Factors that can influence the survival rates of coral snakes (*Micrurus corallinus*) for antivenom production

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ABSTRACT: Envenoming and deaths resulting from snakebites are a particularly important public health problem in rural tropical areas of Africa, Asia, Latin America, and New Guinea. In 2015, *The Lancet* highlighted snake-bite envenoming as a neglected tropical disease and urged the world to increase antivenom production. In Brazil, around 20,000 snakebites occur per year affecting mostly agricultural workers and children, of which 1% is caused by coral snakes (*Micrurus* sp.). Although human envenoming by coral snakes is relatively rare due to their semifossorial habits and nonaggressive behavior, they are always considered severe due to the neurotoxic, myotoxic, hemorrhagic, and cardiovascular actions of their venom, which is highly toxic when compared to the venom of other Brazilian venomous snakes as *Bothrops* sp. (pit vipers), *Crotalus* sp. (rattlesnakes), and *Lachesis* sp. (bushmasters). The production of antivenom serum is an important public health issue worldwide and the maintenance of venomous snakes in captivity essential to obtain high-quality venom. Though more than 30 species of Brazilian coral snakes exist, the specific antivenom serum produced

with the venom of two species, *Micrurus corallinus* and *M. frontalis*, is able to neutralize the accidents caused by the genus in general. *M. corallinus* is considered a difficult species to maintain in captivity and concerned about this difficulty the Laboratory of Herpetology (LH) at Instituto Butantan, over the last 10 yr, has given special attention to its maintenance in captivity. In more than 20 yr of maintenance, LH has made some changes to improve *Micrurus* captive husbandry and welfare. The objective of this study was to verify the factors influencing the survival rates of coral snakes in captivity through data generated from 289 *M. corallinus* from the LH snake facility in the last 10 yr. We observed that survival rates increased significantly with the improvement of nutritional adequacy that included freezing food items before offering them to coral snakes, as well as the development of a new pasty diet to force-feed anorexic animals. Another important factor responsible for increasing life expectancy was the shift of the cage's substrate from *Sphagnum* to bark in 2010, aiding in the eradication of Blister Disease, which used to be responsible for the death of several coral snakes in previous years.

Key words: coral snakes, maintenance, husbandry, nutrition, survival rate

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INTRODUCTION

Envenoming and deaths resulting from snake bites are a particularly important public

health problem in rural tropical areas of Africa, Asia, Latin America, and Papua New Guinea (Williams, 2015). In 2015, the editorial of *The Lancet* highlighted snake-bite envenoming as a neglected tropical disease and urged the world to increase antivenom production (The Lancet, 2015). In 2017, the World Health Organization (WHO) categorized snakebite as a high-priority

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neglected tropical disease, but in May, 2018, WHO resolved to decrease the morbidity and mortality of snake envenoming, and “coordinate global efforts to control snakebite” (Klsbister and Silva, 2018). In Brazil, more than 20,000 snakebites envenoming occur per year (Ministério da Saúde, 2018), affecting mostly young agricultural workers and children from rural areas (Cruz et al., 2009), most of them caused by Viperidae snakebites (*Bothrops* sp., *Crotalus* sp., and *Lachesis* sp.) (Ministério da Saúde, 2018). Although coral snake envenomation (*Micrurus* sp.—Elapidae) is smaller when compared to accidents caused by other Brazilian venomous snakes, 1% and 99%, respectively (Ministério da Saúde, 2018), the wide geographic dispersion of *Micrurus* species and the severity of the accidents obliges the local Ministry of Health to distribute the specific antivenom over the country.

Thirty-five species and subspecies of *Micrurus* are registered for Brazil (Roze, 1996; Campbell and Lamar, 2004; Costa and Bérnills, 2015; Pires et al., 2014; Silva Júnior et al., 2016). Between 2010 and 2016, the Northeast region of Brazil (with nine species) had the greatest number of coral snakebites, 840, followed by the Southeast region (five species) with 306 occurrence. Although the North region of Brazil has the greatest diversity of coral snakes (20 species), 197 accidents occurred during that period, while in Central-West region (11 species) and South (six species), 125 and 93 coral snakebites occurred, respectively, in the same period (Silva Júnior et al., 2016, Ministério da Saúde, 2018).

Even though human envenomation by coral snakes is relatively rare due to their semifossorial habits, nonaggressive behavior and the fact that they generally inhabit sparsely populated areas (Silva and Bucaretychi, 2003; Corrêa-Netto et al., 2011), their venom is highly toxic and always considered severe when compared to Viperidae snakes. Thus, any bite is a medical emergency that requires immediate intervention (Corrêa-Netto, et al., 2011).

The composition of *Micrurus corallinus* venom has been elucidated by several authors through proteomic and transcriptomic approaches (Ho et al., 1995; Leão et al. 2009; Corrêa-Netto et al., 2011; Aird et al., 2017; Morais-Zani et al., 2018). In this context, a recent study conducted by our group showed that the venom of this species is composed by, at least, 13 toxin families, which represents the most complete *M. corallinus* venom proteome described so far in terms of number of toxin families identified (Morais-Zani et al., 2018). Three-finger toxins (3FTx) correspond the most abundant and diversified toxin family, accounting

for ~38% of total venom proteome, followed by L-amino acid oxidases (~12%), snake venom metalloproteinases class PIII (~12%), phospholipases A₂ (PLA₂) (~11%), C-type lectins (~10%) and snake venom serine proteinases (~5%). In addition, phospholipases B, venom nerve growth factor, hyaluronidases, phosphodiesterases, Kunitz-type serine proteinase inhibitors, peptidases, and endonucleases constitute the minor components of *M. corallinus* venom, each one corresponding to ≤2.5% of the total venom proteome.

