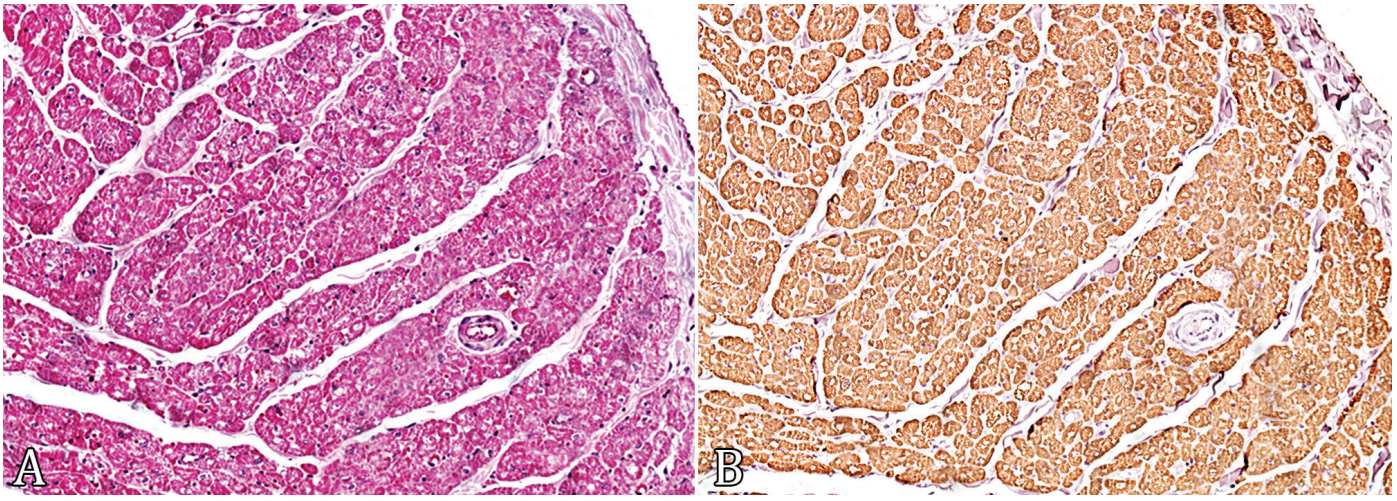

Fig.3. (**A**) Histological section of the heart. Control animal. HE, obj.20x. (**B**) Histological section of the heart with expression for the anti-human cTnC antibody. Control area. IHC, obj.20x .

cats with renal failure without a clear primary heart disease clinical condition. As in the present study, both previously mentioned studies justified the viability and application of serum cTnI in cats diagnosed with primary CKD for possible assistance and verification of early secondary cardiac injuries.

The primary histological lesions found in the hearts of the cats diagnosed with CKD included cardiomyocyte necrosis, foci of fibrosis, and mineralization both of myocytes and of the tunica intima of heart vessels, with both alterations observed in 3/18 animals, in addition to arteriosclerosis, found in only 1/18 cat. Although these were random findings, interstitial and/or perivascular fibrosis was observed secondarily in animals with renal dysfunction. This imbalance can be explained by homeostasis (Bagshaw et al. 2013), where volume overload contributes to increased preload and cardiac output changes. Increased afterload, such as activation of the SNS and RAAS, which lead to the development of systemic arterial hypertension (Bagshaw et al. 2013), and angiotensin II can induce cardiac fibrosis (Kawano et al. 2000).

In this study, both animals that presented foci of interstitial and/or perivascular fibrosis were in stage IV of the IRIS, with classic signs of uremia of advanced CKD. These findings in cats (Langendorf & Pirani 1947) contrast with those reported by D'avila et al. (2016), who found that most dogs with CKD presented interstitial and/or perivascular fibrosis at different IRIS stages of the disease.

The mineralization observed in both myocytes and tunica intima of the vessels, in 3/18 animals can be correlated to the deposition of minerals in tissues submitted to previous necrosis injury, that is, to dystrophic mineralization (Alfrey 2004, Cardoso et al. 2019). D'avila et al. (2016) verified that 100% of dogs evaluated showed endothelial lesions of different intensities, which was observed in only 1/18 of the cats in this study; however, it corroborates the study by Dantas & Kommers (1997), who reported that only 18% of the dogs had extrarenal uremic lesions such as vascular alteration associated with myocardial necrosis and mineralization. Coagulation necrosis and cardiac dystrophic mineralization were also observed in 43.7 and 25% of dogs with CKD, respectively, in the study conducted by Cardoso et al. (2019). Lesions of this nature were attributed to cardiac vessel changes in cats with uremia and/or metabolic and hemodynamic changes caused by CRS, since it reinforces the hypothesis that a dystrophic lesion precedes mineralization in cases of degenerative, inflammatory, or necrotic lesions in uremic dogs.

