

**WASTING SYNDROME IN *Saguinus martinsi* ("MARTIN'S BARE-FACE TAMARIN")-CALLITRICHIDAE – PRIMATES: CASE REPORT**

[Síndrome do emagrecimento progressivo em *Saguinus martinsi* (Callitrichidae – Primates): relato de caso]

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**RESUMO** - O termo síndrome do emagrecimento progressivo em saguis foi inicialmente usado para descrever o emagrecimento e enfraquecimento dos primatas recém-desmamados. Subsequentemente o termo expandiu e passou a incluir saguis de qualquer idade. O caso relatado neste artigo foi observado em uma fêmea de nove anos de *Saguinus martinsi* da colônia do Centro de Primatologia do Rio de Janeiro, Brasil. O sagui apresentou aparecimento súbito de diarreia, fraqueza muscular e rápida perda de peso, o que está de acordo com o escrito nos casos da síndrome do emagrecimento. Foram realizados exames parasitológicos, clínicos, patológicos e histopatológicos buscando evidenciar as causas da doença. Concluiu-se que as lesões histopatológicas observadas nos rins, pulmões, intestinos e fígado foram determinantes do emagrecimento progressivo e com base na literatura consultada presumimos ser o primeiro caso da patologia relatada em primatas da espécie *Saguinus martinsi*.

**Palavras-Chave:** Síndrome do emagrecimento progressivo, sagüis, *Saguinus martinsi*.

**ABSTRACT** - The term wasting marmoset syndrome was initially used to describe the generalized weakness in young marmosets recently weaned. Subsequently, it was expanded to include all callitrichids in all ages. The case related in this study was observed in a nine years old female of *Saguinus martinsi* of the Primatology Center of Rio de Janeiro, Brazil. The tamarin presented sudden symptoms of intermittent diarrhea, weakness and marked weight loss resembling the wasting syndrome. Parasitological, clinical, pathological, bacteriological and histopathological aspects were analyzed. We conclude that the disease was probably the first case in Martin's Bare-face Tamarin and that it was characterized by lesions observed in the histopathology of the kidneys, liver, intestines and lungs.

**Keywords:** Wasting marmoset disease, *Saguinus martinsi*, Martin's Bare-face Tamarin.

## INTRODUÇÃO

Marmoset wasting disease ("wasting marmoset syndrome - WMS") is a devastating problem amongst marmosets and tamarins, since it may spread out on a colony causing devastating losses. Symptoms include weight loss in spite of a voracious appetite, emaciation, muscular weakness, coordination disorders progressing to paralysis of the hindquarters, chronic non-responsive diarrhea, lethargy, rough hair coat, and hair loss on the tail (Gatesman, 1997; Wiisman, 2005). According to Ialegio & Baker (1995), weight loss and diarrhea are the most commonly listed signs. WMS is considered as a major death cause in Callitrichid colonies (Ialegio & Baker, 1995). Main signs are weight loss, alopecia, muscle atrophy, anemia, chronic

diarrhea, weakness and paralysis, and wet greasy fur.

Although various possible causes have been raised for this syndrome, including virus, bacteria, autoimmune disease, nutritional deficiencies or excesses, parasites, stress conditions and various others proposed etiologies like gastrointestinal infections, nephritis, pancreatitis and chronic colitis, the real etiology is still unclear (Wiisman, 2005).

The purpose of this study was to describe the occurrence of a case of WMS in *Saguinus martinsi* and to suggest possible etiologies for this case.

## CASE REPORT

In July of 2004 a nine years old female "Martin's Bare-face Tamarin" (*Saguinus martinsi*) developed

symptoms resembling WMS. Typical signs like feces-smear perineum and hind leg associated with an intermittent diarrhea progressing to cachexia were observed. The animal came to death within four weeks. The gross appearance of the feces presented in the diarrhea was variable ranging from soft to watery, often pale and containing undigested food. No mucus or blood were observed in the feces and, according to the criteria described Ettinger & Feldman (2000), it was classified as originated in the small intestine.

After the detection of the first symptoms, the animal was submitted to a special diet without milk and with an increase in fruit and insect offering for seven days, which did not improve its general condition. Rectal feces and blood from femoral vein were collected and antibiotic therapy with Trimethoprim/sulphonamide associated to enrofloxacin for five days was instituted, with no clinical improvement. Therefore, gentamycin was administered for one week, associated to probiotics (*Saccharomyces cerevisiae*; CHR. Hansen Ind.). Clinical aspect of the animal did not improve and it continued losing weight. Four weeks after the beginning of the symptoms the animal died.

