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Measuring of the salivary immunoglobulin in *Callithrix jacchus* primates in captivity running title: salivary immunoglobulin in marmoset[1]

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ABSTRACT.- Ferraz M.C., Matos A.V.R., Ferreira J.C.P., Queiroz C.M. & Oba E. 2024. **Measuring of the salivary immunoglobulin in *Callithrix jacchus* primates in captivity running title: salivary immunoglobulin in marmoset.** *Pesq. Vet. Bras. 44:e07401, 2024.* Departamento de Reprodução Animal e Radiologia Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Distrito de Rubião Júnior s/n, Cx. Postal 560, Botucatu, SP 18618-970, Brazil. E-mail: myrnacferraz@gmail.com

The primary antibody in the mucous membranes and exocrine glands is a polymetric secretory immunoglobulin A. This immunoglobulin has been used as an indicator of stress in various animals, including nonhuman primates, and can also be used to monitor immune activity. The secretory immunoglobulin A is predominantly found in seromucous secretions such as saliva, tears, colostrum, milk, and liquor, as well as tracheobronchial, intestinal, and genitourinary secretions. This study aimed to measure the salivary IgA levels in *Callithrix jacchus* (common marmoset) by the enzyme-linked immunosorbent assay test (ELISA). Twelve pairs of animals were used, previously conditioned by the operant conditioning technique with positive reinforcement to saliva collection. Samples were collected once a week for six months. In this experiment, the salivary secretory immunoglobulin A concentrations in *Callithrix jacchus* were very low. We suggest new studies using other techniques to quantify the IgA quantities in the saliva of these animals.

INDEX TERMS: Immunoglobulin A, salivary, *Callithrix jacchus*, marmoset, measurement.

RESUMO.- [**Medição de imunoglobulina salivar em primatas *Callithrix jacchus* em cativeiro: imunoglobulina salivar em sagui.**] O principal anticorpo presente nas mucosas e glândulas exócrinas é uma IgA polimétrica denominada sIgA. Esta imunoglobulina vem sendo utilizada como indicadora de estresse em diversos animais incluindo primatas não humanos, podendo também ser utilizada para o monitoramento da atividade imunológica. A imunoglobulina A secretora é encontrada predominantemente em secreções seromucosas, como saliva, lágrimas, colostro, leite, liquor, bem como secreções traqueobrônquicas, intestinais e geniturinárias. O objetivo deste estudo foi mensurar os níveis da IgA salivar em *Callithrix jacchus* (sagui comum) através do teste de imunoabsorção enzimática (ELISA). Doze pares de animais foram previamente condicionados pela técnica de condicionamento operante com reforço positivo à coleta de saliva. As amostras foram coletadas uma vez por semana por seis meses. Neste experimento as concentrações de imunoglobulina salivar A secretora em *Callithrix jacchus* foram muito baixas. Nós sugerimos novos estudos utilizando outras técnicas para quantificar a IgA na saliva desses animais.

TERMOS DE INDEXAÇÃO: Imunoglobulina A, saliva, *Callithrix jacchus*, sagui, medidas.

INTRODUCTION

Callitrichids are used in research that evaluate aspects related to the development of diseases and physiologic parameters (Clarke 1994), aside from being experimental models for nutrition, pharmacology, toxicology, and stress studies (Abbott et al. 2003). Using the common marmoset (*Callithrix jacchus*) in studies, Johnson et al. (1996) showed that this species presents several characteristics that make it a model for social stress studies, for example, the phylogenetic proximity to humans and the fact that this species displays physiologic

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and behavior responses similar to humans in anxiety-inducing situations (Barros & Tomaz 2002).

Immunoglobulin A (IgA) was first identified in 1959 by Heremans, and it is present in all mammals' serum and organic fluids. It represents 15 to 20% of immunoglobulins and is divided into subclasses, IgA 1 (90%) and IgA 2 (10%) (Heremans et al. 1959).