In 9/18 cats with CKD, there was a reduction in the levels and even total loss of cTnC expression in the sarcoplasm of groups of isolated cardiomyocytes or myocytes. At histopathological examination, cardiomyocytes presented a significant decrease in immunoreactivity, which, in histopathology, showed an increase in cytoplasmic eosinophilia, associated with nuclear pyknosis/karyorrhexis. Marked immunoreactivity was observed in the remaining and intact myocytes of both animals with CKD and those used as negative controls. Few studies have reported the use of IHC with anti-troponin antibody to detect cardiac lesions in veterinary medicine. Recently, through IHC for the human cTnC antibody, degenerative-necrotic lesions have been observed in the heart of cattle poisoned by *Amorimia exotropica* (Pavarini et al. 2012, Bandinelli et al. 2014) and of sheep poisoned by *Amaranthus spinosus* (Costa et al. 2016). Foci of hypereosinophilic cardiomyocytes with coagulation necrosis, neutrophilic inflammation, and subtle to mild variation in the staining pattern of some fibers with part of them showing a total loss of troponin I were found in the myocardium of dogs infected with *Dirofilaria immitis* (Carretón et al. 2012). D'avila et al. (2016) performed IHC on the heart fragments of 22 dogs with a previous diagnosis of CKD and found, in the heart of 16 animals, groups of hypereosinophilic cardiomyocytes with homogeneous cytoplasm and, in some cases, loss of striation and pyknosis, and IHC also revealed, in all dogs, several groups of myocytes with significant or absence of immunoreactivity for the cTnC antibody.

This study demonstrates a relationship between increased serum cTnI, increased cytoplasmic cardiomyocyte eosinophilia, and confirmed incipient cardiac injury due to loss of immunoreactivity to cTnC in the sarcoplasm and cardiomyocytes of cats diagnosed with CKD. There was an increase in eosinophilic cardiomyocytes confirmed by the loss of troponin from cardiac myocytes through IHC using human cTnC antibody. These findings corroborate those observed in dogs with CKD and heartworm disease (Carretón et al. 2012, D'avila et al. 2016), sheep with acute and subacute nephrotoxic injury (Costa et al. 2016), and cattle (Pavarini et al. 2012, Bandinelli et al. 2014) with cardiac injuries, which confirm the viability of this technique for these species. Regarding the extent of cardiac injury in cats, small foci of isolated necrosis or individual cardiomyocytes were observed, demonstrating that, even in advanced stages of CKD, cardiac lesions manifest differently in this species, which can be explained by the different pathophysiology and consequent clinical-pathological behavior of the feline species (Bagshaw et al. 2013). There is little evidence of this in the literature, both in dogs and cats (Sampedrano et al. 2006). In humans (Ronco et al. 2008), pathophysiology, especially concerning CKD, is a primary disease. Among the hypotheses addressed, systemic hypertension secondary to kidney disease is one of the leading causes of cardiac injuries. It can affect the heart of cats through injuries adaptive to the increased pressure of the heart load that, in some cases, promotes myocyte ischemia and consequent necrosis (Carr & Egner 2009).

In this study, each heart region was assessed separately to map the main affected areas, and 9/18 animals (50%) presented specific areas with incipient necrosis of cardiomyocytes in routine staining, confirmed by IHC, with the loss of staining for the human cTnC antibody. Among the observations, the apex (41.7%) was the main affected area, followed by left ventricle (25%), right papillary muscle (16.7%), left papillary muscle (8.3%), and right ventricle (8.3%), which indicates a greater predisposition of the species to damage in these regions of the heart. In the study conducted by Bandinelli et al. (2014) when mapping the hearts of cattle poisoned by *Amorimia exotropica*, the most affected area, the left papillary muscle, was found to be predisposed to this disease. In previous studies using of the cTnC antibody in the myocardial fragments of dogs (D'avila et al. 2016) and sheep (Costa et al. 2016), no mapping of the heart areas was performed.

In the present work, the animals were classified into the different IRIS stages by establishing the history, anamnesis, and clinical signs compatible with CKD over more than three months of evolution, as well as laboratory tests and serum measurements of creatinine and urea performed at different times throughout the clinical evolution of each cat. Creatinine

was used as the primary marker for IRIS classification since it is the most used substance for biochemical assessment of glomerular filtration (Junior & Williams 2010). There were 4/18 animals (2.25%) in stage II, 1/18 (5.6%) in stage III, and the vast majority, 13/18 (72.2%), were in stage IV. Clinical signs and laboratory findings for each animal confirm the standards stipulated by IRIS for each category. The predominance CKD stage IV is characterized by the presence of intense azotemia (serum creatinine >5.0 mg/dL), with significant loss of renal function and several systemic manifestations of uremia. However, no correlation was observed between the stages of IRIS and increased serum cTnI and loss of immunoreactivity at IHC by statistical analysis (*p*-value of 0.5252 by the Fisher's exact test), which suggests independence between IRIS staging and the number of regions with decreased expression for cTnC antibody in cardiomyocytes, that is, the number of affected regions is not associated with IRIS staging. In addition to the different pathophysiology of each species for CKD and CRS, Porciello et al. (2008) suggest that this response is individual, which justifies the independence and response to disease and the consequent data for the cardiovascular system for each animal.

CONCLUSIONS

Increased serum cTnI levels occur in cats with compromised renal function regardless of the IRIS stages, and this increase is correlated with the extent of cardiac injury observed at IHC.

The anti-human cTnC antibody demonstrated considerable viability for use in the feline species and IHC proved to be an effective tool to assist with detection and extension of cardiac injury in cats diagnosed with chronic kidney disease (CKD).

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