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Myocardial stereology in captive *Callithrix kuhlii* (Callitrichidae, Primates): healthy animals versus animals affected by wasting marmoset syndrome (WMS)[1]

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ABSTRACT.- Pissinatti T.A., Pissinatti A. & Burity C.H.F. 2007. **Myocardial stereology in captive *Callithrix kuhlii* (Callitrichidae, Primates): healthy animals versus animals affected by wasting marmoset syndrome (WMS)**. *Pesquisa Veterinária Brasileira 27(2):75-79.* Setor de Morfologia, IBC, Universidade do Grande Rio (Unigranrio), Rua Prof. José de Souza Herdy 1160, uque de Caxias, RJ 25071-202, Brazil. E-mail: cburity@unigranrio.com.br

This study comprised 12 hearts of Wied´s black-tufted-ear marmoset, *Callithrix kuhlii* (Coimbra-Filho 1985), 6 with Wasting Marmoset Syndrome (WMS) and 6 non-affected. Biometry was performed after death. After necropsy, the hearts were weighed, dissected, fixed in 10% formalin solution (pH 7.2), and processed for optical microscopy at 5μm sections stained with Haematoxylin-Eosin. Quantitative analysis was performed by stereological techniques. The statistical differences between the biometrical and stereological parameters were assessed by the Mann-Whitney test. The morphometric results showed that WMS causes a significant reduction in body and cardiac weights, and also in the volume density of vessels in those animals. Further studies are necessary to understand some of the results shown here.

INDEX TERMS: Wasting marmoset syndrome, myocardium, stereology, *Callithrix*, Callitrichidae.

RESUMO.- [**Estereologia do músculo cardíaco em *Callitrhix kuhlii* cativos (Primatas, Callithrichidae): animais sadios versus animais afetados pela síndrome do emagrecimento progressivo (SEP)**.] Neste estudo, foram utilizados corações de 12 Sagui-de-Wied, *Callitrhix kuhlii* (Coimbra-Filho 1985), sendo 6 animais afetados pela SEP e 6 animais normais. Após a morte foi realizada a biometria seguida de necropsia. Os corações foram fixados em formol tamponado a 10%, pesados e dissecados, sendo processados através de técnicas histológicas de rotina para microscopia óptica em cortes de 5μm corados por Hematoxilina-Eosina. As análises quantitativas foram feitas com o uso de técnicas estereológicas. As diferenças estatísticas entre os parâmetros biométricos e estereológicos foram avaliadas usando o test Mann-Whitney. Os resultados encontrados através da morfometria mostraram que a SEP causa uma redução significante do peso tanto corporal quanto do músculo cardíaco, e também uma redução no volume dos vasos nestes animais. Novos estudos são necessários para entender alguns dos resultados mostrados aqui.

TERMOS DE INDEXAÇÃO: Síndrome do emagrecimento progressivo, miocárdio, estereologia, *Callithrix*, Callitrichidae.

INTRODUCTION

Brazil has the greatest number of species of nonhuman primates on the planet; however, many of these species are currently endangered by constant deforestation and traffic of the animals. One of these species is *Callithrix kuhlii*, Wied´s black-tufted-ear marmoset (Coimbra-Filho 1985). In 1981, the *kuhlii* taxon was established for the southern region of Bahia, eastern Brazil, as a subspecies of *Callithrix penicillata*. This was not in accordance with had been established by other authors, who considered *Callithrix kuhlii* as a hybrid between *Callithrix penicillata* and *C. geoffroyi* (Rylands et al. 1993). In the past, an extensive area of the Atlantic Forest was the habitat for those

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animals; nowadays, however, they can only be found in a small area of the southeastern Bahia and in captivity in several places worldwide (Coimbra-Filho 1985). In the Atlantic Forest, they may be found in the canopy layer and may also reach the ground to feed on insects. The diet of the Callitrichidae consists basically of fruits, insects, and of plant secretions (Rylands et al. 1993).

The species lives in groups. The alpha animals, the first couple that mates, originate such groups, which may be comprised of up to 10 members. The offspring, after becoming adults, usually abandon the group to form other groups in new territories (Haig 1999).

The species is characterized by the dark brown color of its fur, with reddish-brown areas mainly in the inner part of the thighs, a small frontal white mark, and almost black head, fore and hind paws, and limbs (Coimbra-Filho 1985).

