FULL ARTICLE

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Survey of euthanasia methods used in neotropical chiropterans in São Paulo State, Brazil: critical analyses and proposal for standardization of bat euthanasia methods

Levantamento dos métodos de eutanásia utilizados em quirópteros neotropicais no estado de São Paulo, Brasil: análise crítica e proposta de padronização de métodos de eutanásia em morcegos

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ABSTRACT

Zoonotic Surveillance Divisions (ZSDs) rescue and euthanize bats in contact with humans. The euthanasia procedure should be conducted safely for the animal and caretaker and minimal stress for the animal is important. The objectives of this study were: i. evaluate the methods of euthanasia used by ZSDs in the State of São Paulo; ii. compare the methods with national and international guidelines for animal euthanasia practices; iii. assess the methodologies considering taxonomy and eating habits of the main bat species, and iv. propose standardization of euthanasia procedures. Sixty-five ZSDs locations received an online questionnaire or were contacted by telephone and 33 ZSDs (50.8%) responded and are distributed in 11 mesoregions in the state to remain anonymous. The euthanasia methods were divided into chemical (injectable or inhalation), physical, or mixed methods. Bat specimens (n = 550) were identified and classified to evaluate the main genera found in the state. The location of the ZSD, species, eating habits, and the method of euthanasia used were analyzed. The specimens by bat families were Molossidae (n = 340), Phyllostomidae (n = 171), and Vespertilionidae (n = 39). Chemical methods were used in 25 ZSDs (75.75%), physical in 5 (15.15%), and mixed in 3 (9.09%). There is no uniformity or standardization in bat euthanasia methods used by ZSDs, although most are based on acceptable chemical methods. It was proposed an algorithm to assist the veterinarian in choosing the method of euthanasia for bats that will allow standardizing euthanasia procedures for this species, considering physiological differences, and respecting technical, bioethical, and animal welfare guidelines.

Keywords: Euthanasia in bats. Animal welfare. Bioethics. Rabies control.

RESUMO

As Divisões de Vigilância de Zoonoses (DVZs) coletam e eutanasiam morcegos que tiveram contato com humanos. O procedimento de eutanásia precisa ocorrer de modo seguro para o animal e para o manipulador, sendo importante garantir o mínimo de estresse ao animal. Os objetivos deste estudo foram: i. avaliar os métodos de eutanásia praticados nas DVZ do estado de São Paulo; ii. comparar esses métodos com os guias nacionais e internacionais de práticas de eutanásia animal; iii. avaliar as metodologias aplicadas considerando a taxonomia e os hábitos alimentares das principais espécies de ocorrência de morcegos; e iv. propor a padronização dos procedimentos de eutanásia. Sessenta e cinco DVZs receberam o questionário *online* ou foram contatadas por telefone, dessas, trinta e três DVZs (50,8%) que responderam ao questionário foram incluídas no estudo e distribuídas em 11 mesorregiões para garantir anonimato. Os métodos de eutanásia reportados foram divididos em métodos químicos (injetáveis; inalatórios), físicos ou mistos. Os espécimes de morcegos (n = 550) foram identificados e classificados para avaliar os principais gêneros encontrados no estado. A localização da DVZ, as espécies, os hábitos alimentares e o método de eutanásia utilizado foram analisados. Os espécimes por família de espécies de morcegos foram Molossidae (n = 340), Phyllostomidae (n = 171) e Vespertilionidae (n = 39). Métodos químicos foram utilizados em 25 DVZs (75,75%), físicos em 5 (15,15%) e mistos em 3 (9,09%). Não há uniformidade ou padronização nos métodos de eutanásia de morcegos, embora a maioria seja baseada em métodos químicos aceitáveis. É proposto um algoritmo para auxiliar o veterinário na escolha do método de eutanásia, que

permite a padronização desses procedimentos para os morcegos, considerando as diferenças fisiológicas e respeitando as diretrizes técnicas, bioéticas e de bem-estar animal.
Palavras-chave: Eutanásia em morcegos. Bem-estar animal. Bioética. Controle da raiva.

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Introduction

Bats are the only mammals capable of true flight and represent almost 25% of all mammal species with more than 1,400 described species worldwide (Upham et al., 2022). The order Chiroptera occupies diversified ecological niches worldwide. It has a great variation in morphology and eating habits, which include fruits, nectar, blood, insects, amphibians, fish, and small mammals (Richardson, 2011). In Brazil, nine families are described, consisting of 68 genera and more than 180 species (Abreu et al., 2022) and in the State of São Paulo eight families, 43 genera, and 79 species are recognized (Garbino, 2016).