Cardiotoxic, myotoxic, hemolytic, hemorrhagic, and edematogenic manifestations have been described in human patients (Gutiérrez et al., 1983; Gutiérrez et al., 1986; Arroyo et al., 1987; Barros et al., 1994), but in severe cases death usually occurs as a consequence of muscle paralysis and respiratory arrest due to the action of neurotoxins that act presynaptically (mainly PLA₂) or postsynaptically (mainly 3FTx) (Rosenfeld, 1971; Snyder et al., 1973; Silva and Bucaretychi, 2003; Warrell, 2004; Cecchini et al., 2005; Bucaretychi et al., 2006; Tanaka et al., 2010), with a human lethality rate of 0.41% (Ministério da Saúde, 2018).

Micrurus corallinus and *M. frontalis* are responsible for most of the human elapidic envenomation in Brazil (Leão et al., 2009) producing an irreversible and progressive neuromuscular blockade, reducing evoked acetylcholine (ACh) release, and increasing the spontaneous release of ACh (Silva and Bucaretychi, 2003). The signs and symptoms of the envenomation are a result of this progressive blockade at the neuromuscular endplate and include paresthesia, palpebral ptosis, ophthalmoplegia, paralysis of the jaw, larynx and pharynx muscles, sialorrhea and paralysis of neck and limb muscles (Brazil, 1987; Silva and Bucaretychi, 2003). Besides supportive clinical care, serotherapy with specific antivenom is the only treatment for coral snake bite (Raw et al., 1991; Gutiérrez et al., 2011).

Although the specific antivenom serum is produced with equal amounts of venom of two species of coral snakes, *M. corallinus* and *M. frontalis* (Raw et al. 1991), Ciscotto et al. (2011) demonstrated that the bivalent serum produced at Butantan Institute (São Paulo) and Ezequiel Dias Foundation (Minas Gerais) by hiperimmunization of horses, reacted in western blot with at least 11 different venoms of *Micrurus* sp., though the venom mechanism of only few species has been investigated so far (Silva Júnior et al., 1991). According to Silva and Bucaretychi (2003), biochemical studies of coral snake venoms are scarce due to the difficulty in capturing them

in nature, challenging maintenance in captivity and small amount of venom obtained in extraction.

Venomous snakes used for the production of antivenom serum in the beginning of the twentieth century were received from farmers and rural workers after the successful campaign carried out by the physician and first director of Butantan Institute, Dr. Vital Brazil, “Defense against snakebites”, when the importance of maintaining venomous snakes in captivity for antivenom serum production was emphasized. From this period on, Butantan Institute continued receiving several species of venomous and nonvenomous snakes by the population.

However, over the last 10 yr there has been a decrease in the number of snakes donated to the Institute, probably due to several factors including anthropic action by deforestation; increased population awareness, which avoids removing snakes from their natural habitat; and difficulty in transporting snakes due to more specific and stringent Brazilian legislation (Grego, 2006).

Due to the factors mentioned above, besides the semifossorial habits of *M. corallinus* (Marques and Sazima, 1997) (Figure 1) making their visualization and capture in nature difficult, the number of coral snakes received at the Institute has been declining precipitously over the last 20 yr (personal communication). From 1997 to 2005 the Institute received an average of 177 *M. corallinus* per year, while from 2006 to 2013, 72 specimens per year (40% less). In 2017, the Laboratory of Herpetology (LH) received only 35 *M. corallinus* (Figure 2). Aware of this decrease, the LH began improving husbandry methods to increase the welfare and longevity of the specimens maintained in captivity to avoid decrease in antivenom serum production.

Keeping *M. corallinus* in captivity has been a challenge due to its difficult adaptation in captive

conditions, which seems to be related to its ecological traits and diet specificity (Serapicos and Merusse, 2002). Regarding its microhabitat, *M. corallinus* is a cryptic inhabitant of the forest, deeply associated to the leaf litter that provides shelter and ground to its daily activities (Marques and Sazima, 1997). In captivity, this issue seems to be reflected by the snake’s behavior of staying under the substrate or beneath its water bowl most part of the time. In relation to its diet, most species of coral snakes are small in size and prey primarily on smaller snakes (colubrids and typhlopids) but will also eat small lizards and amphisbaenians (Marques and Sazima, 1997; Urdaneta et al., 2004; Maffei et al., 2009; Tivador et al., 2011).

A critic point in the maintenance of *M. corallinus* for venom obtainment is the small quantity of venom produced when compared to viperid snakes (pit vipers, bushmasters and rattlesnakes). In contrast to viperids, the fangs of coral snakes are short, hollow structures that are permanently fixed in position on the anterior maxillary bones, which is a feature of proteroglyphous dentition (Pardal et al., 2010). So, instead of using a becker in venom milking, as in viperid snakes, *Micrurus* venom extraction is done with tips attached to its proteroglyphous fangs (Figure 3) and in sequence the venom is pipetted to a microtube. To increase the amount of venom milked, since 2008 pilocarpine (an alkaloid that induces exocrine gland secretion) is injected subcutaneously (10 mg kg^{-1}), 10 min prior to the milking (Rosenberg et al., 1985). According to Morais-Zani et al. (2018), the use of pilocarpine in *M. corallinus* increases the amount of venom milked and does not change significantly its composition and activities. Whilst *Bothrops jararaca* maintained at our bioterium produces an average of 83 mg of lyophilized venom per specimen (personal communication), *M. corallinus* produces only 3 mg of lyophilized venom/specimen (Morais-Zani et al., 2018). Given that, a higher number of specimens is needed to produce the necessary amount of specific antivenom serum required for the treatment of coral snakebites in Brazilian territory.

