

## Some Aspects of the Ascarid Parasitism in Rattlesnakes (*Crotalus durissus*)

Castro RA<sup>1</sup>, Marigo J<sup>2</sup>, Loibel SMC<sup>3</sup> and Kathleen Fernandes Grego<sup>1\*</sup>

<sup>1</sup>Laboratory of Herpetology, Instituto Butantan, Avenida Vital Brazil, São Paulo, SP, Brazil

<sup>2</sup>Department of Pathology, Faculdade de Medicina Veterinária e Zootecina, Universidade de São Paulo (USP), São Paulo, Brazil

<sup>3</sup>Universidade Estadual Paulista, DEMA/IGCE, Campus Rio Claro, Rio Claro, SP, Brazil

**\*Corresponding Author:** Kathleen Fernandes Grego, Laboratory of Herpetology - Instituto Butantan, Avenida Vital Brazil, São Paulo, SP, Brazil.

**Received:** August 24, 2020; **Published:** October 09, 2020

**DOI:** 10.31080/ecve.2020.05.00325

### Abstract

**Background:** Snakebite accidents are a public health problem in tropical countries and are listed as a tropical neglected disease by the World Health Organization (WHO). Annually, around 20,000 snakebites occur in Brazil and although the species *Crotalus durissus* is responsible for only 10% of the registered snakebite accidents, it has the highest fatality rate (around 1%). The maintenance of snakes in captivity has become an alternative for obtaining venom for the production of antivenom serum and immunological researches since the 20th century. Snakes coming from nature are usually subjected to various stressors related to captivity and their health conditions can worsen if simple prophylactic management, as deworming, are not performed.

**Objectives:** This study aimed to determine some aspects of the parasitism by ascarids nematodes in *C. durissus* specimens; correlate the findings with corticosterone plasma levels; and determine if parasitized animals are more stressed than non-parasitized ones.

**Methods:** A total of 12 rattlesnakes with and without ascarids had their blood withdrawn to evaluate biochemical and hematological parameters, and to determine plasma corticosterone.

**Results:** Snakes with ascarids presented higher corticosterone basal values, than dewormed animals, showing they were more stressed and, consequently, more susceptible to infections than animals free from ascarids. After the experiment, the rattlesnakes were euthanized to determine macroscopic and histopathologic findings. Parasitized snakes showed more histopathological alterations, than dewormed ones.

**Conclusion:** Preventive medicine, hematological and fecal analysis, and adequate husbandry environment are important issues to keep snakes in healthy and welfare conditions to ensure specimen survival and longevity.

**Keywords:** Nematodes; Parasites; Ascarids; Stress; Captivity

### Abbreviations

LH: Laboratory of Herpetology; SVL: Snout-Vent-Length; TL: Total Length; EPG: Eggs Per Gram of Feces; CTAB: Cetyltrimethylammonium Bromide; NaCl: Sodium Chloride; EDTA: Ethylenediaminetetraacetic Acid; PCR: Polymerase Chain Reaction; DG: Dewormed Group; PG: Parasitized Group; DNA: Deoxyribonucleic Acid; WHO: World Health Organization

## Introduction

Snakebite accidents represent a public health problem in tropical countries [1] and are listed as a tropical neglected disease by the World Health Organization - WHO [2]. About 20,000 snakebite accidents are recorded annually in Brazil [3], with the family Viperidae counting for 99% of the accidents recorded [1,3]. In Brazil there are four important genus of viperids: *Bothrops* sp (pit vipers), *Crotalus durissus* (rattlesnakes), *Lachesis muta* (bushmasters) and *Micrurus* sp (coral snakes). Although the genus *Bothrops* is responsible for approximately 80% of the snake bites, the genus *Crotalus* has the highest mortality rate, around 1.0% [3].

Rattlesnakes are terrestrial animals, robust and not very agile (Figure 1). They have specialized eating habits, preying on small mammals, such as rodents and marsupials since birth [4,5]. Rattlesnake's venom has neurotoxic, myotoxic and coagulant activity [6,7] and at the site of the bite there is almost no reaction, but little oedema, erythema, little or no pain, and a sensation of numbness [6,7]. Only specific antivenom serum therapy is effective in treating venomous snake bite accidents.



**Figure 1:** *Crotalus durissus* specimen

Antivenom serum production in Brazil began in 1901 with the establishment of the Serum Therapy Institute, which soon changed its name to Instituto Butantan, currently one of the leading centers of vaccine and serum production in Brazil [8]. In this scenario, the maintenance of venomous snakes in captivity for antivenom serum production has become an alternative for obtaining venom of high quality, on a scale compatible with the demands of the population, saving thousands of lives each year.

The indoors serpentarium of the Laboratory of Herpetology (LH) at Instituto Butantan maintains over 1,000 of Brazilian venomous snakes in captivity and has a snake's breeding program, in order to supply venom for antivenom serum production and decrease the necessity of capturing wild snakes for this purpose. Regarding the species *Crotalus durissus*, our indoors serpentarium maintains approximately 250 rattlesnakes and, although we breed rattlesnakes efficiently, we still receive some specimens from nature to increase the geographic variability of the colony. Various captive-related stressors can weaken recently-caught snakes' immune system, leading to the development of various diseases [9,10], such as infectious and parasitic ones, which can cause high morbidity and mortality [11]. The main negative effects of parasitism include anemia, anorexia, reduced survival, as well as reduced fertility [12,13]. Furthermore, the presence of endoparasites in captive snakes can interfere in the animals' health and in the quality and quantity of venom yielded [6].

Ascarids are parasites belonging to the nematoda class and are the most frequent ones found in rattlesnakes, which infection can be fatal [14]. Ascarid adult nematodes are found in the snake’s gastrointestinal tract and produce resistant eggs that are eliminated with feces [6,15]. The intermediate host, rodents in general, is infected when eating snakes’ feces with eggs. In the rodent’s gastrointestinal tract, the larvae eclose and incise in the rodent’s muscles, lungs or peritoneum [16,17]. When snakes feed on infected rodents, the ascarids larvae can cause severe injuries during migration through the snakes’ viscera. The adult form parasitizes the gastrointestinal tract, causing anorexia, regurgitation, obstruction and sometimes intestinal perforation [15]. Brazilian snakes are parasitized by five genera of ascarids: *Ascaridia*, *Ophidascaris*, *Hexametra*, *Polydelphis* and *Travassosascaris* [18].