In order to understand the reasons and the most probable etiology of the WMS, first of all we analyzed the possible influence of general causes related to dietary and stress, since those are the most frequently reported causes (Gatesman, 1997; Wormell, 2000; Foster 2001). It is important to notice that no changes were observed in diet, captivity environment or cage mate, and no capture/handling either intra or interspecies tensions. Therefore, we concluded that the primate in this study did not suffer dietary or environmental stresses that could justify the occurrence of the syndrome, what was confirmed by the presence of a healthy male partner kept in the same cage, under the same management conditions. Therefore, several laboratory tests were conducted in order to elucidate the etiology of the WMS in this tamarin, as described:

**Parasitology** - Samples of feces were collected in order to identify endoparasites in parasitological exams according to methods of Faust et al (1938) and Ritchie modified by Young et al. (1979).

**Bacteriology** – The presence of virulent strains of *Escherichia coli* were also investigated. In this study, a sample of feces was collected from the rectum using a sterile swab and a multiplex PCR assay was performed to simultaneous amplification of *eae*, *stx1* and *stx2* genes using specific primers as previously described (China et al. 1999). The identity of DNA amplicons were compared with positive controls included in each reaction and at least two reactions were conducted in order to confirm the results.

Samples of small and large intestines were also processed for *Mycobacterium* sp. recovery. The OIE protocol (World Organization for Animal Health, 2004) was used and the samples were inoculated onto slopes of Herrold's egg yolk agar with mycobactin J (Allied Monitor), with and without antibiotics for *Mycobacterium avium* paratuberculosis growth and onto slopes of Lowenstein-Jensen medium enriched with pyruvate for other *Mycobacteria* growth.

**Serology** - Taking into account the presence of rodents in the nearest area of the Center of Primatology, we also investigated the possibility of Leptospirosis. The serum of the animal was separated by centrifugation and tested for specific antibodies by the microscopic agglutination test (MAT) at a dilution of 1:50 to *L. interrogans* serovars Australis (Ballice), Bataviae (Van Tienen), Canicola (Hond Utrecht IV), Icterohaemorrhagiae (RGA), Copenhageni (M 20), Pomona (Pomona), Pyrogenes (Salinem) and Wolffii (3705), *L. borgpetersenii* serovar Ballum (Mus 127) and *L. kirshneri* serovar Grippotyphosa (Moskva V), grown in Ellinghausen liquid medium (EMJH).

**Haematology and Biochemistry** - Blood samples were collected for both haematology and clinical chemistry. Considering the weakness of the tamarin, we collected a small aliquot of blood, just sufficient for determination of glucose, urea and creatinine in clinical chemistry panel. The haematological evaluations included white blood cells count (total and differential), red blood cells count, haemoglobin, and globular volume, mean corpuscular volume and mean corpuscular haemoglobin concentration.

**Anatomopathology** – A post-mortem exam was carried out and sections of several organs were removed for histology. Samples were kept in

10% formaldehyde and processed by standard histological techniques. The samples were embedded in paraffin, and after cutting (5.0µ sections) and drying they were stained with Haematoxylin-Eosin, and microscopically analyzed.

Parasitological exams were negative for either helminthes or protozoa. The post-mortem macroscopic and microscope evaluation of the intestines confirmed the absence of these parasites.

Thus, infection by *Trichospirura leptostoma*, a parasite known to live in the pancreas of marmosets and frequently reported as a cause of WMS (Pfister et al, 1990; Wiisman, 2005) was discharged in this study. This parasite have already been detected in *Callithrix jacchus* and *Callithrix penicillata* in an area near to the Center of Primatology (Vicente et al., 1997) and also in others regions in Brazil (Resende et al., 1994). Nevertheless, in the present case neither eggs were found in the feces nor damaged pancreatic ducts were observed in the histopathologic examinations.

Stx and eae are representative virulence genes of enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *Escherichia coli* pathotypes which has been isolated from intestinal tract of humans and some other animal species including marmosets (Carvalho et al, 2003, Almeida et al., 2003). The amplicons were not found in the sample analyzed and we conclude that those bacteria cannot be considered as the cause of the observed symptoms. Negative results in Mycobacteria cultures indicated that neither paratuberculosis nor other mycobacteriosis could be incriminated as the cause of the observed case of WMS.

The anti-*Leptospira* antibody titres of all samples with agglutinating activity were below to 1:100 dilutions. Therefore, we concluded that no serovar could be considered infective and that leptospirosis may not be considered as a cause of the observed syndrome.

In relation to haematological and biochemical analysis, data are depicted in Table 1.