Considering the difficulties listed above, the LH compared different *M. corallinus* husbandry protocols used over the last 20 yr, most of which related to feeding protocols and substrate used in the cages, to verify how different protocols influenced the welfare and survival rate of the animals.

MATERIALS AND METHODS

We collected longevity data from 289 *M. corallinus* kept at the LH from 1997 to 2013. Snakes

Figure 1. *Micrurus corallinus* (snout-vent length: 52 cm). Specimen in captivity for 6 yr. Note the bark substrate.

Figure 2. Decrease in the number of *Micrurus corallinus* received at Butantan Institute over the last 20 yr.

Figure 3. Tips attached to the fangs to facilitate venom milking.

recently arrived at the LH undergo prophylactic procedures such as immersion bath in 0.2% Neguvon (thriclorfon, Bayer Veterinary Products, São Paulo, Brazil) to eliminate ectoparasites; and deworming with Ivomec (1% ivermectin, Merial Products, São Paulo, Brazil, 0.2 mg kg⁻¹) to eliminate most helminths. After this protocol, the snakes are sent to quarantine where they stay for a minimum period of 30 d before entering the colony. Data were clustered according to the husbandry and nutritional protocols used at three different periods, as follows.

Group I (1997 to 1999, n = 210)

In this group, snakes were kept in individual boxes with *Sphagnum* moss as substrate and water ad libitum. Food consisted of live animals, including snakes (Viperidae, Colubridae, and Dipsadidae), amphisbaenas (*Amphisbaena alba* and *A. microcephalum*), and lizards (*Ophiodes fragilis*), depending on their availability. Food was offered for two consecutive weeks and followed by a 15-d break after which the snakes were milked. In this group, food items collected in nature were offered

alive to coral snakes without any prior prophylactic treatment, except immersion in 0.2% thriclorfon solution 1 wk before. Coral snakes that did not eat voluntarily were force-fed by gavage with Reptomin (chelonian commercial food, Tetra, São Paulo, Brazil) softened in saline, in a percentage of 10% to 20% of the coral snakes' weight. In this period, pilocarpine was not yet administered to the snakes prior to venom milking.

Group II (2010, n = 26)

In this group, snakes were kept in individual boxes with *Sphagnum* as substrate and water ad libitum. In this period, the coral snakes were fed for three consecutive weeks, followed by a 15-d break after which the snakes were milked. Food items were obtained from nature (mostly Colubridae, Dipsadidae and Viperidae) and from the Viperidae Breeding Program of the LH. Preys were euthanized in a container saturated with CO₂ and frozen for a minimum of 7 d before being offered to coral snakes. An animal facility was set up to host a Breeding Program of Cornsnake (*Pantherophis guttatus*) to start offering live items (newborns of cornsnakes) to *M. corallinus* that refused to eat thawed food. Coral snakes that did not eat voluntarily were force-fed by gavage with a recipe developed at the LH consisting of 1 L of saline + 15 g of commercial food for rodents (Purina, São Paulo, Brazil) + 500 g of thawed snakes (*B. jararaca* and *Crotalus durissus*). Snakes were boiled with saline for 60 min and then mixed in blender. After this process, the mixture was sifted and frozen at -20 °C for posterior use. Before offering the pasty diet, vitamin complex (Vitagold, Agrolina, São Paulo, Brazil) was added and the amount of 10% to 20% of pasty was given for all anorexic snakes. In this period, pilocarpine was already used prior to venom milking.

Group III (2011 to 2013, n = 53)

In this period, coral snakes were milked and maintained in the same way as group II, but the substrate used was bark previously treated with chlorine solution, instead of *Sphagnum* moss.

To compare the snakes' longevity between different groups, we used the survival analysis. To accomplish this, we calculated the nonparametric Kaplan–Meier estimator, also known as the product limit estimator, to estimate the survival function from lifetime data. Adjusted regression models were also used having as response the lifetime and, as covariates, food frequency; kind of substrate used; and freezing or not the preys offered. The adjusted models in this study using R software were Weibull, log-normal, logistic, log-logistic, Rayleigh and extreme value. The log-normal model was selected by the estimator Akaike information criterion as the best model. To select the covariates according to their importance in the model, we used the likelihood ratio test (Vanzolini, 1993; Colosimo and Giolo, 2006). The significance index adopted was $P < 0.01$.

RESULTS

The statistical analysis showed a significant difference among the survival probability curves of the groups ($P < 0.01$, Figure 4). In group I after 150 d in captivity, only 10% of the animals were alive, while in group II, 40% were still alive. The statistical difference between groups I and II is related mainly to improvements done on nutritional and prophylactic management. In the same way, there was a significant increase in the survival rate of snakes in group III, when compared to the other groups, representing the substrate switch from *Sphagnum* moss

Figure 4. Kaplan–Meier estimator for survival curves of *Micrurus corallinus* in the different groups. group I (●)—1997 to 1999; group II (■)—2010 and group III (▲)—2011 to 2013.

to bark. In this group, after 150 d of captivity, about 75% of the animals remained alive and healthy. Still observing the graphic (Figure 4), we can affirm that in the 90s (group I) the probability of maintaining a coral snake in captivity for more than 500 d was zero; in 2010 (group II), this probability increased to 5%, while in the period from 2011 to 2013 (group III), the probability of maintaining a coral snake for more than 500 d in captivity increased to 40%. As groups II and III had very similar protocols, we can suggest that the important factor increasing coral snakes' survival rate in the last group was the bark substrate.