This study came from the necessity to better understand how ascarids can affect rattlesnake’s health, impairing their maintenance in captivity.

**Materials and Methods**

**Animals**

Twelve rattlesnakes (*Crotalus durissus*) from São Paulo State, newly caught from nature and parasitized by ascarids, were used in this study (approved by the Animal Ethics Committee, number 2362041215) in a period of 10 months. After confirming the parasitism and determining the degree of infestation by stool ova test, the animals were divided into two groups consisting of males and females. The Dewormed Group (DG) was composed of 6 adults (3 males and 3 females) treated with ivermectin 1% diluted 1:10 in propylene glycol (0.2 mg Kg<sup>-1</sup>, SC, repeated after 15 days) and the Parasitized Group (PG) was composed of 6 adults (3 males and 3 females), naturally infected by ascarids, but not dewormed. The weight, snout-vent-length (SVL) and total length (TL) of the snakes before the beginning of the experiment, are shown in table 1 for the DG, and in table 2 for the PG. The animals were fed with slaughtered rodents once per month (10 to 20% of the snake’s body weight) and were kept individually in plastic cages with corrugated cardboard as substrate and a plastic bowl containing fresh and clean water ‘*ad libitum*’. Snakes were kept in rooms with temperature around 25°C, humidity around 50%, and in a 12:12h light and dark cycle.

Snake	Gender	Weight (g)	SVL-TL (cm)
Cdt 1	F	589g	86,5 - 92 cm
Cdt 2	M	605g	93 - 103 cm
Cdt 3	F	748g	90 - 96 cm
Cdt 4	M	638g	90 - 96 cm
Cdt 5	F	660g	84 - 89 cm
Cdt 6	M	572g	85 - 95 cm

**Table 1:** Biometry of snakes of the DG in time 0.

Snake	Gender	Weight (g)	SVL-TL (cm)
Cdt 7	F	589g	86,5 - 92 cm
Cdt 8	M	605g	93 - 103 cm
Cdt 9	F	748g	90 - 96 cm
Cdt 10	M	638g	90 - 96 cm
Cdt 11	F	660g	84 - 89 cm
Cdt 12	M	572g	89 - 97 cm

**Table 2:** Biometry of snakes of the PG in time 0.

### Coproparasitological data collection

Prior to the beginning of the experiment, fecal samples from all animals were collected. The stool ova tests used to detect eggs in feces were the Direct Fresh Fecal Smear Technique; the Flotation Technique (Sheather's Procedure); and the Ether-Water Sedimentation Technique. After qualitative confirmation of the carrier state, the quantitative determination was performed with McMaster technique, which expressed the amount of eggs per gram of feces (EPG). The last technique served as an indicator of the intensity of ascarid infestation. Egg counts greater than 500 are generally considered moderate infestations and those greater than 1000, considered massive infestation [19,20]. Fecal exams were performed monthly.

### Hematological data collection

Blood samples were taken from all animals at Time 0 (prior to the beginning of the experiment) and, afterwards, at every month. The animals were physically contained with the aid of Lutz's loop and blood samples were obtained by the puncture of the caudal vein using 20 x 0.55 mm gauge needles and disposable plastic syringes of 3 mL, without the addition of anticoagulant. At each sample, a volume up to 1% of the animal's weight was obtained. Blood smears were made immediately after collecting the samples for leukocyte differential count. One aliquot of blood was placed in plastic tubes without anticoagulant to obtain serum to evaluate biochemical parameters; while another was placed in heparinized plastic tubes with 5000 IU/mL of sodium heparin for evaluation of hematological parameters, such as total erythrocyte, leukocyte and thrombocyte count and hematocrit and hemoglobin determination. Plasma was used to measure corticosterone levels.

Monitoring of the snakes' health was also performed by biochemical tests, such as glucose, calcium, phosphorus, total protein, albumin, uric acid, alanine aminotransferase, aspartate aminotransferase, cholesterol, alkaline phosphatase, creatine kinase (CK-NAC) and creatinine. These parameters were performed as indicated by the manufacturer of Labtest® biochemical kits, in a semi-automatic Termolab® biochemical device.

Determination of corticosterone concentrations in plasma was performed using the DetectX® Corticosterone Enzyme Immunoassay Kit (Arbor Assays™, Ann, MI, USA) using the manufacturer's suggested protocol. Sample preparation and ELISA plaque reading were performed at the Department of Pathology at the Faculty of Veterinary Medicine and Zootechnics at São Paulo University (USP).

Immediately upon physical restraint we collected blood from the caudal vein (time to bleed = 180 seconds from the moment the snake's cage was opened) to dose basal corticosterone levels of both groups. This detail is important, as plasma corticosterone levels increase rapidly to the response of stressful stimulus, that can be triggered by physical restraint [21,22].

### Anatomical and pathological examination

At the end of the experiment all animals were euthanized (approved by the Animal Ethics Committee, number 2362041215) and necropsy performed for macroscopic evaluation; collection of parasites for molecular identification of ascarids; and collection of tissue fragments for histopathological evaluation.

### Molecular identification of ascarids

DNA extraction was performed using a classic protocol with phenol and chloroform [26], extraction buffer solution with cetyltrimethylammonium bromide (CTAB) (2% CTAB, 1M Tris pH 8.0, 0.5M EDTA pH 8.0, 5M NaCl) [23,24] and commercial kits (QIAamp DNA Mini Kit QIAGEN®). Small pieces of nematodes were placed in a lysis solution for DNA extraction [25]. The polymerase chain reaction (PCR) was performed using oligonucleotides, previously described in the literature for nematode spacer regions (ITS1) (NC5F, NC13R) [26].

ITS1 sequences were analyzed in relation to the quality of the Phred-Phrap program (<http://asparagin.cenargen.embrapa.br/phph/>) and edited in the Chromas Lite 2.01® program to generate a consensus sequence. Once the consensus nucleotide sequences of each sample were determined, they were aligned with the aid of the Clustal W program contained in the BioEdit Sequence Alignment Editor suite [27], based on homologous sequences available on GenBank. Sequence alignment matrices were built for each of the markers with the aid of the MEGA 6 program [28] to obtain phylogenetic trees.