Immunoglobulin A plays an important role in neutralizing and eliminating local antigens (Stokes et al. 1975) and modulating immune factors, aside from being studied as a stress indicator in several animals, including chicken and primates (Pearson 1998, Florence et al. 1999, Carver & Hau 2000). The main biological function of IgA is the protection against invading microorganisms, such as viruses and bacteria, on the mucous surfaces, inhibiting their adherence mechanism to epithelial cells (Turner & Owen 1991, Granger et al. 2007, Peterson & Loring 2014).

The measurement of secretory IgA in conjunction with the other physiological biomarkers is another study tool to correlate physiological stress with immune response and overall health. Monitoring SIgA together with glucocorticoid monitoring may be useful in better understanding the results of studies involving health and welfare. Thus, SIgA may be an important tool for evaluating the responses of various species to a new environmental enrichment, as well as to new social partners and a variety of challenges encountered in captivity (Staley et al. 2018).

IgA does not fixate complement; because of that, it can act against microorganisms without starting the inflammatory process cascade, which damages the surface of epithelial cells. Besides that, it promotes a lowering in absorption of a variety of antigens of allergenic agents, inhaled or ingested, which can cause immune responses (Schäfer et al. 1999).

Secretory IgA (SIgA) is the dimeric form of IgA, bound to another protein named secretory piece, produced by epithelial cells. This component facilitates the transport and impedes the proteolytic action on IgA, making it an important defense mechanism of the mucosa. The SIgA is predominantly found in seromucous secretions such as saliva, tears, colostrum, milk, and liquor, as well as tracheobronchial, intestinal and genitourinary secretions (Turner & Owen 1991, Bakema et al. 2006).

MATERIALS AND METHODS

Animal Ethics. All procedures were registered on the System for Authorization and Information on Biodiversity according to the "Instituto Chico Mendes de Conservação da Biodiversidade" (SISBIO-ICMBIO Protocol No. 24055-1) The experiment had the approval of the Ethics Committee on Animal Use (CEUA) of the "Universidade Estadual Paulista 'Júlio de Mesquita Filho'" (Unesp), Botucatu/SP, Brazil, warrant number 126/2010.

This study was performed in the Wildlife Medicine and Research Center of the "Faculdade de Medicina Veterinária e Zootecnia" (FMVZ), Unesp, Botucatu/SP, Brazil, 22°53'09" S, 48°26'42" W, with an annual mean temperature of 22°C.

Twelve adult *Callithrix jacchus* couples aged between 3 and 7 years were used. The animals had either been apprehended by authorities from illegal owners and transferred to the "Parque Ecológico do Tietê", São Paulo/SP, Brazil (6 males, 6 females), or had been housed at the "Parque Zoológico Municipal Quinzinho de Barros", Sorocaba/SP, Brazil (6 males, 6 females). The animals were housed in cages measuring 60cm height x 120cm width x 60cm depth, kept in natural conditions of temperature, humidity and light-dark cycle. The animals used were born in captivity and were already being kept in cages of the same size before the experiment and under the same conditions of diet and temperature before, during and after the study. Two meals were offered per day, composed of fruits, vegetables and eggs in the morning and a protein paste in the afternoon (ground Alcon® feed with banana, honey and Sustagem®). As a complement, mealworms (*Tenebrio molitor*) were offered once a week.

The animals were conditioned using instrumental conditioning with positive reinforcement to the collection of saliva before the beginning of the experiment (Ferraz et al. 2013) (Fig.1).

Saliva collections were performed once a week, always in the afternoon (3:00-4:00 p.m.), for six months in the warmer months, from September to February. After collecting, the swabs used were placed in Eppendorf® tubes and stored in a -20°C freezer until the moment of dosing.

Before dosing, the 350 samples were centrifuged at 2.500G for 10 minutes so that the saliva would concentrate on the bottom of the Eppendorf® tube.

The samples were measured in duplicate. The measurement of IgA was performed using the enzyme-linked immunosorbent assay (ELISA) by Alpha Diagnostic International, Texas, USA, for IgA detection in nonhuman primates. This kit is based on the IgA antibody bindings to two samples, one immobilized in a microtiter, and the horseradish peroxidase (HRP) conjugates. Later, a washing stage was added, and the chromogenic substrate and color were evidenced by the HRP enzyme reaction on the substrate, which is directly proportional to the amount of IgA in the samples. The absorbance reaction was measured using the ELISA microtiter reader with a 450nm filter. The IgA concentration on the samples and controls was calculated from a standard curve with known concentrations of the immunoglobulin.