In 1976, Wasting Marmoset Syndrome (WMS) was reported for the first time as a disease with high morbidity and mortality (Sá 1999). That syndrome is characterized by clinical alterations, such as progressive emaciation, muscle atrophy, paralysis of the hind limbs, alopecia of the tail, nephritis, and intermittent diarrhea. It affects animals kept in captivity, which has been associated with immunologic reactions and components of the diet (Leite 2002). Nevertheless, WMS is not recognized as one specific syndrome or disease due to the great variety of factors that may influence its onset (Potkay 1992). Shimwell et al. (1979) and Murgatroyd & Chalmers (1980) reported no hereditary influence.

Regarding food, the Callitrichidae are considered a very demanding species. Several food formulations have been proposed for the captivity by Coimbra-Filho & Maia (1977), Coimbra-Filho et al. (1981), Clapp & Tardif (1985), Tardif et al. (1988), and Barnard et al. (1988), aiming at the species' good growth, reproduction, and longevity. In addition, such formulations improve their capacity to resist to pathogenic agents and stress (Knapka et al. 1995, Oftedal & Allen 1996, Wormell 2000).

Other studies correlate the appearance of WMS with gastrointestinal diseases (Chalifoux et al. 1982, Lewis et al. 1987), glucose intolerance (McNees et al. 1983) or primary deficiency of antioxidants (Gutteridge et al. 1986), parasitosis of the pancreatic ducts (*Trichospirura leptostoma*) (Pfister et al. 1990, Pissinatti et al. 1985), and other pathologies (Ialegio & Backer 1995).

MATERIALS AND METHODS

Samples

Sampling for this study comprised 12 hearts of Wied´s black-tufted-ear marmoset (*Callithrix kuhlii*), 6 of which belonged to animals that died due to complications of WMS, such as marked cachexia, caused by intermittent diarrhea, and also limb paralysis, which make the survival of the animals difficult. The other 6 animals died due to different causes that did not affect the cardiomyocytes, such as fights within the animals' enclosures and intense intestinal parasitosis. Those animals originated from the Center of Primatology of Rio de Janeiro (Centro de Primatologia do Rio de Janeiro, CPRJ-FEEMA) and were kept in outdoor enclosures, thereby being exposed to the natural conditions of the Atlantic Forest. Three walls of the enclosures were made of wire mesh, and the fourth was made of concrete to provide protection and avoid visual contact with other animals in the colony, reducing stress.

Food and water were provided twice daily, and the diet consisted basically of bread, banana, egg, seeds, meat, dietary supplements, and *Tenebrio molitor* larvae. Both the healthy and affected animals lived under those same management conditions.

After died in the colony, the animals underwent necropsy, were classified and became part of the collection of the Museum of Primatology of the CPRJ-FEEMA, (Coimbra-Filho *et al*. 1986). Their hearts were immediately weighed, dissected, and fixed in 10% formalin solution (pH 7.2). This study was approved by the Ethical Committee (CETA, Plínio Leite University Center - UNIPLI) and received the protocol number 001/04, although no animal was sacrificed. Several myocardial fragments were obtained, sent to the histology laboratory of the School of Veterinary Medicine (UNIPLI, Itaboraí, RJ), and were processed according to the method "orthotrip", because the cardiac muscle is an anisotropic tissue. This procedure allowed randomization, and anisotropic sections were obtained (Matfeld et. al. 1985).

The myocardial fragments were then embedded in paraffin, sectioned at 5ìm, and stained with Hematoxylin and Eosin (HE).

Stereology and statistical analysis

The stereological studies were carried out at the Morphology Laboratory of the Institute of Biosciences, (Unigranrio, Duque de Caxias, RJ).

Fifteen random fields were counted for each specimen, according to the M42 testing system, which was superimposed over the computer screen coupled to a photomicroscope. The M42 testing system consists of 21 line segments and 42 points in a testing area of 36.36 d^2 (Weibel 1979, Weibel et al. 1966). (Fig.1)

For the purpose of stereological quantification, the myocardium was considered as consisting of cardiomyocytes and cardiac interstitium. The volume density (V_V) was determined by points of the testing system lying above those structures, as long as they did not intercept the forbidden line or its extensions (Gundersen 1977, Matfeld et al. 1985). The V_V myocyte included the cardiomyocyte and its nucleus. The V_V vessels included the profile of vessels, and the V_V connective included the connective tissue and cells other than myocytes and nerves (Weibel 1979).