Since 1911, the role of vampire bats (*Desmodus rotundus*) in maintaining the sylvatic rabies cycle has been recognized in Brazil. This led the Ministry of Agriculture, Livestock and Food Supply to establish, in 1966, the National Program for the Control of Herbivores Rabies, with guideline techniques for epidemiological surveillance and population control of the vampire bat (Brasil, 2009). However, epidemiological surveillance of rabies also includes insectivorous and frugivorous bats (Scheffer et al., 2007), and staff that manages these species should always be trained and vaccinated for rabies (Mullineaux & Keeble, 2016).

The interaction between these mammals and humans is unusual, but it occurs more frequently during the blood meal of vampire bats, by bat invasion of human habitation, or by the uncommon consumption of bat meat, with very few reports in Brazil (Lawson et al., 2019; Li et al., 2019; Setz & Sazima, 1987). Despite infrequent contact, this interaction can be important and enough to transmit rabies to humans and domestic animals (Benavides et al., 2020; Castilho et al., 2017). In this sense, it is up to the Zoonotic Surveillance Division (ZSD) of the municipalities to respond to any call from citizens who report having found a bat. After removal or rescue, those animals still alive are euthanized to obtain biological samples used in epidemiological monitoring of rabies in official laboratories (Dezorzi, 2020).

The term "euthanasia", of Greek origin in the terms "*eu*" (good) and "*thanatos*" (death), refers to the process of inducing "good death", without pain and with minimal stress as a humane way of killing the animal (Brasil, 2018). Animal euthanasia is a circumstantially necessary procedure in veterinary medicine, but it must follow recognized protocols such as those described in the Guide for Good Animal Euthanasia (Brasil, 2018; Conselho Federal de Medicina Veterinária, 2013). Despite being aimed at animal species used in teaching and animal experimentation, the guide represents a national technical guideline, establishing methodologies that are acceptable or unacceptable for each animal species (Brasil, 2018; Conselho Federal de Medicina Veterinária, 2013).

However, there are no official technical guidelines for euthanasia methods for bats in Brazil, which represents a gap in knowledge and a potential for accidents. Thus, it was established the hypothesis that the methods of euthanasia for bats are the same used in small mammals, such as rodents and domestic animals, in the State of São Paulo, and it was proposed to survey the methods of bat euthanasia applied by ZSDs in the state. Additionally, it was conducted a survey of the species of bats with the highest occurrence in the state. From the comparison and analysis of the methods recommended for animal euthanasia, it was proposed an algorithm to standardize the methods of euthanasia to be used in bats.

Materials and Methods

An epidemiological survey was conducted on the methods of bat euthanasia employed in ZSDs in the State of São Paulo, Brazil, through an open questionnaire sent by e-mail to 65 municipal ZSDs in São Paulo, in two stages, conducted from October 2017 to August 2018 and from January to February 2019. The ZSDs were asked to respond, voluntarily and anonymously, as it was not collected the

name of the person who provided the answer and, after that, it was associated the ZSDs with mesoregions of de State of São Paulo, the electronic questionnaire is available on the Google Forms virtual platform (available at: shorturl.at/qrxAV). The questionnaire contained a short introductory explanatory text and response fields with the contact email address (only for internal control), what condition the bats were received (alive or dead), and which euthanasia method was used. The second stage was conducted through telephone contact and included the same questions to the ZSDs, that had not been answered electronically. The ZSDs were associated with the mesoregions of the State of São Paulo for analysis (Instituto Brasileiro de Geografia e Estatística, 1990).

The methods of euthanasia practiced were categorized into chemical method (injectable and inhaled agent), physical (hypothermia or traumatic brain injury/cervical traction), or mixed (mixture of injectable and inhaled agents, or a chemical and a physical agent).

The survey of the species of bats occurring in the São Paulo State was based on the database of 550 bats sent by the ZSDs of São Paulo for the diagnosis of rabies at the Pasteur Institute (Health Secretary, São Paulo State) in the same period. This study was approved by the Ethic Committee in the Use of Animals of School of Veterinary Medicine and Animal Science (CEUA - FMVZ/USP) under protocol N° 9836240517.