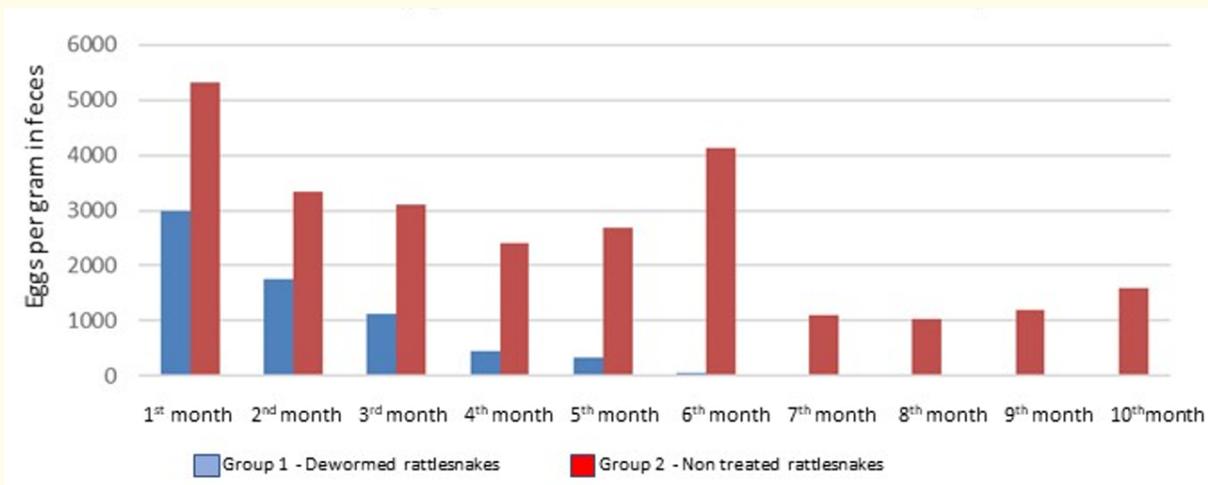
### Statiscal analysis

To analyze differences within the same group on different dates, repeated-use ANOVA or Friedman's test and Dunn's method were used. The differences between both groups were tested with unpaired t-test or Welch's ANOVA when the data presented normal distribution. When data were not normally distributed, Mann-Whitney test were used.

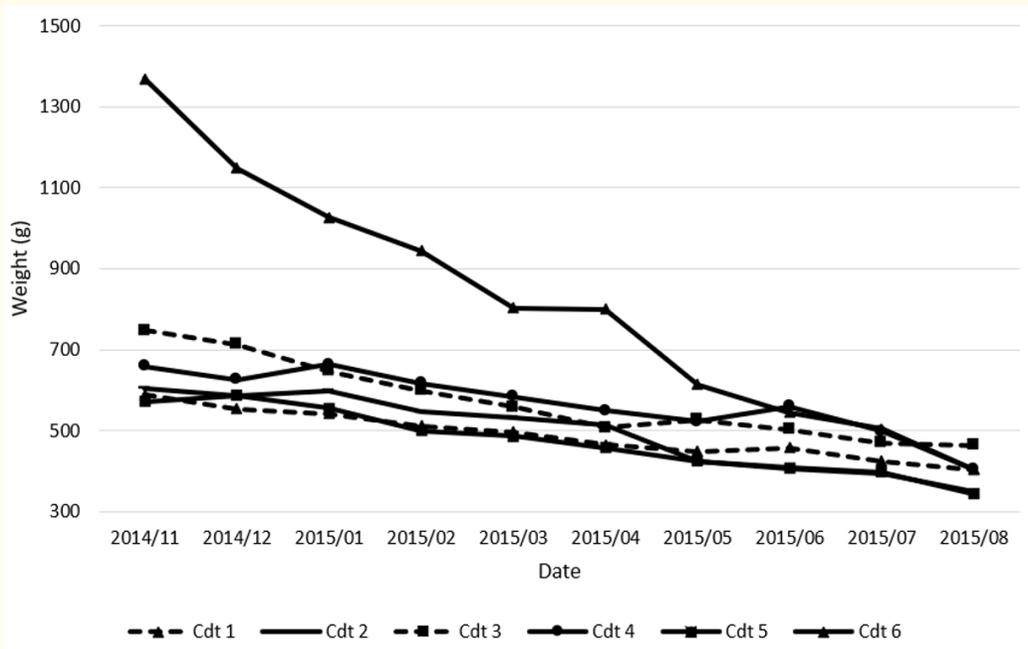
Regarding serum corticosterone concentrations, the difference within the same group were tested using repeated measures ANOVA test and Bonferroni test; or Friedman's test with Dunn's post-test, if distribution was not normal. When comparing differences between both groups, a one-way ANOVA and Turkey test were used for normal distribution data, while Kruskal-Wallis test with Dunn post-test were used, if data did not respect the normality assumptions.

### Results

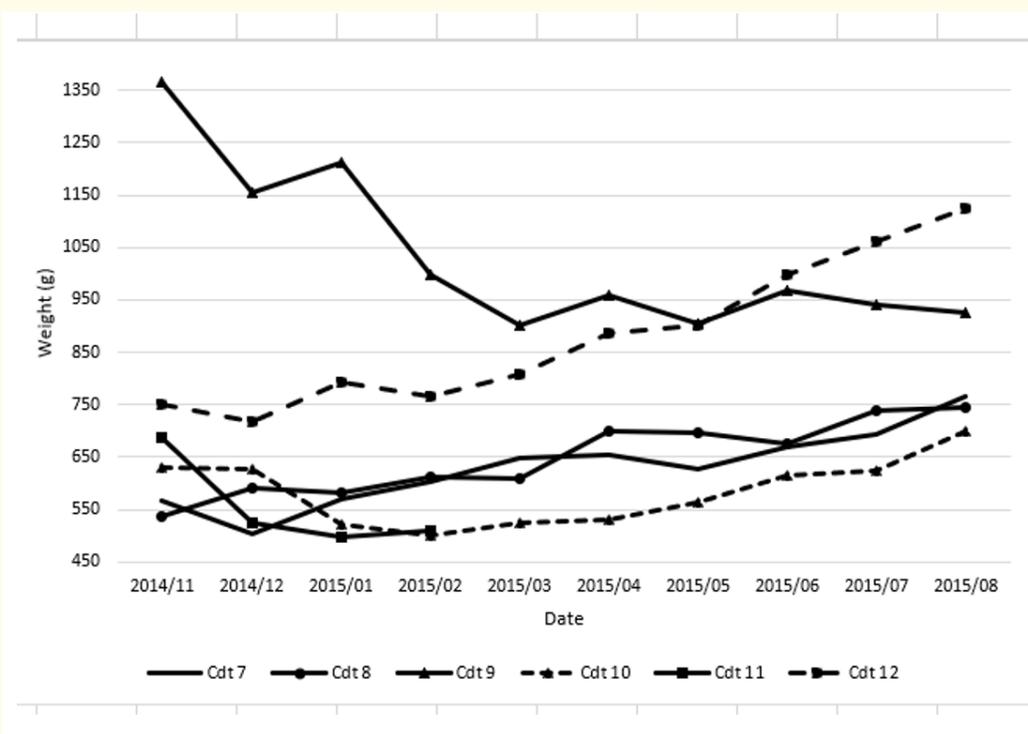
Stool samples were collected from both groups throughout the study to verify the efficacy of the antiparasitic treatment in the DG, and to monitor the parasitic load and symptoms in the PG. The PG showed higher EPG rates throughout the experiment, as can be seen in graph 1. Four animals of the PG had postprandial regurgitation and three animals stopped feeding for three consecutive months and had to be force-fed with pre-slaughtered rodents, lubricated with polyvitamin. All animals of this group lost, at least, 30% of their initial weight at the end of the experiment, as can be seen in graph 2, while most snakes of the DG gained at least, 15% of their initial weight during the study (Graph 3), with the exception of one snake of this group that lost 30% of its body weight, and of a snake that died in the fourth month of study.