In relation to the venom yielded, unfortunately in group I the range of venom obtained per animal was not recorded at that time, however, for comparative purposes, we may consider the oldest data recorded before the changes introduced in group II. In this context, the average of venom yielded in 2006 and 2007 was 2.3 mg of freeze-dried venom per animal, without using pilocarpine prior to milking. In group II, the range of venom yielded was 2.9 mg of freeze-dried venom per animal, while in group III, 3.0, 3.6, and 3.7 mg of freeze-dried venom per animal in 2011, 2012, and 2013, respectively, using pilocarpine before milking the snakes, as in group II. It can be verified an increase of 27% from 2010 to 2013.

DISCUSSION

One of the most important factor in maintaining snakes in captivity is their nutritional adequacy (Chacón et al., 2012). Contrary to *M. corallinus*, some species such as *M. surinamensis*, *M. lemniscatus*, and *M. spixii* have a higher plasticity regarding their diet, also feeding on eel-shaped fishes (Olamendi-Portugal et al., 2008; Bernarde and Abe, 2010). According to our knowledge, fishes of the species *Synbranchus marmoratus* (marbled swamp eel) are generally well accepted by semi-aquatic fish-eating species of coral snakes already adapted to captivity, which facilitates their maintenance. Other species of *Micrurus* have very specific diets essentially composed by ectothermic vertebrates such as Gymnophiona, Amphisbaenidae, and other Squamata, although fishes have also been reported as prey for some of them (Marques and Sazima, 1997; Urdaneta et al., 2004; Maffei et al., 2009; Tivador et al., 2011). Marques et al. (2017) registered an *M. frontalis* feeding on a rotten pit viper in the field. Chacón et al. (2012) reported success maintaining *M. nigrocinctus* with thawed tilapia fillets (*Oreochromis* sp.) supplemented with calcium

and vitamin D, which according to the authors is an alternative diet easy to obtain and that increased the gain of weight and longevity of the snakes.

The acceptability of alternative diets in captivity seems to vary among different species of *Micrurus*. Unfortunately, in our experience, *M. corallinus* does not accept diet based on fish meat, even when eel-like fishes are offered. The natural diet of *M. corallinus* consists mainly of elongated preys such as amphisbaenids, some lizards (specially legless ones), caecilians, and some species of semifossorial snakes (Marques and Sazima, 1997), which represents a challenge in its maintenance in captivity given the fact that the majority of these items are difficult to keep and reproduce in captivity as live food.

The use of live preys, despite providing a remarkable food stimulus to coral snakes, presents several problems from the health point of view, mainly if the items come from nature, since wild snakes, lizards, and amphisbaenians can carry a huge number of harmful pathogens, as well as parasites encysted in their muscles, liver and serous, serving as intermediate or paratenic hosts for various diseases (Grego, 2004; Schumacher, 2006; Radhakrishnan et al., 2009; Wang et al., 2011). Freezing food items before consumption can be the most reasonable control measure for parasites (Adams et al., 1997; Molina-Garcia and Sanz, 2002), besides reducing the numbers of some enteric viruses and bacteria (Georgsson et al., 2006; Butot et al., 2008). Therefore, freezing food items (-20°C) after euthanasia, before offering them to coral snakes, seems to be an important prophylactic measure to control parasitic and some microbiological diseases.

Usually, newly arrived *M. corallinus* often refuses to eat thawed preys, even when the item makes part of its regular diet. For this reason, offering live preys is important to trigger coral snake's predatory behavior. In this context, another fact that seemed to have had an important effect on the increased survival rates of group II, when compared to group I, was the use of captive bred cornsnakes offered as live food, emerging as a viable solution to feed coral snakes that refused to feed on thawed preys. *Pantherophis guttatus* is an exotic prolific species, easy to maintain and to reproduce in captivity and whose offspring are born with appropriate size to feed young and adult specimens of *M. corallinus*.

Despite efforts, some specimens still refuse to eat voluntarily, what is often critical as anorexic snakes can be more susceptible to pathogens (Rossi, 2005b; Scullion and Scullion, 2009; Grange, 2014).

In these cases, force-feeding by gavage is a recommended method widely used by several institutions, zoos and snake keepers to maintain the body condition of the animals (Mishima and Lin, 1972; Panizzutti et al., 2001). Winter et al. (2017) had success in feeding *Micrurus altirostris* by gavage with a pasty diet composed by chicken liver, eggs, vitamin, and mineral supplementation, administered every 15 d, getting a median longevity of 1,485 d and a maximum of 1,940 d for this species in captivity.

Our first protocol of force-feeding (group I) used a pasty diet made of commercial reptile food (Reptomin, Tetra, São Paulo, Brazil), given in a proportion of 10% to 20% of the snake's body weight. However, this pasty caused diarrhea in most coral snakes. Although we were not able to determine the causes of this diarrhea, the feces seemed to be composed mainly by undigested material. The pasty developed and used at LH since 2010 (group II) has a content similar to what the coral snakes would feed in nature; ceased the diarrhea and, furthermore, increased coral snakes' weight. This new recipe using as main ingredient thawed snakes has brought the highest success rates in relation to maintenance and gain of weight in anorexic animals.