The information provided by the ZSDs was tabulated and analyzed using descriptive statistics. The analysis considered the taxonomic classification and eating habits of the bats described in the literature, in addition to the location of the ZSD in the mesoregion of the state and the method of euthanasia used. Based on the comparative analysis of national and international guidelines on animal euthanasia, an algorithm for choosing the euthanasia protocol was proposed to standardize the euthanasia methods for bats.

Results

Of the 65 ZSDs, 37 (57%) responded to the questionnaire, and of these, four (10.8%) reported not performing euthanasia because they received bats already dead. The evaluated data were based on information reported by 33 (50.8%) ZSDs distributed in 11 mesoregions in the state: 1 - São José do Rio Preto; 2 - Ribeirão Preto; 3 - Araçatuba; 5 - Araraquara; 6 - Piracicaba; 7 - Campinas; 9 - Marília; 11 - Itapetininga; 12 - São Paulo Metropolitan Macro; 13 - Vale do Paraíba Paulista and 15 - Metropolitan of São Paulo (Figure 1).

Fourteen (42.42%) ZSDs indicated the use of inhaled chemical agents; 11 ZSDs (33.33%) reported using methods of euthanasia by an injectable chemical agent, with or without drug associations; five (15.15%), used physical methods, and three (9.09%), mixed methods. Figure 2 shows the distribution of euthanasia methods used in ZSDs.

Eighteen ZSDs (54.54%), corresponding to six mesoregions (2, 6, 7, 12, 13, and 15) and 18 municipalities, routinely employ acceptable methods with injectable drugs (ketamine + thiopental + potassium chloride [KCl]; phenobarbital and thiopental + KCl), inhaled (isoflurane) and mixed (isoflurane + thiopental and isoflurane + thiopental + KCl). The use of isoflurane was reported by four ZSDs (12.12%), occurring in four mesoregions (2, 6, 7, and 15), corresponding to eight municipalities. In 13 (39.39%), corresponding to eight mesoregions (1, 2, 3, 7, 9, 11, 13, and 15), with 13 municipalities, unacceptable methods such as ether inhalation, formalin + hypothermia were reported; inhalation of formaldehyde or alcohol combined with hypothermia; association of thiopental + lidocaine; traumatic brain injury and the compound mebezonium iodide, embutramide and tetracaine hydrochloride. Mixed methods of euthanasia were routinely practiced in ZSDs

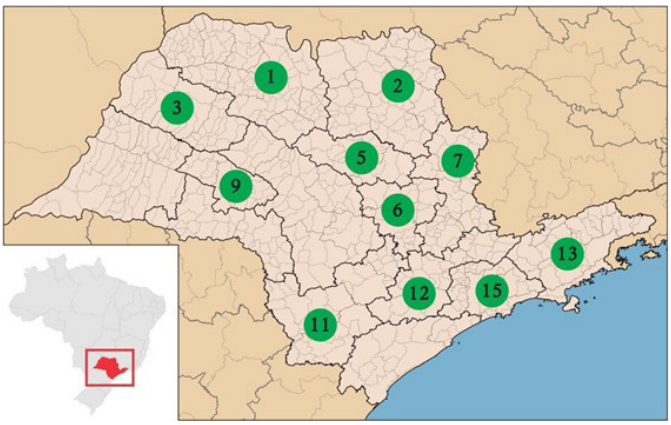

Figure 1 – Map of the State of São Paulo. Geographical distribution of the 11 mesoregions of the State of São Paulo included in the study. Source: Adapted from Abreu (2006).

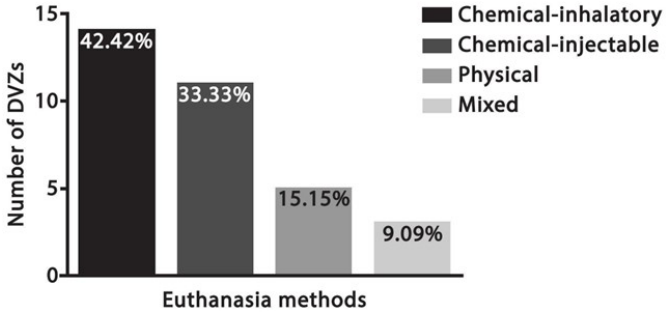

Figure 2 – Distribution of the euthanasia methods. Methods of euthanasia practiced in bats in Zoonotic Surveillance Divisions (ZSDs) in São Paulo, Brazil, distributed according to absolute and relative numbers (%). Source: Lauri (2019).

corresponding to mesoregion 15 and two municipalities and include the complementary application of KCl in duly anesthetized animals. In the study, two ZSDs (6.06%), corresponding to two mesoregions (5 and 6) and two municipalities, used cervical traction alone. Four ZSDs (12.12%), corresponding to four mesoregions (2, 3, 7, and 15) and five municipalities, indicated the isolated use of ether inhalation in euthanasia.