**Graph 1:** Average quantity of EPG in DG and PG, in 10 different sample dates. São Paulo, 2018.



**Graph 2:** Weight (g) monitoring of PG at different dates. São Paulo, 2018.



**Graph 3:** Weight (g) monitoring of DG at different dates. São Paulo, 2018.

Dewormed snakes fed spontaneously on rodents and never regurgitated, while non-treated snakes usually did not accept preys, and six out of seven underwent some force-feeding and/or regurgitation event.

Regarding blood analysis, no significant differences between the groups or intragroup were observed during the experiment in relation to the parameters studied: Total erythrocytes count, total leucocytes count, total trombocytes count, hematocrit value, hemoglobin value and differential leucocyte count.

Comparing the biochemical parameter results in both groups at equal moments, statistical differences were seen only for total protein concentration ( $p < 0,05$ ) and for alanine aminotransferase test ( $p = 0,0084$ ). In relation to plasma corticosterone values, no statistical difference was observed between both groups in time 0 (before the beginning of the experiment), but from there on a statistically significant difference ( $p < 0.0092$ ) was observed in almost all samples collected (Table 3). At the end of the experiment, basal plasma corticosterone values for the PG were, at least, three times higher than those found in animals of the DG.

Corticosterone (ng/mL)	DG	PG
Time 0	51,5 ± 11,0	53,1 ± 14,5
1 <sup>st</sup> blood sample	43,4* ± 16,0	87,2* ± 36,7
2 <sup>nd</sup> blood sample	60,5* ± 25,7	79,8* ± 32,0
3 <sup>rd</sup> blood sample	101,8 ± 42,4	92,7 ± 44,5
4 <sup>th</sup> blood sample	36,6* ± 15,7	86,9* ± 61,3
5 <sup>th</sup> blood sample	51,8* ± 24,0	202,7* ± 156,0
6 <sup>th</sup> blood sample	57,3* ± 17,5	119,2* ± 17,0
7 <sup>th</sup> blood sample	32,9* ± 5,9	103,1* ± 32,0

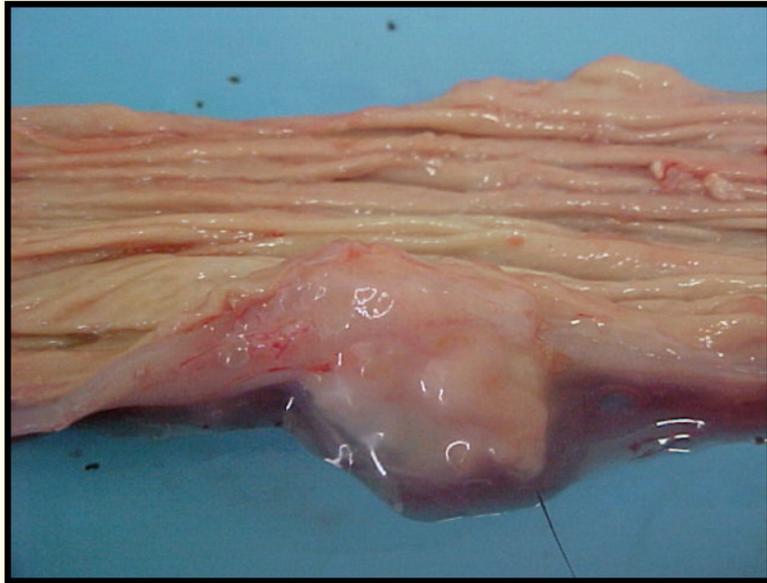
**Table 3:** Mean basal corticosterone plasma values (± standard error) for snakes of the DG and PG, at different moments. Sao Paulo, 2018.

Legend: The \* means statistical difference between the DG and PG in the same sample.

The snake Cdt 11 (DG) died on the 4<sup>th</sup> month of study, and at necropsy we observed pale mucous membranes, presence of parasitic granuloma in the stomach (Figure 2) and congested adrenal glands. On the other hand, the necropsies of the euthanized snakes of the DG were in good health condition and no ascarids were found on the gastrointestinal tract, although some parasitic cysts and larvae could be seen on pulmonar serous and on the liver capsule (Figure 2). Main histopathological findings included micro and macrovacuolar degeneration of hepatocytes (Figure 3) and mild gastric edema.

Notwithstanding, most snakes of the PG were cachectic, with pale oral mucosa, had intestinal and gastric wall thickening, presence of ascarids in the gastric lumen (Figure 4); gastritis and gastric parasitic granulomas (Figure 5). Main histopathological findings included severe gastric edema, gastritis, gastric parasitic granuloma (Figure 6), gastric hemorrhage, histiocytic granuloma in stomach and liver and heterophilic infiltration in the lung (Figure 7).

