v. 2, n. 5, 2022

Journal of
Agricultural
Sciences
Research

TECHNIQUES FOR CHEMICAL CONTAINMENT IN CHELONIANS

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Abstract: Chemical restraint for wild animals is necessary to perform procedures such as: physical examination, collection of biological material and imaging exams. Little is known about the effects of anesthetics on chelonians, in part, because of the rare studies and investigations. This investigation aimed to demonstrate three techniques of chemical containment for chelonians, in addition, to evaluate latency period, duration of drug effect and the degree of chemical containment. We used 6 animals, 2 of each species: Kinosternon scorpioides, Chelonoidis carbonaria and Phrynops geoffroanus, from services provided by Hovet/UEMA. Three protocols were used: the association of (Ketamine 10mg/Kg and Midazolam 1mg/Kg intramuscularly - Protocol 1), (Propofol at a dose of 35 mg/Kg intracoelomically - Protocol 2) and (Butorphanol 1 mg/kg kg intramuscularly - Protocol 3). The following criteria were evaluated: spontaneous locomotor activity, muscle relaxation and manipulation of limbs, tail, head and mouth. Protocol 1 had a mean latency period of 5 minutes and the duration of the drug's effect lasted for 95 minutes. The species Phrynops geoffroanus was more sensitive to this protocol, showing marked signs of sedation. In protocol 2, the species Chelonoidis carbonaria had more intense signs of sedation. The latency period was 3 minutes on average and the duration of the effect lasted for 80 minutes. Protocol 3 did not cause signs of chemical containment.
Keywords: Sedation, Chelonians, Propofol, Ketamine, Midazolam.

INTRODUCTION

Anesthesia or chemical restraint in reptiles is necessary to facilitate the performance of clinical examinations and obviously surgical procedures (Furtado & Sobral, 2020), as there is a great possibility of bites, scratches or poisoning, in this way, many species require, in addition to anesthesia, the use of PPE - Personal Protective Equipment (de Freitas et al., 2020).

Chemical restraint or anesthesia in chelonians must take into account the knowledge of their physiology and biology, because, as they are ectothermic, they will react unpredictably to drugs in different environmental conditions (Frye, 2007), with a generally slower metabolism than mammals or birds, moreover, pathological processes are not normally observed until it is in a very advanced state (Luçolli et al., 2017). Because they are ectothermic animals, their response to administered drugs depends on the ambient temperature.

The anesthetic protocol must produce immobilization, analgesia, and muscle relaxation for good sedation, in addition to being safe and easily reversible. 2014).

For the chemical containment of chelonians, several anesthetic protocols can be used, such as ketamine, or the association tiletamine-zolazepam, however there are reports of the application of propofol, both by the intramuscular and intracoelomatic routes (Bragagnoli et al., 2020), so this is The investigation aimed to demonstrate three chemical containment techniques for chelonians through the evaluation of the latency period, the duration of the drug's effect and the degree of chemical containment.

MATERIAL AND METHODS

The experiment was submitted to the Ethics Committee in the Use of Animals of the State University of Maranhão (CEUA – UEMA) (Protocol No. 28/2021) and was carried out at the Francisco Edilberto Uchoa Lopes University Veterinary Hospital (HVU-UEMA), and 6 animals were used, 2 of each species: Kinosternon scorpioides, Chelonoidis carbonaria and Phrynops geoffroanus.

The animals of the species Kinosternon Scorpiodis came from the Scientific Breeding Center of UEMA (authorization by IBAMA - 1899339/2008). The animals of the species Chelonoidis carbonaria and Phrynops geoffroanus, came from clinical consultations performed at the Veterinary Hospital.

The animals underwent biometry and data were collected such as: weight (Kg), Maximum straight plastron length (CMRP), Maximum straight plastron width (LMRP), Maximum straight carapace length (CMRC) and Maximum straight carapace width (LMRC)) and height in centimeters.

The management was carried out with water and food fasting for 24 hours. In addition, the animals remained in pens and were removed 1 hour before for temperature adaptation and placed in plastic boxes, kept at a temperature of 28°C throughout the experiment. For chemical containment, 3 protocols were performed: Protocol 1 – association of Ketamine (10 mg/Kg) and Midazolam (1 mg/Kg) intramuscularly; Protocol 2 – Propofol (35 mg/Kg) intracoelomically; and Protocol 3 – Butorphanol (1 mg/Kg) intramuscularly (Figure 1). For each of the protocols, 1 animal of each species was used, with the same animals used in the protocols, but with a 3-day interval between the procedures.