The analyzed population of bats was divided into three families: Molossidae (n = 340), Phyllostomidae (n = 171), and Vespertilionidae (n = 39). This population of bats included 29 species, the most frequent *Molossus molossus* (n = 162), *Eumops glaucinus* (n = 76), *Glossophaga soricina* (n = 69), *Artibeus lituratus* (n = 60), *Molossus rufus* (n = 36), *Cynomops planirostris* (n = 22), *Myotis nigricans* (n = 20), *Desmodus rotundus* (n = 16), *Eptesicus furinalis* (n = 13), *Nyctinomops laticaudatus* (n = 13), *Sturnira lilium* (n = 12), *Eumops perotis* (n = 11), *Platyrrhinus lineatus* (n = 9), *Eumops auripendulus* (n = 6), and *Tadarida brasiliensis* (n = 6). The remaining ones add up to 19 individuals, which are distributed in three or fewer specimens by the species *Anoura caudifer*, *Artibeus fimbriatus*, *Artibeus planirostris*, *Cynomops greenhalli*, *Eumops perotis*, *Histiotus* sp., *Lasiurus ega*, *Myotis albescens*, *Nyctnomops aurispinosus*, and *Phyllostomus discolor*, among other specimens belonging to the Molossidae family. Table 1 shows the distribution of the most frequent families and species of bats by mesoregion, as well as the predominant eating habits, with mollossids being considered insectivores, phyllostomids being phytophagous and hematophagous, and vespertilionids being insectivores. Table 2 shows the distribution of reported euthanasia methods, according to mesoregion and families of bats.

Table 1 – Bat families and species included in the study, distributed according to predominant feeding habits and mesoregions of greater occurrence in the State of São Paulo

Family	Frequent species of bats	Predominant eating habit[a]	Occurrence mesoregion(s)
Molossidae	*Cynomops greenhalli*	INS	7
	Cynomops planirostris	INS	2, 6 and 7
	Eumops auripendulus	INS	5
	Eumops glaucinus	INS	1, 2, 3, 5, 6, 7 and 9
	Eumops perotis	INS	2, 5 and 7
	Molossus molossus	INS	1, 2, 3, 5, 6, 7, 9, 11, 12, 13 and 15
	Molossus rufus	INS	1, 2, 3, 5, 6, 7, 9, 13 and 15
	Nyctinomops aurispinosus	INS	2
	Nyctinomops laticaudatus	INS	2 and 7
	Tadarida brasiliensis	INS	7 and 15
Phyllostomidae	*Anoura caudifer*	FTG	12
	Artibeus fimbriatus	FTG	5
	Artibeus lituratus	FTG	2, 5, 6, 7, 13 and 15
	Artibeus planirostris	FTG	7
	Desmodus rotundus	HTF	3 e 5
	Glossophaga soricina	FTG	2, 5, 6, 7, 12 and 15
	Phyllostomus discolor	FTG, INS, AM	2
	Platyrrhinus lineatus	FTG	2 and 7
	Platyrrhinus sp.	FTG	2
	Sturnira lilium	FTG	2, 7, 12, and 15
Vespertilionidae	*Eptesicus furinalis*	INS	2, 7 and 15
	Eptesicus perotis	INS	5
	Histiotus sp.	INS	6
	Lasiurus ega	INS	2 and 6
	Myotis albescens	INS	7
	Myotis nigricans	INS	2, 7, 12 and 15

[a]INS = insectivore; FTG = phytophagous; HTF = hematophagous; AM = amphibian.

Table 2 – Bat euthanasia methods reported by 33 ZSDs in the State of São Paulo, distributed according to mesoregion and main families of bats.