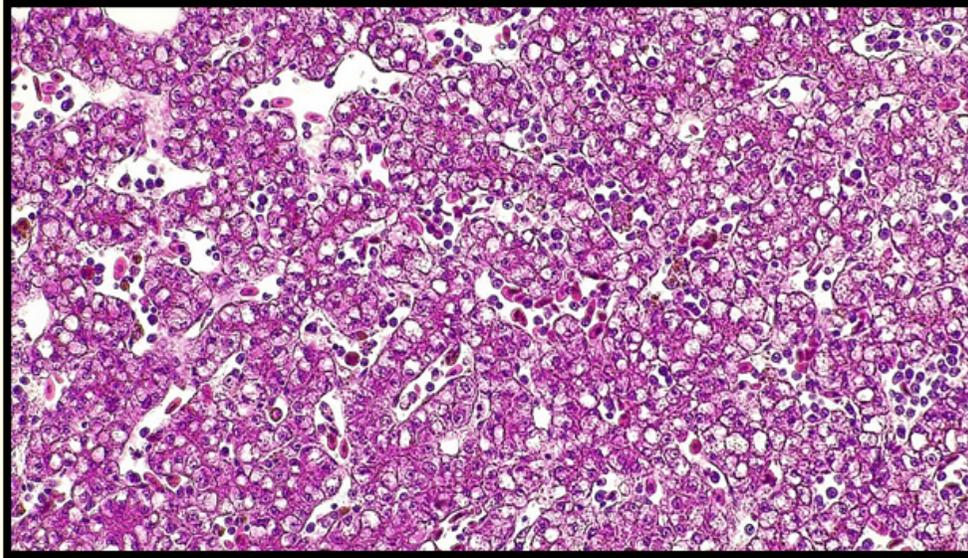
The molecular identification of ascarids found in necropsied snakes of the DG was difficult as there are few snake parasites available at Genbank. The sequence obtained in this study have been grouped with the only available *Ophidascaris* sequence and separated from other represented nematode genera, such as, for example, *Raphidascaris acus* and *Rhabdias fuscovenosa*. Due to the lack of sequences of



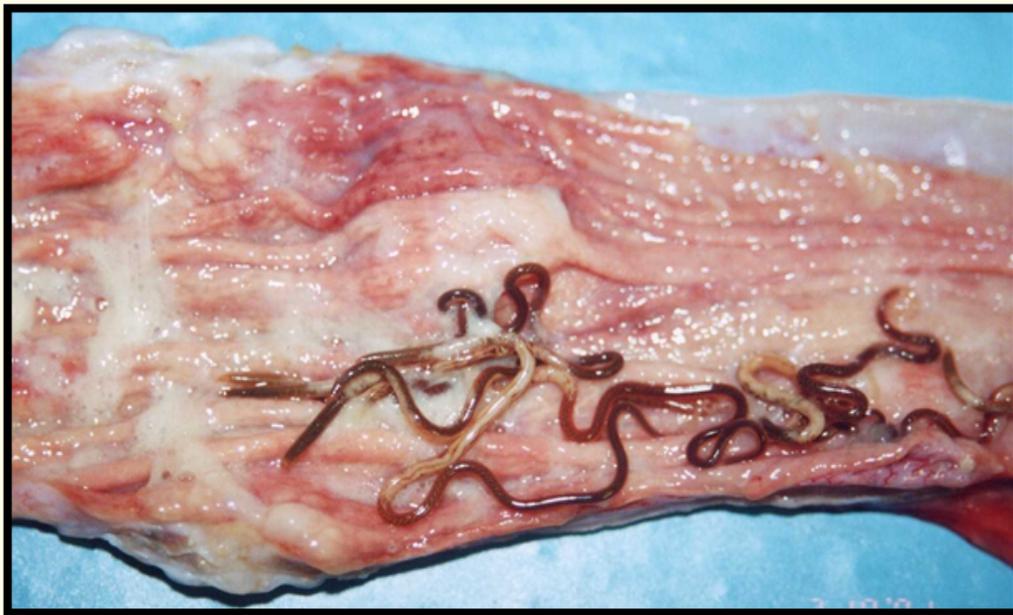
**Figure 2:** Parasitic granuloma in the stomach of *Crotalus durissus* (Cdt 11 - DG).



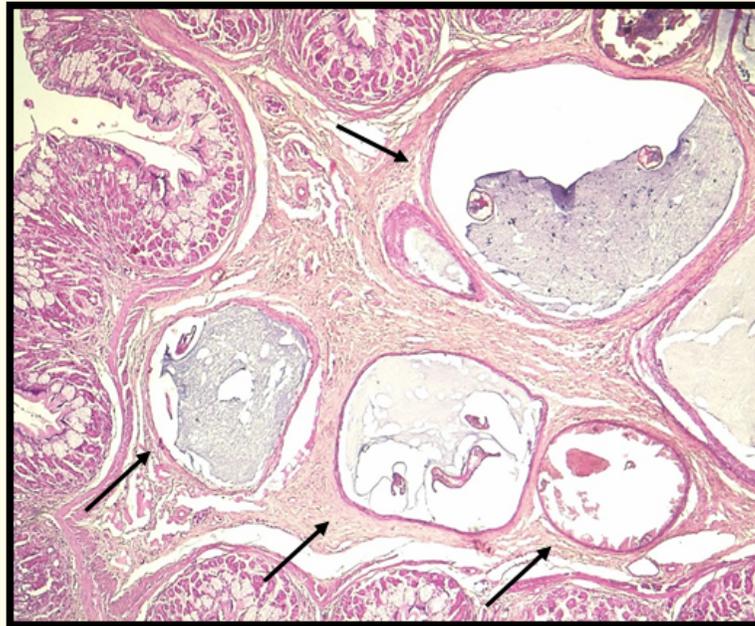
**Figure 3:** Parasite larvae between liver parenchyma and liver capsule (Cdt 7 - DG).