Besides the new pasty recipe, freezing prey items to control diseases before offering them to coral snakes; in addition to the employment of captive bred corn snakes as live food, highly increased the survival rates of group II, when compared to group I ($P < 0.01$). Although a significant difference between the survival curves of groups I and II was observed, mainly due to nutritional changes, the greatest increase in longevity occurred in animals of group III, probably related to substrate change.

Over the years, different types of substrates had been tested at our *Micrurus* facility (soil, xaxim powder, and *Sphagnum* moss) and though all of them resembled the natural forest floor of the coral snake's habitat, they were not efficient in maintaining optimum humidity levels. In general, the substrates mentioned above tended to store too much water, leaving the environment excessively humid and prone to bacterial colonization. Therefore, snakes kept on these substrates were frequently affected by blister disease (Frye, 1991; Rossi, 2005a; Jacobson, 2010), a bacterial disease that affects reptiles housed in high humidity environments (Hoppmann and Barron, 2007). Blister disease consists of an epidermal blistering throughout the body, which may progress to epidermal ulceration, caseous necrosis, subcutaneous abscess, and septicemia (János et al., 2012). Snakes affected by this

disease usually come to death quickly and it seems that *M. corallinus* is especially prone to this condition (personal communication).

It is noteworthy that after using bark as substrate, no other cases of blister disease were identified in our colony. Besides the provision of good shelter, the main advantage of bark over the other substrates is related to the capacity of this material to retain humidity without keeping the environment soaked, maintaining abiotic conditions more conducive to *M. corallinus*. According to Owen et al. (2008), bark substrate provides an excellent aeration with less water holding capacity. Moreover, the spaces between the wood pieces resemble the leaf-litter air circulation of the snake's natural habitat.

Although the survival rate of group II had increased when compared to group I ($P < 0.01$), there was still a huge incidence of blister disease, even when the coral snakes were feeding regularly and gained weight. As the only husbandry difference between groups II and III was the change of substrate, probably this single measure was responsible for the huge increase in the survival rate of *M. corallinus*.

As milking in group I was not performed with the aid of pilocarpine, comparison between the venom yielded in this period and the other groups was not feasible. However, it is possible to observe an increase of 27% in the venom yielded in group III, when compared to group II, probably due to the improvement in snakes' welfare after the last husbandry protocol implemented, culminating in the snakes' gain of weight and expanded lifespan. According to Carvalho et al. (2014), working with different species of Brazilian coral snakes, there was a strong tendency of positive correlation between body size and amount of venom extracted. Nowadays, 22% of our coral snakes have more than 1,915 d in captivity (1,915 to 3,160 d), 28% more than 1,104 d (1,104 to 1,550 d) and 50% have less than 820 d in captivity. Our eldest *M. corallinus* was born in captivity in February, 2010 and has more than 3,100 d old (8 yr and 7 mo).

The advances were of fundamental importance to reach adequate production of antivenom serum, both in quantity and quality. The next step of our group will be the reproduction of this species in captivity.

CONCLUSION

In summary, results of this study indicate that measures implemented in *Micrurus* facility as the use of bark to avoid excessive moist; captive bred cornsnakes offered as live food, instead of using

live prey items from nature that can be vectors of several diseases; the use of thawed snakes to eliminate or decrease some microorganisms and parasites encysted; and finally, the development of a pasty diet offered to anorexic snakes similar to what coral snakes would eat in nature, proved to be efficient in providing an increase in coral snakes' welfare and, consequently, in their life expectancy, weight gain and increase in venom obtainment, contributing to national public health over this neglected tropical disease. Furthermore, the administration of pilocarpine prior to milking increased the quantity of venom obtained in extraction.

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LITERATURE CITED

- Adams, A. M., K. D. Murrell, and J. H. Cross. 1997. Parasites of fish and risks to public health. *Rev. Sci. Tech.* 16:652–660.
- Aird, S. D., N. J. da Silva, L. Qiu, A. Villar-Briones, V. A. Saddi, M. Pires de Campos Telles, M. L. Grau, and A. S. Mikheyev. 2017. Coralsnake venomics: analyses of venom gland transcriptomes and proteomes of six Brazilian taxa. *Toxins* 9:187. doi:10.3390/toxins9060187.
- Arroyo, O., J. P. Rosso, O. Vargas, J. M. Gutiérrez, and L. Cerdas. 1987. Skeletal muscle necrosis induced by a phospholipase A2 isolated from the venom of the coral snake *Micrurus nigrocinctus nigrocinctus*. *Comp. Biochem. Physiol. B.* 87:949–952. doi:10.1016/0305-0491(87)90417-2
- Barros, A. C., D. P. Fernandes, L. C. Ferreira, and M. C. Dos Santos. 1994. Local effects induced by venoms from five species of genus *Micrurus* sp. (coral snakes). *Toxicon* 32:445–452. doi:10.1016/0041-0101(94)90296-8
- Bernarde, P. S., and A. S. Abe. 2010. Food habits of snakes from Espigão do Oeste, Rondônia, Brazil. *Biota Neotropica* 10:167–173. doi:10.1590/S1676-06032010000100017
- Brazil, O. V. 1987. Coral snake venoms: mode of action and pathophysiology of experimental envenomation. *Revista do Instituto de Medicina Tropical de São Paulo* 29:119–126. doi:10.1590/S0036-46651987000300001
- Bucarety, F., S. Hyslop, R. J. Vieira, A. S. Toledo, P. R. Madureira, and E. M. de Capitani. 2006. Bites by coral snakes (*Micrurus* spp.) in Campinas, state of São Paulo, southeastern Brazil. *Rev. Inst. Med. Trop. São Paulo* 48:141–145. S0036-46652006000300005
- Butot, S., T. Putallaz, and G. Sánchez. 2008. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *Int. J. Food Microbiol.* 126:30–35. doi:10.1016/j.ijfoodmicro.2008.04.033
- Campbell, J. A., and W. W. Lamar. 2004. The venomous reptiles of western hemisphere. Ithaca and London: Cornell University Press.
- Carvalho, A. V., C. F. David, A. M. Pessoa, and N. J. Silva Jr. 2014. Um estudo do rendimento do veneno de cobras corais brasileiras e seu uso na avaliação do soro antielapídico. *Sci. Med.* 24:142–149. doi:10.15448/1980-6108.2014.2.16119