The first dosages revealed very low values of IgA, and an adjustment of the curve was necessary (dilution of the standards x50).

A dose of 10µl from each sample was used at a 1:10 dilution. The assays were performed according to the manufacturer's protocol (Alpha Diagnostic International, Texas, USA).

RESULTS AND DISCUSSION

Under the conditions of this study, the kit was not effective as to measure the IgA in the saliva of the *Callithrix jacchus.*

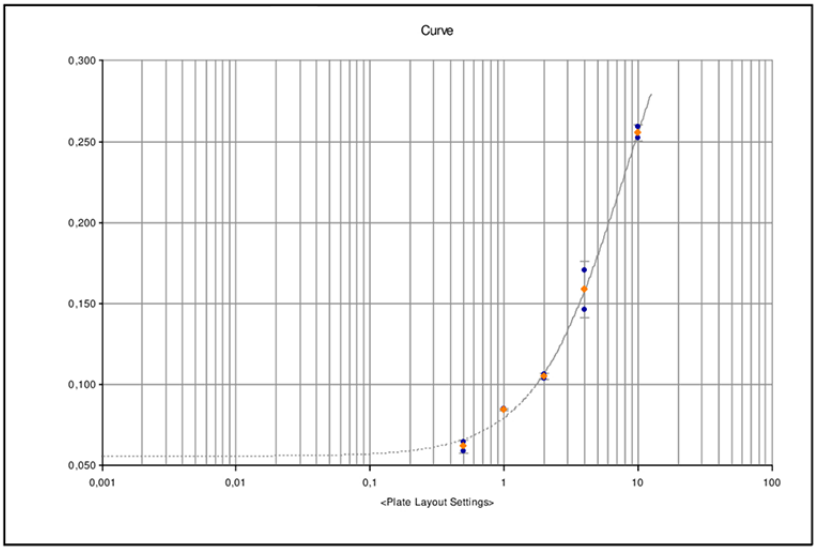
Fig.1. Saliva collection in *Callithrix jacchus* after conditioning.

The concentrations in the samples were low, and most were outside the curve (Fig.2). Even after the adjustment, only 52 of the 350 samples dosed were inside the curve (Fig.3).

The concentrations given by the commercial kit for old-world nonhuman primates in different dilutions (1:60K and 1:240K) varied from 432ng/mL to 50ng/mL, much lower than the ones reported in other species. This can be linked to the low volume of saliva obtained in the samples, which can be related to the small size of the individuals or the fact that the protein structure is different among species.

The same result was observed by Higham et al. (2010). These authors also used ELISA as a method for measuring IgA in non-human primates. In this study, no rhesus samples responded to the SIgA assay at any concentration. Human saliva responded to the assay as anticipated. These authors, as well as our study, demonstrate that it is the rhesus saliva itself that is unsuitable for the assay.