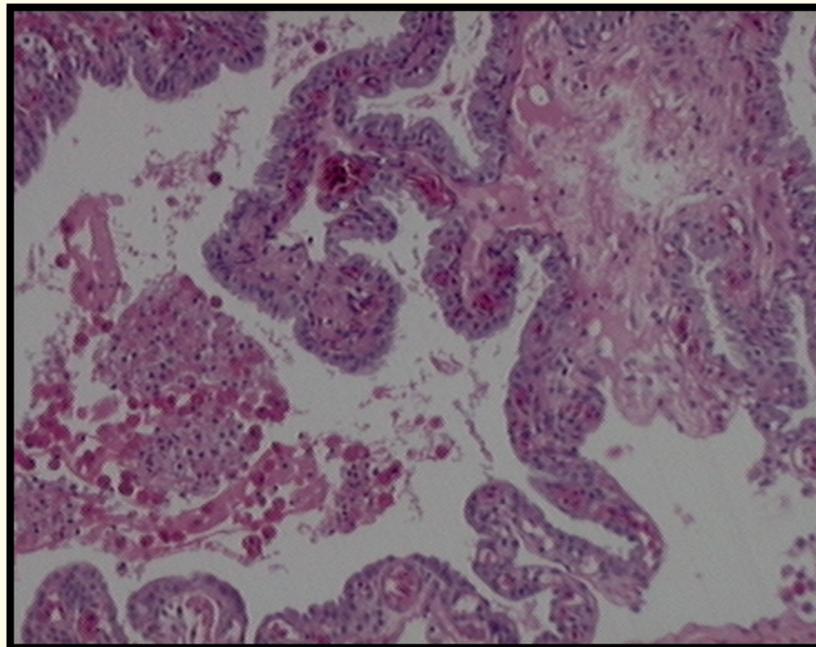
**Figure 4:** Micro and macrovacuolar hepatocyte degeneration (Cdt 8 -DG).



**Figure 5:** Ascarids in the stomach lumen of *Crotalus durissus* (Cdt 4 - PG). Note the congested gastric mucosa and catarrhal secretion.



**Figure 6:** Gastric parasitic granuloma in Cdt 2 (PG), with segments of ascarids (black arrows).



**Figure 7:** Pneumonia with heterophilic infiltration in Cdt 3 (PG).

more species and specimens for confirmation, we could not conclude what genus was infecting our snake, but are sure they belonged to the Family Ascarididae.

### Discussion

Although parasitism can alter host physiology [29], development [30], behavior [31] and reproduction [32], few studies have analyzed the interaction of parasites on snakes' basal corticosterone levels to verify how negatively they can affect the animals. In this study we verified that the snakes infected by ascarids showed corticosterone basal levels at least three times higher than the basal levels encountered in the DG. These higher basal levels of corticosterone may be indicative of chronic stress. Studies on chronic stress in reptiles have shown that high levels of serum corticosterone are associated with reproductive failure, immune suppression and a reduction or lack of growth [11,33]. Chronically stressed captive animals with high parasitic load are much more likely to succumb to infections, have a shorter life expectancy, are more susceptible to diseases and, in general, have an unhealthy appearance [11,34,35]. The PG (Parasitized Group) lost weight probably due to the disruption of the nutrient absorption caused by the ascarid nematodes, that utilize all nutrients passing through the intestinal tract, as well as causing inflammation and/or infection of the gastrointestinal tract [11,36]. Four animals of the PG showed postprandial regurgitation, as a result of inflammation of the gastric mucosa or the presence of parasitic granulomas, that can cause mechanical obstruction, blocking the passage of food content from the esophagus to the stomach [6]. According to Jacobson [36], the body of some ascarids forms a loop through the gastric wall, with the anterior and posterior ends extending into the lumen, while their mid-portion is fit deep into the submucosa causing inflammation [36]. Three animals of the PG stopped feeding for 3 consecutive months and had to be force-fed with pre-slaughtered rodents lubricated with polyvitamin. Anorexia, postprandial regurgitation, gastric obstruction, and malnutrition are associated with classic ascarid nematode infestation [16,37].

Most snakes of the DG (Dewormed group) gained weight during the study, with the exception of the Cdt 9 that lost 30% of its initial weight by the end of the study. Although this specimen accepted prey and had no signs of illness, it did not gain weight during the study. It is worth considering that animals showing neither physiological nor behavioral indicators of stress, may still be experiencing distress [38]. All animals of the PG lost weight, corroborating the study made with *Pantherophis gloydi* in which reproductive performance and body score were affected by parasitic condition [39]. According to this article, the heterophil/lymphocyte ratio is an indirect marker of stress [39]. Lymphocytes are involved in immune responses and their numbers decrease in response to chronic stress and malnutrition [39]. Notwithstanding, in our study no increase were seen in the heterophil/lymphocyte ratio in the PG with high levels of corticosterone, corroborating the work done with *Bothrops jararaca*, *Crotalus durissus* and *Boa constrictor* species [40] in which the authors also had not seen an increase in the heterophil/lymphocyte ratio in snakes with high levels of corticosterone.

Heavy ascarids nematodes infestation may cause gastric/intestinal blockage by the bolus of worms; and obstruction of the bile and pancreatic duct by adult or young worms. Infestation can aggravate nutritional deficiencies, but due to the slow turnover of erythrocytes and their extended lifespan, up to 800 days, it is difficult to verify anemias caused by the action of ascarids [41]. Most hematological and biochemical parameters in our study did not show significant differences between both groups, and the results were similar to those observed by other authors working with newly arrived rattlesnakes from nature [42], with the exception of the concentrations of Total Protein and ALT. These biochemical tests showed lower concentration in the PG, although lower levels of ALT have no pathological significance.

It is known that anorexia, gastritis, parasitic disruption of nutrient absorption, and liver disorders can decrease Total Protein in parasitized animals [43]. The Total Protein Concentration for reptiles in general is 3 to 8 mg/dL [44]. In our study, the values obtained in the PG revolved around the lower limit. Hypoproteinemia in reptiles is related to malnutrition, malabsorption, enteropathies and chronic kidney and liver diseases [45] that can be aggravated in endoparasite infection.

In our study, the gross and histopathological findings in the PG were severe gastric edema, gastritis, gastric parasitic granuloma, gastric hemorrhage and histiocytic granuloma, that are in accordance with the lesions found in other studies [46-48]. These lesions may decrease the absorption of nutrients due to gastric epithelial destruction; gastric obstruction by parasitic granuloma; the action of parasite fixation on gastric mucosa [36,48]; or to food regurgitation caused by the thickening of the gastric wall [6], causing nutritional deficiencies [49,50]. On the necropsies of the euthanized snakes of the DG, no ascarids were found in the gastrointestinal tract, although some parasitic cysts and larvae could be seen on pulmonar serous and on the liver capsule (hypobiosis). The Snake Cdt 11 (DG) died on the 4<sup>th</sup> month of study, and at necropsy we observed pale mucous membranes; presence of parasitic granuloma in the stomach, without parasites; and congested adrenal glands. This animal could have died of the Syndrome of Captivity Maladaptation. Main histopathological findings in the DG included micro and macrovacuolar degeneration of hepatocytes and mild gastric edema.