- Cecchini, A. L., S. Marcussi, L. B. Silveira, C. R. Borja-Oliveira, L. Rodrigues-Simioni, S. Amara, R. G. Stábeli, J. R. Giglio, E. C. Arantes, and A. M. Soares. 2005. Biological and enzymatic activities of *Micrurus* sp. (coral) snake venoms. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 140:125–134. doi:10.1016/j.cbpb.2004.11.012
- Chacón, D., S. Rodríguez, J. Arias, G. Solano, F. Bonilla, and A. Gómez. 2012. Maintaining coral snakes (*Micrurus nigrocinctus*, Serpentes: Elapidae) for venom production on an alternative fish-based diet. *Toxicology.* 60:249–253. doi:10.1016/j.toxicol.2012.04.332
- Ciscotto, P. H., B. Rates, D. A. Silva, M. Richardson, L. P. Silva, H. Andrade, M. F. Donato, G. A. Cotta, W. S. Maria, R. J. Rodrigues, et al. 2011. Venomic analysis and evaluation of antivenom cross-reactivity of South American *Micrurus* species. *J. Proteomics* 74:1810–1825. doi:10.1016/j.jprot.2011.07.011
- Colosimo, E., and S. R. Giolo. 2006. *Análise de sobrevivência Aplicada*. 1st ed. São Paulo: Edgard Blucher; p. 367.
- Corrêa-Netto, C., I. L. M. de Junqueira-de-Azevedo, D. A. Silva, P. L. Ho, M. Leitão-de-Araújo, M. L. M. Alves, and J. J. Calvete. 2011. Snake venomomics and venom gland transcriptomic analysis of Brazilian coral snakes, *Micrurus altirostris* and *M. corallinus*. *J. Proteomics.* 74:1795–1809. doi:10.1016/j.jprot.2011.04.003
- Costa, H. C., and R. S. Bérnils. 2015. Répteis brasileiros: lista de espécies 2015. *Herpetologia Brasileira.* 4:75–93.
- Cruz, L. S., R. Vargas, and A. A. Lopes. 2009. Snakebite envenomation and death in the developing world. *Ethn. Dis.* 19 (1 Suppl. 1):S1–42.
- Frye, F. L. 1991. *Reptile care: an atlas of diseases and treatments*. Neptune City, NJ: TFH Publications.
- Georgsson, F., A. E. Thörnqvist, M. Geirsdóttir, J. Reiersen, and N. J. Stern. 2006. The influence of freezing and duration of storage on *Campylobacter* and indicator bacteria in broiler carcasses. *Food Microbiol.* 23:677–683. doi:10.1016/j.fm.2005.10.003
- Grange, F. L. 2014. Causes, diagnosis and treatment of reptile anorexia. *World Small Animal Veterinary Association, World Congress Proceedings, Johannesburg City Parks and Zoo; Parkview, Johannesburg, South Africa.* accessed December 15, 2018. <https://www.vin.com/apputil/content/defaultadv1.aspx?pId=12886&id=7054670>.
- Grego, K. F. 2006. Determinação dos níveis de corticosterona e hormônios esteróides sexuais, induzidos pelo estresse da contenção física e da extração de veneno, em *Bothrops jararaca* (Ophidia: Viperidae). PhD Diss. Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Brazil.
- Grego, K. F., C. H. Gardiner, and J. L. Catão-Dias. 2004. Comparative pathology of parasitic infections in free-ranging and captive pit vipers (*Bothrops jararaca*). *Vet. Rec.* 154:559–562. doi:10.1136/vr.154.18.559
- Gutiérrez, J. M., O. Arroyo, F. Chaves, B. Lomonte, and L. Cerdas. 1986. Pathogenesis of myonecrosis induced by coral snake (*Micrurus nigrocinctus*) venom in mice. *Br. J. Exp. Pathol.* 67:1–12.
- Gutiérrez, J. M., G. León, and T. Burnouf. 2011. Antivenoms for the treatment of snakebite envenomings: the road ahead. *Biologicals.* 39:129–142. doi:10.1016/j.biologicals.2011.02.005