The volume densities (V_V) of myocytes, vessels, and connective tissue were calculated as follows:

$$Vv = \frac{Pp}{P_T}\ \% \qquad \text{Where } P_P \text{ is the number of points lying above structure, and } P_T \text{ is the total number of points of the testing system (in this case, 42 points).}$$

The numeric densities per area of the cardiomyocytic nucleus ($Q_{A[n]}$) and vessels ($Q_{A[v]}$) were calculated as follows:

$$L/\mu m^2, \text{ where } A_T \text{ is the testing area equivalent to } 36.36d^2 \text{ in the } M_{42} \text{ testing system.}$$

The length density of vessels ($L_V[v]$) was calculated as follows:

$$Lv = 2Q_A \qquad \mu m/\mu m^3$$

The surface density of cardiomyocytes ($S_V[m]$) and vessels ($S_V[v]$) was determined as follows:

$$Sv = \frac{2.I}{L_T} \qquad \mu m^2/\mu m^3, \text{ where I is the intersection of the myocardial components with the testing line, and } L_T \text{ is the length of the testing line (21.d in the } M_{42} \text{ testing system).}$$

The statistical differences between healthy and affected animals were tested by use of the Mann-Whitney *U*-test (á = 0.05) (Zar 1999).

RESULTS

The results are shown in Tables 1 and 2 and Figures 2 to 5. *Callithrix kuhlii*, as other primates, has a structure in the myocardium that is very common in mammals, a bundle of myocytes separated by an interstitium of connective tissue. In animals affected by WMS (Fig.3), a greater amount of interstitium among myocytes may be qualitatively observed when compared with that of healthy individuals (Fig.2).

Regarding the biometry performed in the animals, body weight was 63.43% lower in those affected by WMS, and a statistically significant difference was observed between the mean weights in healthy and affected animals (P = 0.003). (Table 1)

The cardiac weight in the animals affected by WMS was 65.7% smaller than that in the healthy animals, and their mean values also differed significantly (P = 0.016).

Considering the stereological parameters, the Q_A nucleus values in the affected animals were approximately 5.0% smaller than those in the healthy animals. The Q_A vessel values, however, were 5.5% greater in the affected animals, although they were not statistically confirmed (Table 2).

The V_V myocyte values of the *Callithrix kuhlii* studied are shown in Figures 4 and 5. The volume density of the myocyte, V_V myocyte, was 2.2% smaller in the affected animals, and that of the connective tissue, V_V connective, was 2.7% greater.

The volume density of the vessel, V_V vessel, was 50% smaller in the animals affected by WMS (Table 1). According to the Mann-Whitney test, the V_v vessel values were statistically significant when comparing healthy and affected animals (Table 2).

The S_V nucleus values were exactly the same in both groups, while the S_V vessel values were approximately 30% smaller in affected animals. Those values, however, were not statistically confirmed (Table 2). The L_V vessel value in the affected group was 3.43% greater than that in the healthy group.

Table 1. Descriptive statistics and comparisons of the biometric parameters studied in *Callithrix kuhlii* healthy and affected by Wasting Marmoset Syndrome (WMS)

Biometric parameters	Healthy	Affected	Mann-Whitney U-test	Significance p
Body weight (grams)	405.67±72.82	257.33±26.33	0.001	0.003
Cardiac weight (grams)	2.77±0.62	1.82±0.55	3.000	0.016
Age (grams)	4.0±3.48	5.58±2.54	12.50	0.378

Table 2. Descriptive statistics and comparisons of the cardiac parameters studied in *Callithrix kuhlii* healthy and affected by Wasting Marmoset Syndrome (WMS)

Biometric parameters	Healthy	Affected	Mann-Whitney U-test	Significance p
Q_A nucleus ($1/\mu m^2$)	64.30±25.57	61.16±15.13	16.00	0.748
Q_A vessel ($1/\mu m^2$)	29.15±16.30	30.86±10.95	13.50	0.470
V_V vessel (%)	1.0±0.5	0.5±0.2	5.00	0.034
V_V connective tissue (%)	30.8±5.8	33.5±5.0	13.00	0.423
V_V myocyte (%)	68.2 ± 5.2	66.0±5.1	15.00	0.630
S_V nucleus ($\mu m^2/\mu m^3$)	3.49±2.35	3.49±1.05	16.50	0.809
S_V vessel ($\mu m^2/\mu m^3$)	2.07±1.21	1.46±0.96	12.00	0.334
L_V vessel ($\mu m^2/\mu m^3$)	58.30±32.61	61.73±21.91	13.50	0.470