### Conclusion

The maintenance of animals in which prophylactic treatments are not performed is much more laborious and costly, due to the interventions that may need to be performed on these animals, in addition to a higher morbidity and mortality in the serpentarium. Parasitized animals becomes a risk to the serpentarium due to the spread of parasites, especially of those with direct life cycles; associated to the stress of captivity that can suppress the animal's immune response, predisposing them to infections and diseases caused by viruses, bacteria, yeasts and fungi.

The purpose of a maintenance facility is to keep animals in healthy and welfare conditions and in appropriate reproductive conditions. Given that, preventive and prophylactic medicine should be very well established and routinely employed both to newcomers' snakes and those already on the snake farm.

With the results obtained in this study we consider safe to confirm that parasitized animals are much more stressed and, consequently, more susceptible to opportunistic infections in captivity, than dewormed animals. Besides, parasitized snakes feed poorly, lose weight, and have higher basal corticosterone levels than non-parasitized animals exposed to equal environmental conditions. It is clear that the adoption of preventive medicine is of fundamental importance for the proper maintenance of captive snakes to obtain venom of good quality, both for the production of antivenom serum and for immunobiological researches.

Preventive medicine and adequate husbandry environment are the key words to keep snakes in healthy and welfare conditions to ensure specimen survival and longevity.

### Acknowledgments

This paper would not have been possible without the support of the staff of the Laboratory of Herpetology at Instituto Butantan; and the generosity of Dr. Catão-Dias for letting us use the facilities of the Pathology Department at the University of São Paulo/SP. We would also like to thank the Central Bioterium of Rodents at Instituto Butantan for the preys used for feeding the snakes.

This article is the result of a master's degree defended at the Department of Veterinary Clinic of the Faculty of Veterinary Medicine and Zootechnics of the University of São Paulo in 2016.

### Bibliography

1. Oliveira RC., *et al.* "Epidemiologia dos acidentes por animais peçonhentos". In: Cardoso JL, Haddad JV, França FOS, Wen FH, Malaque C, editors. *Animais Peçonhentos no Brasil: biologia, clínica e terapêutica dos acidentes*. 2<sup>nd</sup> edition, São Paulo: Sarvier (2009): 6-21.

2. WHO. Selection and veterinary health care of animals used for production of antivenoms. In: Guidelines for the production, control and regulation of snake antivenom immunoglobulins". Replacement of Annex 2 of WHO Technical Report Series 964 (2016).
3. Sistema de Informação de Agravos de Notificação. Acidentes por animais (2018).
4. Campbell J and Lamar WW. "The venomous Reptiles of the Western Hemisphere". 2<sup>nd</sup> edition. Campbell J, Lamar WW, editors. New York: Comstock pub (2004).
5. Melgarejo AR. "Serpentes Peçonhentas do Brasil". In: In: Cardoso JL, Haddad JV, França FOS, Wen FH, Malaque C, editors. "Animais Peçonhentos no Brasil: biologia, clínica e terapêutica dos acidentes. 2<sup>nd</sup> edition, São Paulo: Sarvier (2009): 33-61.
6. Jorge MT and Ribeiro LA. "The epidemiology and clinical picture of an accidental bite by the South American rattlesnake (*Crotalus durissus*)". *Revista do Instituto de Medicina Tropical de São Paulo* 34.4 (1992): 347-354.
7. Azevedo-Marques MM., et al. "Animais Peçonhentos no Brasil: biologia, clínica e terapêutica dos acidentes". In: Cardoso JL, Haddad JV, França FOS, Wen FH, Malaque C, editors. 2<sup>nd</sup> edition, São Paulo: Sarvier (2009): 91-98.
8. Canter HM. "100 anos de Butantan". In: 100 anos de Butantan (2000): 74.
9. Heat KB. "Fatal parasitic anemia in a green vine snake". *Veterinary Medicine, Small animal clinician*. 78.7 (1983): 1095-1096.
10. Wellehan JFX and Walden HDS. "Parasitology (including hemoparasites)". In: Divers SJ and Stahl SJ, editors. *Mader's Reptile and Amphibian Medicine Surgery*. Saint Louis, Missouri (2019): 281-300.
11. Kolesnikovas CKM., et al. "Microbiological findings in Brazilian rattlesnakes (*Crotalus durissus*)". *Boletín de la Asociación Herpetológica Española* 12.1 (2001): 25-28.
12. Francisco LR., et al. "Class Reptilia, Order Squamata (Ophidia): Snakes". In: Fowler M and Cubas ZS, editors. *Biology, Medicine, and Surgery of South American Wild Animals*. Iowa state university press (2001): 40-50.
13. Rataj AV., et al. "Parasites in pet reptiles". *Acta Veterinaria Scandinavica* 53 (2011): 33.
14. Wilson SC and Carpenter JW. "Endoparasitic disease of reptiles". *Seminars in Avian and Exotic Pet Medicine* 5.2 (1996): 64-74.
15. Sprent JFA. "Studies on ascaridoid nematodes in pythons: speciation of *Ophidascaris* in the Oriental and Australian regions". *Parasitology* 59 (1969): 937-959.
16. Frye F. "Biomedical and surgical aspects of captive reptiles husbandry". Krieger Publishing Company, Flórida (1991).
17. Anderson RC. "Nematode parasites of vertebrates: their development and transmission". 2<sup>nd</sup> edition. Wallingford, England: CAB International (2000): 650.
18. Pinto., et al. "Nematode parasites of the neotropical rattlesnake, *Crotalus durissus* Linnaeus, 1758 (Ophidia, Viperidae) from Brazil: new records and general aspects" (2010).
19. Castilho VLP., et al. "Exame parasitológico quantitativo das fezes: estudo comparativo entre os métodos de McMaster, Stoll-Hausheer e Kato-Katz". *Revista da Sociedade Brasileira de Medicina Tropical* 17.4 (1984): 209-212.