The following criteria were evaluated: spontaneous locomotor activity, muscle relaxation and manipulation of limbs, tail, head and mouth. The response was evaluated through a subjective score, where 0 - no manipulation possible, 1 - minimal effect, 2 - reasonable effect and 3 - maximum effect. For Spontaneous Locomotive Activity, the score was: 0 – animal can move around, 1 – animal cannot move.

To aid in the evaluation, a chart was used and all parameters were analyzed every 5 minutes (Chart 1).

After recovery, a summation was performed for each protocol. A sum greater than or equal to 9 would indicate a contained animal.

RESULTS AND DISCUSSION

Protocol 1 had a mean latency period of 5 minutes and a mean duration of restraint of 95 minutes, corroborating the work carried out by Bienzle & Boyld (1992), in the association of Ketamine (20mg/Kg) and Midazolam (2mg/Kg) in Chelydra Serpentina, whose latency period was similar to that of this protocol.

In parakeets (Psittacula krameri), the use of Midazolam (3.65mg/Kg) alone or associated with Ketamine (40-50mg/Kg), intranasally, presented a latency period of 2.7 minutes with production of sedation and permission for manipulation for small procedures (Versal & and Eskandari, 2006).

In Alligator mississippiensis, Terpin (1978), observed a latency period of 5 to 20 minutes after the application of 45 to 70 mg/kg of ketamine.

The most sensitive species submitted to this protocol was Phrynops geoffroanus, which showed marked signs of sedation and relaxation, obtaining a maximum sum of 15, corroborating Santos et al. (2009), in the administration of Midazolam 2mg/kg via IM and obtained a good muscle relaxation response in the species.

The use of ketamine alone, at a dose of 10 mg/kg, Leonardo (2012) considered its action inefficient because it did not produce pharmacological containment in the species Podocnemis expansa. Likewise, Alves Junior (2006) regarding the association of Ketamine 20mg/Kg with Midazolam 2mg/kg and Ketamine 60mg/Kg with Midazolam 2mg/Kg, the effect of muscle relaxation and the ease of handling the animals was also not observed in the Podocmemis species. expands.

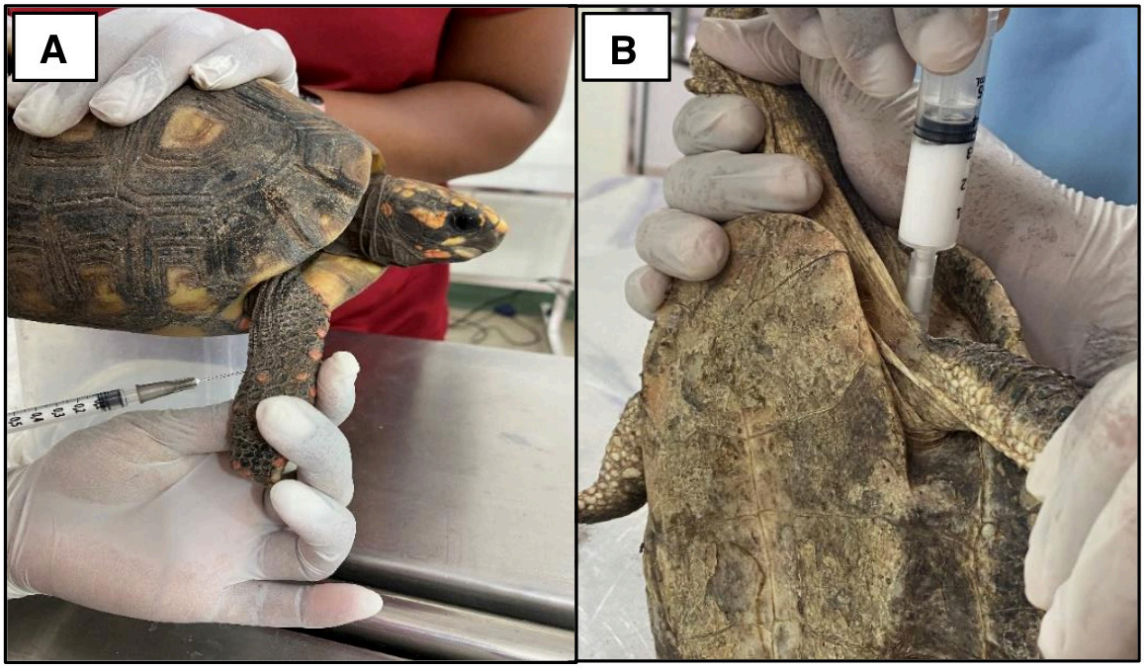

Figure 1: Intramuscular application of drugs in the thoracic limb of Chelonoidis carbonaria (A), intracoelomatic application of propofol, parallel to the fusion of the plastron with the carapace, medially to the forelimbs and laterally to the neck of Phrynops geoffroanus (B).