Although reference values are suggested for arious species of tamarins and include a wide range of values, we could conclude that there was not significant reflex of the disease on the haematological picture. An important finding was the hypoglycaemia, which can be related to the low absorption of nutrients by the intestinal

mucosa.

**Table 1** – Hematological and chemical data of a *Saguinus martinsi* with wasting marmoset syndrome (WMS) in the reported case.

	<i>Saguinus martinsi</i> with WMS	Reference values.*
Globular Volume (%)	36	38.3 - 55.8
Hemoglobin (g/dL)	10.7	12.3 - 17.6
Eritrocyte (x10 <sup>9</sup> /mm <sup>3</sup> )	5.2	5.20 - 7.01
MCV (fL)	68	70.0 - 84.0
MCHC (%)	29,7	30.5 - 33.3
Leukocytes (x10 <sup>3</sup> /mm <sup>3</sup> )	14	5.4 - 16.7
Segmented neutrophils (x10 <sup>3</sup> /mm <sup>3</sup> )	7.98	1.5 - 12.2
Bands (x10 <sup>3</sup> /mm <sup>3</sup> )	0.14	0.0 - 0.1
Lymphocytes (x10 <sup>3</sup> /mm <sup>3</sup> )	4.2	0.8 - 5.7
Monocytes (x10 <sup>3</sup> /mm <sup>3</sup> )	7	0.0 - 1.6
Eosinophils (x10 <sup>3</sup> /mm <sup>3</sup> )	0.98	0.0 - 0.5
Basophils (x10 <sup>3</sup> /mm <sup>3</sup> )	0.0	0.0 - 0.5
Glucose (mg/dL)	60	75 -1 69
Urea (mg/dL)	12	10 - 32
Creatinine (mg/dL)	0,4	0.5 - 0.9

\*Values are from The New England Regional Primate Research Center (Savage, 1995)

Although reference values are suggested for various species of tamarins and include a wide range of values, we could conclude that there was not significant reflex of the disease on the haematological picture. An important finding was the hypoglycaemia, which can be related to the low absorption of nutrients by the intestinal mucosa.

The bone marrow cytology showed moderated myeloid hyperplasia, mild megakaryocytic and lymphoplasmacytic hyperplasia and increased iron stores in the marrow. Increase in myeloid series is often related to inflammatory, immune-mediated or neoplastic diseases (Harvey, 2001). Hyperplasia of lymphoid series, as observed in the present case, can also be caused by immune-mediated diseases and antigenic stimulation of infection and/or inflammation diseases. These results were compatible with the histopathology findings, which revealed inflammatory process in several organs.

The increase in iron stores in the marrow observed in this case is probably related to a sequestration of this element into macrophages observed in inflammatory conditions (Harvey, 2001).The most prominent lesions in the lungs were congestion of alveolar capillaries, atelectasis, emphysema, degeneration and/or necrosis in some areas. It could be concluded that these lesions contributed to the development of the sickness and subsequent death. No significant histologic lesions were observed in the heart, adrenal and thyroid glands, pancreas or spleen.

The histopathology of the kidneys revealed diffuse vacuolar renal degeneration, chronic and diffuse interstitial nephritis and multifocal renal necrosis. In this case, the lesions were more severe those cited by Brack & Rothe (1981) in *Callithrix jacchus*. In the liver the lesions were diffuse congestion peri-portal hepatitis with infiltration of mononuclear (lymphocytic-plasmacytic) inflammatory cells. The large intestine presented lymphoid reactive follicles and in the small intestine signs of enteritis were observed.

Although we could not observe signs of the colitis as cited by Morin (1983) and by Logan & Khan (1996), the observed lesions could justify the chronic diarrhea in the subject animal, probably caused by malabsorption or increased secretion of water and ions into the small intestine lumen. In agreement to Ettinger & Feldman (2000) the preservation of mucosa, both epithelial cells and tight junctions enabled us to conclude that no viral or bacterial process was involved in the enteropathy. Those findings are compatible with the bacteriological culturing results.

## CONCLUSION

In the present case, we observed chronic diarrhea and weight loss condition associated with renal, hepatic, and pulmonary lesions, sufficient conditions to refuse the hypothesis that wasting marmoset disease occurred due to common poor husbandry and nutritional level.

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## REFERÊNCIAS

Almeida PMP, Silveira RH, Vargas TLZ, Joaquim RM, Andrade JRC, Cerqueira AMF. 2003. Ocorrência e caracterização de *Escherichia coli* produtora da lesão “Attaching And

Effacing” (Aeec) em animais no Estado do Rio de Janeiro In. Anais do XXII Congresso Brasileiro de Microbiologia, Florianópolis-Brazil. 2003.