20. Urquhart GM., *et al.* "Parasitologia Veterinária". 2<sup>nd</sup> edition. Monteiro SG, ed. Santa Maria (RS), Universidade Federal de Santa Maria (2007).
21. Grego KF. "Determinação dos níveis séricos de corticosterona e hormônios esteroides sexuais, induzidos pelo estresse da contenção física e da extração de veneno, em *Bothrops jararaca* (Ophidia: Viperidae) [dissertation]". São Paulo (SP): Universidade de São Paulo (2006).
22. Sapolsky RM., *et al.* "How do glucocorticoids influence stress response? Integrating permissive, suppressive, stimulatory, and preparative actions". *Endocrinology Review* 21 (2000): 55-89.
23. Sambrook J., *et al.* "Molecular Cloning: A laboratory manual". 2<sup>nd</sup> edition. Cold spring harbor laboratory press. New York: Soft Cover (1989).
24. Dzido J., *et al.* "Selected mitochondrial genes as species markers of the Artic *Contraecum osculatum* complex". *Journal of Helminthology* 86.2 (2012): 252-258.
25. Timi JT., *et al.* "Molecular identification, morphological characterization and new insights into the ecology of larval *Pseudoterranova cattani* in fishes from the Argentine coast with its differentiation from the Antarctic species, *P. decipiens* sp. E (Nematoda: Anisakidae)". *Veterinary Parasitology* 199.1-2 (2014): 59-72.
26. Zhu X., *et al.* "Assessing sequence variation in the intestinal transcribed spacers of ribosomal DNA within and among members of the *Contraecum osculatum* complex (Nematoda: Ascaridoidea: Anisakidae)". *Parasitology Research* 86.8 (2000): 677-683.
27. Hall TA. "BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT Nucleic Acids Symposium Series" (1999).
28. Tamura K., *et al.* "Mega 6: Molecular evolutionary genetics analysis version 6.0". *Molecular Biology and Evolution* 30.12 (2013): 2725-2729.
29. Grego KF., *et al.* "Comparative pathology of parasitic infections in free-ranging and captive pit vipers (*Bothrops jararaca*)". *Veterinary Records* 154.18 (2004): 559-562.
30. Rataj AV., *et al.* "Parasites in pet reptiles". *Acta Veterinaria Scandinavica* 53.1 (2011): 33.
31. Davis JR., *et al.* "Snake parasitism in an urban old-growth forest". *Urban Ecosystems* 15 (2012): 739-752.
32. Uhrig EJ. "Reproductive implications of parasitic infections and immune challenges in garter snakes". Oregon State University (2015).
33. Tokarz RR., *et al.* "Stress and reproduction in reptiles. Hormones and reproduction of vertebrates". Academic Press (2011): 169-213.
34. Rataj AV., *et al.* "Parasites in pet reptiles". *Acta Veterinaria Scandinavica* 53.1 (2011): 33.
35. Martínez S. "How to assess stress in reptiles". *Journal of Exotic Pet Medicine* 23.3 (2014): 240-243.
36. Jacobson ER. "Parasites and parasitic diseases of reptiles". In: Jacobson E, ed. *Infectious Diseases and Pathology of Reptiles*. 1st edition. Boca Raton: Taylor and Francis Group (2007): 731.

37. Pinto RM, *et al.* "Nematodes parasites of the neotropical rattlesnake, *Crotalus durissus* Linnaeus, 1758 (Ophidia, Viperidae) from Brazil: new records and general aspects". *Neotropical Helminthology* 4.2 (2010): 137-147.
38. Arena PC and Warwick C. "Miscellaneous factors affecting health and welfare". In: Health and welfare of captive reptiles (1995): 263-283.
39. Xuereb A, *et al.* "Relation between parasitism, stress, and fitness correlates of the Eastern foxsnake (*Pantherophis gloydi*) in Ontario". *Journal of Herpetology* 46.4 (2012): 555-561.
40. Carvalho MPN. "Functional analysis of peripheral blood leucocytes of snakes (Boidae and Viperidae) during the process of adaptation to captivity [dissertation]". São Paulo (SP); Universidade de São Paulo, (2018).
41. Duguay R. "Numbers of blood cells and their variation". *Biology of the Reptilia* 3 (1970): 93-109.
42. Troiano JC, *et al.* "Haematological reference intervals of the South American rattlesnake (*Crotalus durissus terrificus*, Laurenti, 1768) in captivity". *Comparative Haematology International* 7.2 (1997): 109-112.
43. Zanotti LCRA. "Aspectos hematológicos, bioquímicos, morfológicos e citoquímicos de células sanguíneas em Viperídeos neotrópicos dos gêneros *Bothrops* e *Crotalus* mantidos em cativeiro. Tese de Doutorado". Universidade de São Paulo (2007).
44. Redrobe S and MacDonald J. "Sample collection and clinical pathology of reptiles". *Veterinary Clinics of North America: Exotic Animal Practice* 2.3 (1999): 709-730.
45. Almosny NRP and Monteiro ADO. "Patologia clínica. Tratado de Animais Selvagens: medicina veterinária". Roca, São Paulo (2007): 939-966.
46. Elbihari S and Hussein MF. "*Ophidascaris filaria* (Dujardin, 1845) from the African Rock Python, *Python sebae*, in the Sudan, with a note on associated gastric lesions". *Journal of Wildlife Diseases* 9 (1973): 171-173.
47. Brar RS, *et al.* "Maintaining rear-fanged snakes for venom production: an evaluation of mortality and survival rates for *Philodryas olfersii* and *P. patagoniensis* in captivity". *Journal of Venomous Animals and Toxins including Tropical Diseases* 18 (2012): 164-172.
48. Mello EM, *et al.* "Gross and histologic features of gastritis due to *Ophidascaris andi* in tropical rattlesnakes (*Crotalus durissus*)". *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 69.2 (2017): 415-421.
49. Garcia D, *et al.* "Alterações patológicas causadas por nematoides parasitas de jararaca (*Bothrops jararaca* Wied, 1824) criadas em cativeiro. Pathological aspects of nematodes of jararaca (*Bothrops jararaca*) reared in captivity". *Revista Brasileira de Ciência Veterinária* 11.1-2 (2014).
50. Wilson SC and Carpenter JW. "Endoparasitic diseases of reptiles". *Seminars in Avian and Exotic Pet Medicine* 5 (1996): 64-74.

**Volume 5 Issue 11 November 2020**

**©All rights reserved by Kathleen Fernandes Grego, *et al.***