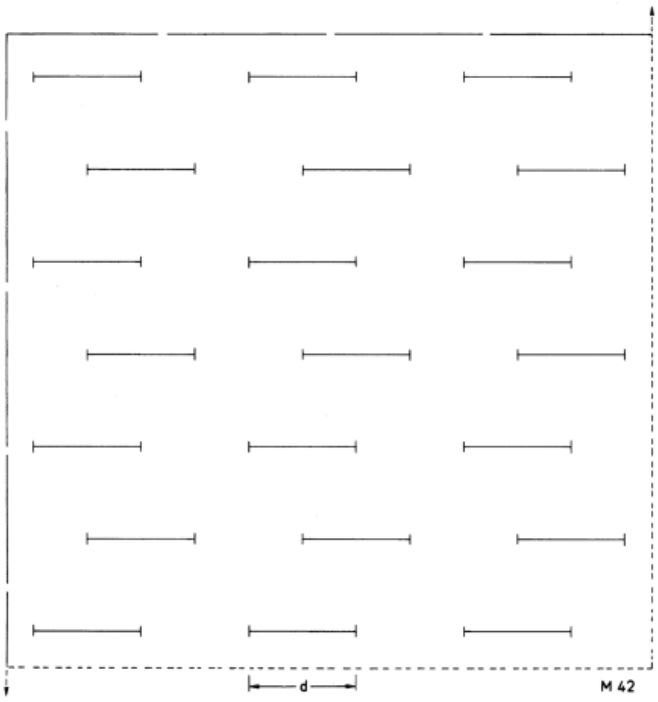

Fig.1.Diagram of the M42 testing system incorporating the forbidden line (dotted line). The elements intercepted by that line should not be considered to avoid the bias of over estimation (Gundersen 1977).

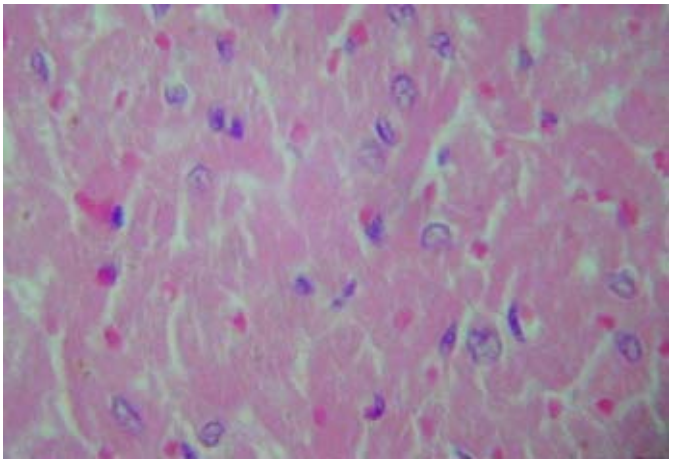

Fig.2. Heart muscle of a healthy *Callithrix kuhlii.* HE, obj.40.

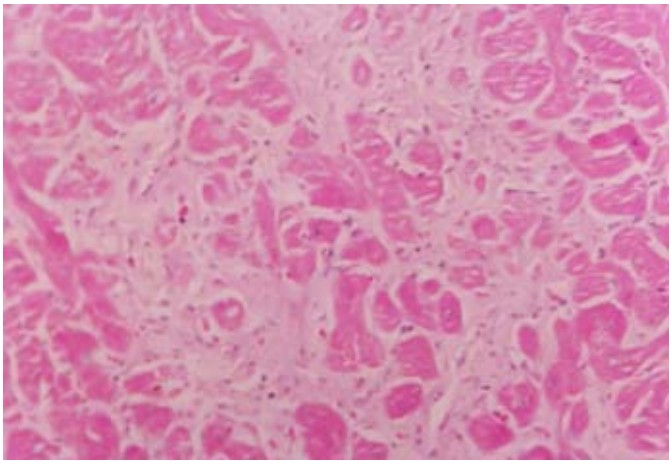

Fig.3. Heart muscle of by Wasting Marmoset Syndrome affected *Callithrix kuhlii*. HE, obj.20.

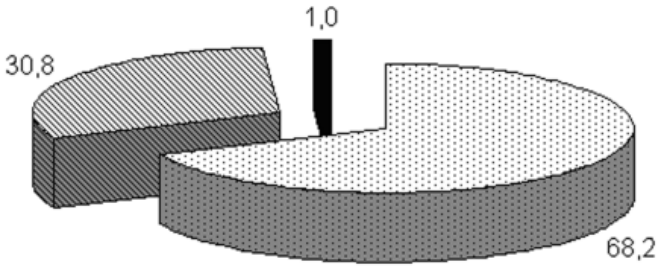

Fig.4. Graphs depicting the myocardial volume densities, V_V (%), for myocytes (dotted area), connective tissue (lined area), and vessels (solid color area) of the healthy animals.