- Gutiérrez, J. M., B. Lomonte, E. Portilla, L. Cerdas, and E. Rojas. 1983. Local effects induced by coral snake venoms: evidence of myonecrosis after experimental inoculations of venoms from five species. *Toxicon* 21:777–783. doi:10.1016/0041-0101(83)90066-1
- Ho, P. L., M. B. Soares, T. Yamane, and I. Raw. 1995. Reverse biology applied to *Micrurus corallinus*, a South American coral snake. *J. Toxicol.* 14:327–337. doi:10.3109/15569549509019467
- Hopmann, E., and H. W. Barron. 2007. *Dermatology in reptiles.* J. Exotic Pet Med. 16:210–224. doi:10.1053/j.jepm.2007.10.001
- Jacobson, E. 2010. *Infectious diseases and pathology of reptiles: color atlas and text.* Boca Raton, FL: CRC Press.
- János, D., K. Imre, V. Herman, and C. Nicolae. 2012. A case of blister disease to boa constrictor. *Vet. Med.*:88–91.
- Klsbister, G., and A. Silva. 2018. Addressing the global challenge of snake envenoming. *The Lancet.* 392:619–620. doi:10.1016/S0140-6736(18)31328-X
- Leão, L. I., L. H. Paulo, L. M. I. Junqueira-de-Azevedo. 2009. Transcriptomic basis for an antiserum against *Micrurus corallinus* (coral snake) venom. *BMC Genomics.* 10:112. doi:10.1186/1471-2164-10-112
- Maffei, F., G. R. Nascimento, and D. G. Neto. 2009. Predation on the lizard *Ameiva ameiva* (Sauria: Teiidae) by a coral snake *Micrurus frontalis* (Serpentes: Elapidae) in Brazil. *Herpetol. Notes.* 2:235–237.
- Marques, O. A., R. Z. Coeti, P. A. Braga, and I. Sazima. 2017. A rotten choice: feeding attempt by a coral snake (*Micrurus frontalis*) on a dead pitviper (*Bothrops jararaca*) that had swallowed a bulky rodent. *Herpetol. Notes.* 10:137–139.
- Marques, O. A., and I. Sazima. 1997. Diet and feeding behavior of the coral snake, *Micrurus corallinus*, from the Atlantic forest of Brazil. *Hepetol. Nat. Hist.* 5: 88–93.
- Ministério da Saúde, Sistema de Informação de Agravos de Notificação—Sinan Net. 2018. Acidente por animais peçonhentos, serpentes peçonhentas. Notificações registradas: banco de dados. – accessed July 29, 2018. <http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinanet/cnv/animaisbr.def>.
- Mishima, S., and Y. Lin. 1972. Experimental studies on the extraction and force-feeding of some venomous snakes. *The Snake.* 4:51–58.
- Molina-Garcia, A. D., and P. D. Sanz. 2002. Anisakis simplex larva killed by high-hydrostatic-pressure processing. *J. Food Prot.* 65:383–388.
- Morais-Zani, K., C. Serino-Silva, N. D. C. Galizio, L. J. Tasima, J. F. Pagotto, M. M. T. D. Rocha, J. R. Marcelino, S. S. Sant’Anna, A. K. Tashima, A. M. Tanaka-Azevedo, et al. 2018. Does the administration of pilocarpine prior to venom milking influence the composition of *Micrurus corallinus* venom? *J. Proteomics* 174:17–27. doi:10.1016/j.jprot.2017.12.010
- Olamendi-Portugal, T., C. V. Batista, R. Restano-Cassulini, V. Pando, O. Villa-Hernandez, A. Zavaleta-Martínez-Vargas, M. C. Salas-Arruz, R. C. Rodríguez de la Vega, B. Becerril, and L. D. Possani. 2008. Proteomic analysis of the venom from the fish eating coral snake *Micrurus surinamensis*: novel toxins, their function and phylogeny. *Proteomics* 8:1919–1932. doi:10.1002/pmic.200700668
- Owen, J. S. Jr., S. L. Warren, T. E. Bilderback, D. K. Cassel, and J. P. Albano. 2008. Physical properties of pine bark substrate amended with industrial mineral aggregate. *Acta Horticult.* 779:131–138. doi:10.17660/actahortic.2008.779.14