Parameters	TIME (minutes)																							
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	80	85	90	95	100	105	110	115	120
Spontaneous Locomotive Activity (ALE)																								
Muscular Relaxing																								
Moving the head																								
Moving the tail																								
Moving the body members																								
Moving the mouth																								
Sum																								

Table 1: Table used to evaluate the parameters every 5 minutes.

For chemical containment and tracheal lavage, Silveira et al. (2014) used Ketamine 30mg/Kg and Midazolam 2mg/Kg in Chelonoidis carbonaria. And Cordeiro (2019) in Phrynops geoffroanus, used in an experiment, varied doses of Ketamine and midazolam and found that the protocol with Ketamine (40mg/Kg) and Midazolam (3mg/Kg), intravenously, was effective for chemical containment. and performing minimally invasive procedures.

Benzodiazepines, when applied alone, have minimal sedation effect in most reptiles. Therefore, the association with dissociative drugs such as ketamine (10 to 60mg/Kg) (Valente et al.,2013).

Protocol 2 (Figure 2) had a mean latency period of 3 minutes and the duration of retention was on average 80 minutes, which differs from the results obtained by Bragagoli et al. (2020), who found a mean latency period of 16 minutes in Phrynops geoffroanus when using intracoelomatic propofol at a dose of 35mg/kg. Differently, by the intravenous route, propofol (5 mg/kg) had a latency period of 52 seconds in Caiman crocodilus (Silva Júnior, 2013).

The intracoelomic route is easily accessible and can be used as a route of administration of propofol, as long as it is used in higher doses. Furthermore, high doses of Trachemys scripta elegans produced muscle relaxation and decreased animal-environment interaction (Mouriño & Vasconcelos, 2003).

The species most sensitive to the protocol was Chelonoidis carbonaria, which had a sum of 14 (Figure 3). Bellentini et al. (2004), evaluating different doses of intramuscular and intracoelomatic propfol in Trachemys scripta, provided a good anesthetic quality in 50% of the animals using it intracoelomically at a dose of 20mg/kg. In addition, he found that even with higher doses of propofol by this route, there is no change in myorelaxation.

The association of acepromazine (0.5mg/kg via IM) and propofol (5mg/kg via IV) or acepromazine (0.5mg/kg via IM) and propofol (10mg/kg via IV) in Podocnemis expansa produced satisfactory sedation for physical examinations, collection of biological material and even less invasive surgeries (Alves Júnior, 2012).

In Lithobates catesbeianus, propofol at a dose of 20mg/Kg intracoelomate did not produce analgesia, but allowed chemical containment and the execution of clinical procedures such as collection of biological samples (Cardoso, 2012).

In protocol 3, the signs of relaxation were minimal in the 3 species, in addition, the degree of sedation was insufficient to produce chemical restraint. For the three species, the sum did not exceed 9. According to research carried out by Read (2004), butorphanol is widely used in reptiles, at doses ranging from 0.02 mg/kg to 25 mg/kg. Sladky et al. (2007) tested the analgesic efficacy and respiratory effects of butorphanol in turtles at doses of 2.8 mg/kg and 28 mg/kg. Cubas et al. (2014) suggest doses between 0.5 mg/kg to 2mg/kg, which promote minimal sedation in chelonians.

CONCLUSION

The techniques used in protocols 1 and 2 were effective in chemical containment of chelonians and allowed manipulation for experimental procedures. While protocol 3 did not provide chemical containment, which makes its use at the recommended dose difficult.

Figure 2: Kinosternon scorpioides, Chelonoidis carbonaria and Phrynops geoffroanus (left to right) 15 minutes after the application of Protocol 2. Observe the degree of sedation.

Figure 3: Chelonoidis carbonaria at 20 minutes after intracoelomic application of Propofol 35mg/kg.

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