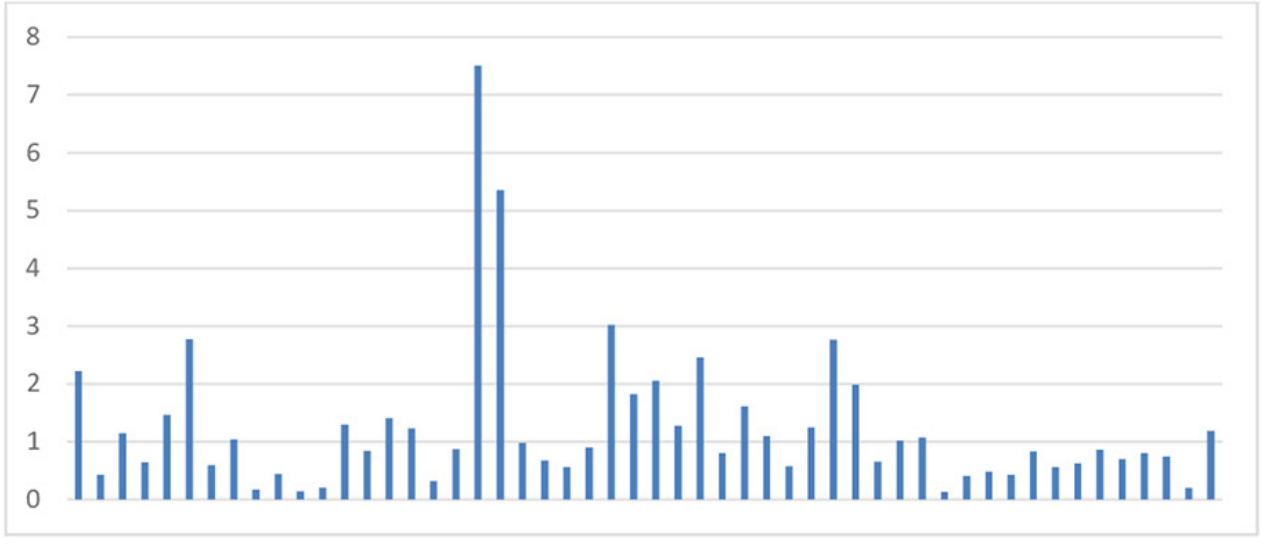
The secreted SigA quantification in the saliva can be determined by using its concentration or secretion rate in clinical and laboratory trials. However, the concentration is



Curve Name	Curve Formula	A	B	C	D	R2
Curve	Y = (A-D)/(1+(X/C)^B) + D	0.0561	1.25	8.32	0.414	0.998

2

Fig.2. Adjusted curve obtained in the assay measuring the salivary IgA of *Callithrix jacchus.*



3

Fig.3. Salivary IgA concentrations (ng/mL) of common marmosets in the 52 samples inside the curve.

relative to the solvent and the amount of saliva produced, or salivary flow, directly influencing SIgA concentrations. There could even be a negative correlation between these values (Ganhao et al. 1984), which generates doubts about the interpretation of the results that use concentration as a parameter (Herbert & Cohen 1993). According to Wicher & Fries (2010), the structure of proteins such as haptoglobulin varies between species, even close ones, probably due to evolution. This structural difference between the molecules could explain the low affinity of salivary IgA from these primates with the antibodies in the kit used. Staley et al. (2018) suggested that whether a commercial antibody can be applied to a species other than the species against which it was produced will depend on cross-reactivity, as determined by amino acid and structural conservation. This will influence both the detectability of IgA and the ability to discern changes in IgA concentrations. In cases where commercially available antibodies show weak signals or no cross-reactivity to the IgA of the species of interest, there are avenues to produce custom detection antibodies.

Another factor that could be responsible for the low readings in the assay is that salivary IgA concentrations in this species' saliva are lower than those seen in old-world primates. Diniz & Da-Costa (1995), in a study using clinical and laboratory data from 265 *Callithrix jacchus* in captivity, suggested that one of the greatest problems seen in these animals is pneumonia. There is a connection between stress and respiratory tract infections (Cohen et al. 1993, Bellar et al. 2017). Over 95% of infections begin on the mucous surfaces (Bosch et al. 2002). Such surfaces are protected by immunoglobulins, mainly IgA (Garret & Kidd 1976). Thus, the low IgA concentrations in the saliva could justify the high occurrence of pneumonia in *C. jacchus* colonies in captivity; besides that, animals kept in zoos and conservation and research centers frequently undergo challenging situations that could also justify the low levels seen in this study. According to Garret & Kidd (1976), psychological stress could cause a lowering in IgA concentrations in the saliva, increasing susceptibility to infections.

In general, acute stress is associated with increases in salivary IgA, whereas prolonged stress has been linked to decreases in salivary IgA (Engeland et al. 2019).

CONCLUSION

In this study, salivary IgA concentrations in primates *Callithrix jacchus* were very low. This species has been used as a biological model in several studies. IgA immunoglobulin is important in checking the status of the immune system and as an indicator of stress in various animals, including non-human primates. Given this, we suggest new studies using other techniques for the quantification of IgA present in the saliva of these animals.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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