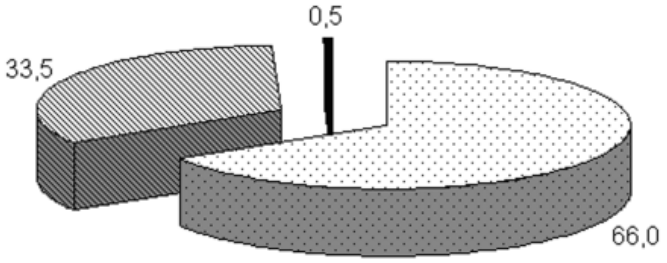

Fig.5. Graphs depicting the myocardial volume densities, V_V (%), for myocytes (dotted area), connective tissue (lined area), and vessels (solid color area) of by the Wasting Marmoset Syndrome (WMS) affected animals.

DISCUSSION

The relation between cardiac and body weights may not be a significant factor in WMS; however, the presence of cardiac interstitial fibrosis may render the heart vulnerable to ischemia.

The necropsy findings and microscopic confirmation of structural alterations, in several organs, even slight to moderate, contribute to the reduction in the metabolic efficiency in the affected primates.

Although the etiology of WMS is unknown, most changes in that disease have been attributed to protein malnutrition and muscle loss, which, according to Logan & Kanwar (1996) and Barnard et al. (1988), may be reverted with nutritional intervention. The size of the muscle fibers vary on the age and diet of the animals; therefore, in animals affected by WMS, inappropriate ingestion of nutrients and amino acids occurs, resulting in muscle dystrophy and inhibition of muscle growth (Gurtler et al. 1987). In regard to biometry, the differences in body weight, cardiac weight, and V_v vessel were statistically significant, confirming the muscle mass loss in affected animals. That syndrome is known to cause exacerbated weight loss. According to our findings, a decrease in the volume density of the vessels occurs, which we suppose may result in a decreased blood flow to the myocardium and consequent general decrease in myocardial mass and metabolism of cardiac cells. Such biometric differences could not be related to differences in the animals' ages, because statistically significant differences were not detected by the Mann-Whitney test for the parameter "age".

Although not statistically confirmed ($p > 0.05$), in animals affected by WMS, Q_A showed a decrease in the number of nuclei per unit of area, as well as a lower volume density of myocytes (V_V myocyte). This means that the myocardium had a numerical and volumetric reduction in cardiomyocytes. Such numerical trend was found.

Stereology of the myocyte showed that animals with WMS had the same number of myocytes, whose sizes, however, were smaller than those in healthy animals. However, with the significance level adopted, those data were not confirmed.

Burity et al. (1996) studied the myocardium of *Callithrix jacchus* and *C. penicillata*, and the following V_V values were established for the myocardium: V_V myocyte = 68.6%, and V_v connective tissue = 31.45%. These values are similar to those obtained in the present study for *Callithrix kuhlii* within the same group, the "Jacchus" group.

Pissinatti et al. (2003) studied the myocardium of 4 taxa of *Leontopithecus* (*L. rosalia*, *L. chrysomelas*, *L. c. chrysopygus*, and *L. c. caissara*), and reported that V_V myocyte varied from 79.6 to 88.4, while the V_V connective tissue 10.2 to 17.4. These values are important for comparison with those of this study and are similar because the animals, although from different species, belong to the same family, Callitrichidae.

Rodrigues (2003) reported that dogs undergoing cardiac denervation, which causes physiological alterations, had an increase in L_V vessel and a decrease in V_V vessel, as compared with a group of dogs that did not undergo cardiac denervation. These alterations may be compared with those found in animals affected by WMS as compared with healthy animals.

Nowadays, this is the first study to correlate body mass loss and cardiac stereology in animals with WMS. Therefore, the inexistence of previous results makes the comparison with ours difficult.

In this study, morphometry (biometry and stereology) showed that WMS affects animals, including their myocardium, generating a significant reduction in body weight and cardiac weight, and a reduction in the volume density of vessels. Such facts, although difficult to understand, may be important in the differential diagnosis with other kinds of disease. Further studies are required.

CONCLUSIONS

• Wasting marmoset syndrome caused body mass loss in affected animals. It also caused a decrease in the cardiac mass in affected animals and a significant decrease in the volume density of vessels in the myocardium.

• This condition may render the heart vulnerable to ischemia due to the metabolic decline of cardiac muscle cells.

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