Brack M, Rothe, H. 1981. Chronic tubulointerstitial nephritis and wasting disease in marmosets (*Callithrix jacchus*). *Vet Pathol* 18:45-54.

Carvalho VM, Gyles CL, Ziebell K, Ribeiro MA, Catão-Dias JL, Senhorini IL, Otman J, Keller R, Trabulsi LR, Pestana De-Castro, AF. 2003. Characterization of Monkey Enteropathogenic *Escherichia coli* (EPEC) and Human Typical and Atypical EPEC Serotype Isolates from Neotropical Nonhuman Primates *J Clin Microbiol* 41:1225-34.

China B, Goffaux F, Pirson V, Mainil J. 1999. Comparison of eae, tir, espA and espB genes of bovine and human attaching and effacing *Escherichia coli* by multiplex polymerase chain reaction. *FEMS Microbiol Letters* 178:177-182

Ettinger SJ; Feldman EC. 2000. Textbook of Veterinary Internal Medicine – Diseases of the dog and cat, v. 1. 5<sup>ed</sup>. WB Saunders: Pennsylvania

Faust EC, D'Antoni JS, Odon V, Miller MJ, Perez C, Sawitz W, Thomen LF, Tobie J, Walker JH. 1938. A critical study of clinical laboratory technics of the diagnosis of protozoan cysts and helminthes eggs in feces. I - Preliminary communication. *Am J Trop Med* 18:169,

Foster K. 2001. Diet and Nutrition of pied Tamarins *Saguinus bicolor bicolor*, at Jersey Zoo. *Lab Anim* 22:300-331

Gatesman TJ. 1997. Veterinary care of Callitrichidae: a short summary. In: Marmosets and Tamarins in Biomedical Research. Proc. Workshop European Marmoset Research Group PRYCE et al Eds., DSSD Imagery: Salisbury, UK p. 78-101.

Harvey JW. 2001. Atlas of veterinary hematology: blood and bone marrow of domestic animals. Philadelphia: W. B. Saunders Company. 228p.

Ialegio M, Baker AJ. 1995. Results of a preliminary survey into wasting marmoset syndrome in callitrichid collections. Proceedings of the First Annual Conference of the National Advisors Group of the American Zoo & Aquarium Association p. 148-158.

- Logan AC; Khan, KNM. 1996. Clinical pathologic changes in two marmosets with wasting syndrome. *Lab Anim Pathol* 24:707-709
- Morin ML. 1983. A different approach in examining a wasting syndrome. *Lab Anim* 12:36-41,
- Pfister R, Heider K, Illgen A, Berlinger R. 1990. *Trichospirura leptostoma*: a possible case of wasting disease in marmoset. *Z Versuchstierkd* 33:157-161
- Resende DM, Pereira LH, Melo AL, Tafuri WL, Moreira NIB, Oliveira CL. 1994. Parasitism by *Primasubulura jacchi* (Marcel, 1857) Inglis, 1958 and *Trichospirura leptostoma* Smith and Chitwood, 1967 in *Callithrix penicillata* marmosets, trapped in wild environment and maintained in captivity. *Memorias do Instituto Oswaldo Cruz* 89:123-125
- Sainsbury AW, Cunningham AA, Hawkey CM, Garden OA. 1993. Conditions causing loss of weight in marmosets. *Proc. Symp Assoc Br Wild Anim Keepers* 17, 16-22
- Savage A. 1995. *The Cotton-top Tamarin Husbandry Manual*, Roger Williams Park Zoo, Providence, RI.  
<http://www.csew.com/cottontop/SSP/Enghome.htm> [Accessed 26 October 2005]
- Vicente JJ, Rodrigues HO, Gomes DC, Pinto RM. 1997. Nematóides do Brasil. Parte V: nematóides de mamíferos. *Revista Brasileira de Zoologia* 14:161-162
- Wiisman, M. 2005. Marmoset Wasting Disease: A Cause Found At Last?  
<http://www.monkeyzone.com/wastingdisease.html>. [Accessed 2 September 2005]
- World Organization for Animal Health. 2004. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*.  
[http://www.oie.int/eng/normes/mmanual/A\\_00045.htm](http://www.oie.int/eng/normes/mmanual/A_00045.htm) [Accessed 26 September 2004]
- Wormell, D. 2000. Management and Guideline for Pied Tamarins *Saguinus bicolor bicolor*. *Dodo* 36:87
- Young KH, Bullock SL, Melvin DM, Spruill CL. 1979. Ethyl Acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. *Journal of Clinical Microbiology*. 10:852-853