Mesoregion	Bat families[a]	Euthanasia methods[1] (classification)[b]
1	Mol	formalin + hypothermia; hypothermia (U)
2	Mol and Ves	isoflurane (A); ether (U)
	Phy	ketamine + thiopental + KCl (A)
3	Mol	ether (U)
5	Mol and Phy	cervical traction (R)
6	Mol and Ves	isoflurane (A)
	Phy	cervical traction (R)
7	Mol and Ves	isoflurane (A); ether (U)
	Phy	phenobarbital (A); thiopental (A); thiopental + lidocaine (U)
9	Mol	traumatic brain injury (U)
11	Mol	compound mebezonium iodide, embutramide, and tetracaine hydrochloride (U); formaldehyde or alcohol (U)
12	Mol	thiopental (A)
	Phy	ketamine + xylazine (A)
13	Mol and Phy	thiopental + KCl (A); compound mebezonium iodide, embutramide, and tetracaine hydrochloride (U)
15	Mol, Phy, and Ves	ether (U); hypothermia (U); isoflurane (A); isoflurane + thiopental (A); isoflurane + thiopental + KCl (A); thiopental (A)

[1]KCl: potassium chloride; [a]Mol: Molossidae; Phy: Phyllostomidae; Ves: Vespertilionidae; [b]Classification of euthanasia method used: A: acceptable, R: acceptable with restrictions; U: unacceptable.

Discussion

In São Paulo State, the ZSDs use isolated physical or chemical methods to mixed methods for the euthanasia of bats, but the procedures were identified as non-uniform, non-standard, and different from the recommended methods used in rodents and domestic animals. The lack of standardization of euthanasia protocols for bats may be partly related to the fact that bats are not specifically included in the euthanasia guides for experimental animals or field studies, as is the case with rodents and domestic animals and partly due to the lack of indication and criteria in the literature on how to choose the method (Brasil, 2018).

Most ZSDs indicated the use of injectable chemical agents (ketamine + thiopental + KCl; phenobarbital or thiopental + KCl), isolated inhalers (isoflurane), or mixed (isoflurane + thiopental and isoflurane + thiopental + KCl) to perform the euthanasia of bats, regardless of taxonomic classification and/or eating habits. These methods were considered acceptable and comparable to national and international guides (American Veterinary Medical Association, 2020; Conselho Federal de Biologia, 2019; Conselho Federal de Medicina Veterinária, 2013). The use of injectable or inhaled sedatives before the euthanasia procedure reduces animal stress and the risk of accidents with humans (Brasil, 2018). Death is achieved with the subsequent intravenous (IV) or intracardiac (IC) application of KCl (Cooney, 2020), associated or not with a neuromuscular blocker or with the use of a carbon dioxide, carbon monoxide, or another inert gas chamber (Conselho Federal de Medicina Veterinária, 2013; American Veterinary Medical Association, 2020). But it was expected some side-effects with the animal being aware, in the case of injections for sedation, or even with the animal unconscious, as agonizing breath, that can be technically unacceptable and uncomfortable to the operator (Robertson, 2020). Although the use of injectable drugs, such as barbiturates (pentobarbital, sodium thiopental), propofol, ketamine, and the association of ketamine with xylazine has been indicated as a way of wild species of native fauna containment (Conselho Federal de Biologia, 2019), others have seen the safety margin of those as too narrow for be used in bats, especially in sedation situations (Bexton & Couper, 2010) since its uses go off-label for all species that are not canines and so they are not approved by FDA (Cooney, 2020).

However, as the application of ketamine as a sedative can cause an excitation phase in insectivorous bats (Lollar, 2010), the use of this drug needs to be applied with caution given the lack of precise information regarding the anesthesia and euthanasia methods of chiropterans. Inhaled agents require additional consideration as isoflurane has a pungent odor and should be avoided by animals capable of holding their breath (American Veterinary Medical Association, 2020). It is known that this drug works well in the *Pteropus* spp. (Irving et al., 2020; Jonsson et al., 2004) but future studies need to be done to confirm the apnea ability in other species. Halothane, on the other hand, is considered ideal for those animals because it has a pleasant odor and lower cost, but it can be toxic to human operators (Brasil,

2018). Thus, the choice of the inhalation agent needs to be judicious, and the veterinarian must consider the favorable and unfavorable points in each situation.