- Panizzutti, M. H. M., M. M. D. Oliveira, J. L. Barbosa, P. L. X. Cavalcanti, O. Rocha-Barbosa. 2001. Evaluation of a balanced fresh paste diet for maintenance of captive neotropical rattlesnakes used for venom production. *J. Am. Vet. Med. Assoc.* 218:912–914. doi:10.2460/javma.2001.218.912
- Pardal, P. P., J. S. Pardal, M. A. Gadelha, L. D. A. S. Rodrigues, D. T. Feitosa, A. L. Prudente, and H. W. Fan. 2010. Envenomation by *Micrurus coral* snakes in the Brazilian Amazon region: report of two cases. *Rev. Inst. Med. Trop. Sao Paulo* 52:333–337.
- Pires, M. G., N. J. Silva, D. T. Feitosa, A. L. C. Prudente, G. A. P. Filho, and H. Zaher. 2014. A new species of triadal coral snake of the genus *Micrurus* Wagler, 1824 (Serpentes: Elapidae) from northeastern Brazil. *Zootaxa*. 4:569–584. doi:10.11646/zootaxa.3811.4.8
- Radhakrishnan, S., S. P. Kurup, and P. S. Banerjee. 2009. Endoparasitism in captive wild-caught snakes indigenous to Kerala, India. *Zoo Biol.* 28:253–258. doi:10.1002/zoo.20231
- Raw, I., R. Guidolin, K. Higashi, E. M. A. Kelen. 1991. Antivenins in Brazil. In: A. T. Tu, editor, *Handbook of natural toxins*, vol. 5. New York: Marcel Dekker Inc.; p. 557–581.
- Rosenberg, H. I., A. Bdolah, and E. Kochva. 1985. Lethal factors and enzymes in the secretion from Duvernoy's gland of three colubrid snakes. *J. Exp. Zool.* 233:5–14. doi:10.1002/jez.1402330103
- Rosenfeld, G. 1971. Symptomatology, pathology and treatment of snake bites in South America. In: B. Wolfgang and E. Buckley, editors, *Venomous animals and their venoms*, vol. II. Cambridge, MA: Academic Press; p. 345–384.
- Rossi, J. V. 2005a. Dermatology. In: S. J. Divers and R. M. Mader, editors, *Reptile medicine and surgery*. Philadelphia: Elsevier Health Sciences; p. 104–117.
- Rossi, J. V. 2005b. General husbandry and management. In: S. J. Divers and R. M. Mader, editors, *Reptile medicine and surgery*. Philadelphia: Elsevier Health Sciences; p. 25–41.
- Roze, J. A. 1996. Coral snakes of the Americas: biology, identification, and venoms. Malabar, FL: Krieger Publishing; p. 328.
- Schumacher, J. 2006. Selected infectious diseases of wild reptiles and amphibians. *J. Exotic Pet Med.* 15:18–24. doi:10.1053/j.jepm.2005.11.004
- Scullion, F. T., and M. G. Scullion. 2009. Gastrointestinal protozoal diseases in reptiles. *J. Exotic Pet Med.* 18:266–278. doi:10.1053/j.jepm.2009.09.004
- Serapicos, E. D. O., and J. L. B. Merusse. 2002. Variação de peso e sobrevida de *Micrurus corallinus* sob diferentes condições de alimentação em biotério (Serpentes, Elapidae). *Iheringia: Série Zool.* 92:105–109.
- Silva, N. J., and F. B. Bucarechi. 2003. Mecanismo de ação do veneno elapídico e aspecto clínicos dos acidentes. In: J. L. C. Cardoso, F. F. O. Siqueira, F. H. Wen, C. M. S. Málaque, and H. J. Vidal, editors, *Animais Peçonhentos no Brasil: biologia, clínica e terapêutica dos acidentes*. 1st ed. São Paulo, SP: Sarvier; p. 99–107.
- Silva Júnior, N., P. R. Griffin, and S. D. Aird. 1991. Comparative chromatography of Brazilian coral snake (*Micrurus*) venoms. *Comp. Biochem. Physiol. B.* 99:287–294.
- Silva Júnior, N. J., M. G. Pires, and D. T. Feitosa. 2016. Diversidade das cobras-corais do Brasil. In: N. J. Silva Júnior, editor, *As cobras-corais do Brasil: biologia, taxonomia, venenos e envenenamentos*. Goiânia, GO: Editora da PUC; p. 79–161.
- Snyder, G. K., H. W. Ramsey, W. J. Taylor, and C. Y. Chiou. 1973. Neuromuscular blockade of chick biventer cervicis nerve-muscle preparations by a fraction from coral snake venom. *Toxicon* 11:505–508.
- Tanaka, G. D., M. d. e. F. Furtado, F. C. Portaro, O. A. Sant'Anna, and D. V. Tambourgi. 2010. Diversity of *Micrurus* snake species related to their venom toxic effects and the prospective of antivenom neutralization. *PLoS Negl. Trop. Dis.* 4:e622. doi:10.1371/journal.pntd.0000622
- The Lancet. 2015. Snake bite - the neglected tropical disease. *The Lancet*. 386:1110. doi:10.1016/S0140-6736(15)00247-0
- Tivador, E. J. III, S. Bonge, S. Rodriguez, J. R. Woniak, and W. I. Lutterschmidt. 2011. A photographic record of a rare ophidian predation event. *Southeastern Nat.* 10:561–562. doi:10.1656/058.010.0317
- Urdaneta, A. H., F. Bolaños, and J. M. Gutiérrez. 2004. Feeding behavior and venom toxicity of coral snake *Micrurus nigrocinctus* (serpentes: elapidae) on its natural prey in captivity. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 138:485–492. doi:10.1016/j.cca.2004.08.018
- Vanzolini, P. E. 1993. Métodos estatísticos elementares em sistemática zoológica. São Paulo, SP: Editora Hucitec.
- Wang, F., L. Zhou, S. Gong, Y. Deng, J. Zou, J. Wu, W. Liu, and F. Hou. 2011. Severe infection of wild-caught snakes with *Spirometra erinaceieuropaei* from food markets in Guangzhou, China involves a risk for zoonotic sparganosis. *J. Parasitol.* 97:170–171. doi:10.1645/GE-2519.1
- Warrell, D. A. 2004. Snakebites in Central and South America: epidemiology, clinical features and clinical management. In: J. A. Campbell and W. W. Lamar, editors, *The venomous reptiles of the western Hemisphere*. Ithaca, NY and London: Comstock Publishing Associates; p. 709–761.
- Williams, D. J. 2015. Snake bite: a global failure to act costs thousands of lives each year. *Br. Med. J.* 351:h5378. doi:10.1136/bmj.h5378
- Winter, A. B., M. L. Araújo, R. B. Oliveira, and M. L. M. Alves. 2017. Recorde de longevidade em cativeiro da coral-pampeana, *Micrurus altirostris* (COPE, 1860). *Anais do Congresso Brasileiro de Herpetologia*. 1:61.