Methods based on inhalation of ether, formaldehyde, or alcohol + hypothermia, the association of thiopental + lidocaine; traumatic brain injury, and the application of the compound mebezonium iodide, embutramide, and tetracaine hydrochloride are reported as unacceptable methods (American Veterinary Medical Association, 2020; Brasil, 2018; Conselho Federal de Medicina Veterinária, 2013; Lollar, 2010; Michigan Rabies Working Group, 2018). Although the use of ether and anticoagulant paste are among technical indications for the euthanasia of vampire bats (Brasil, 2009; Costa & Esbérard, 2011), as well as nitrogen, argon, and nitric oxide are accepted methods with restrictions in the euthanasia of rodents and small mammals (Conselho Federal de Medicina Veterinária, 2013). Drugs composed of mebezonium iodide, embutramide, and tetracaine hydrochloride, although used for euthanasia in dogs, contain a curariform that culminates in muscle paralysis and, consequently, cardiorespiratory collapse before the loss of consciousness and should be avoided as a single agent, as its application in the euthanasia of bats causes death without loss of consciousness (Conselho Federal de Medicina Veterinária, 2013; Lollar, 2010). Hypothermia is not indicated in bats, as although Brazilian species do not hibernate, animals could wake up when body temperature reaches levels close to lethal (Lollar, 2010).

Methods of euthanasia accepted with restriction, such as cervical traction (Brasil, 2018), were reported by the ZSDs. Such methods are restricted because they do not meet all ideal criteria. For example, they are methods that require specific knowledge, skills, and techniques to be performed accurately (American Veterinary Medical Association, 2020; Conselho Federal de Medicina Veterinária, 2013).

The routes of application of IV, IC, intramuscular (IM), and intraperitoneal (IP) drugs need to be considered with reservations, since their use requires the skill and knowledge of the handler to avoid accidents during the execution of euthanasia in bats, due to their small size (Bexton & Couper, 2010; Brasil, 2018). The IM and IP routes are feasible in chiropterans, compared to the IV and IC, which are not recommended due small size of bats. Even without the presence of someone connected emotionally with the species of animal, as happened with the euthanasia of a dog or cat, it is necessary for the welfare of the handler that the animal has a quick and effective death. In the IP route, the absorption takes place in the serosal membranes, avoiding those barbiturates with phenytoin that affects the heart first before unconsciousness occurs (Cooney, 2020). In addition to the pharmacological methods, it is important to reduce stress on the bat or to reduce tension in the person who is performing the euthanasia so they're better able to perform it correctly (Shearer, 2020).

The generalization of the euthanasia method may not be suitable for any bats, as was highlighted by some authors due to the physiological characteristics and, indirectly, eating habits of these animals can make it difficult to perform euthanasia ethically, with the safety of the performer and guarantee of animal welfare at the time of death (Brasil, 2018; Lollar, 2010). Knowledge of the family, species, and eating habits can be critical for choosing the euthanasia method for bats, as it points to their physiological particularities. The Phyllostomidae family has predominantly phytophagous bats and it is indicated for their euthanasia the use of IM ketamine sedation, followed by IP barbiturate overdose, or the isolated use of the carbon dioxide trap, which are forms of euthanasia recognized by other authors (Close et al., 1996; Conselho Federal de Biologia, 2019; Michigan Rabies Working Group, 2018; Reilly, 2001).

In the euthanasia of insectivorous bats of the Molossidae and Vespertilionidae families, the use of the above methods is not indicated. Ketamine causes the excitatory phase before the loss of consciousness (Lollar, 2010), and the carbon dioxide trap is also contraindicated for insectivores, as there is evidence of tolerance to this gas and the animals present respiratory distress for one to three minutes, with the return of sedation during the process (Lollar, 2010). Thus, the suggested method for insectivorous bats is the use of an inhaled agent, such as isoflurane or halothane, in overdose or for a long period until definitive respiratory arrest occurs (Lollar, 2010) or the use of thiopental overdose and KCL IP.

The recognized morphological diversity of bats in Brazil, associated with the difficulty of correct taxonomic identification and data limitations on the geographic distribution of species occurring in the State of São Paulo, as well as in other regions of the country, are factors that hinder the establishment of a default standardized method of euthanasia to be applied to all bat species in the state, or in Brazil. The necessary technical knowledge to ensure adequate euthanasia is added as a limiting factor, that is, to guarantee loss of consciousness before death and the safety of the operator (Conselho Federal de Medicina Veterinária, 2013; Pires & Fábian, 2013). However, although the pharmacological responses to chemical agents used for euthanasia among neotropical bat species are not understood, there are three mechanisms known of general action of

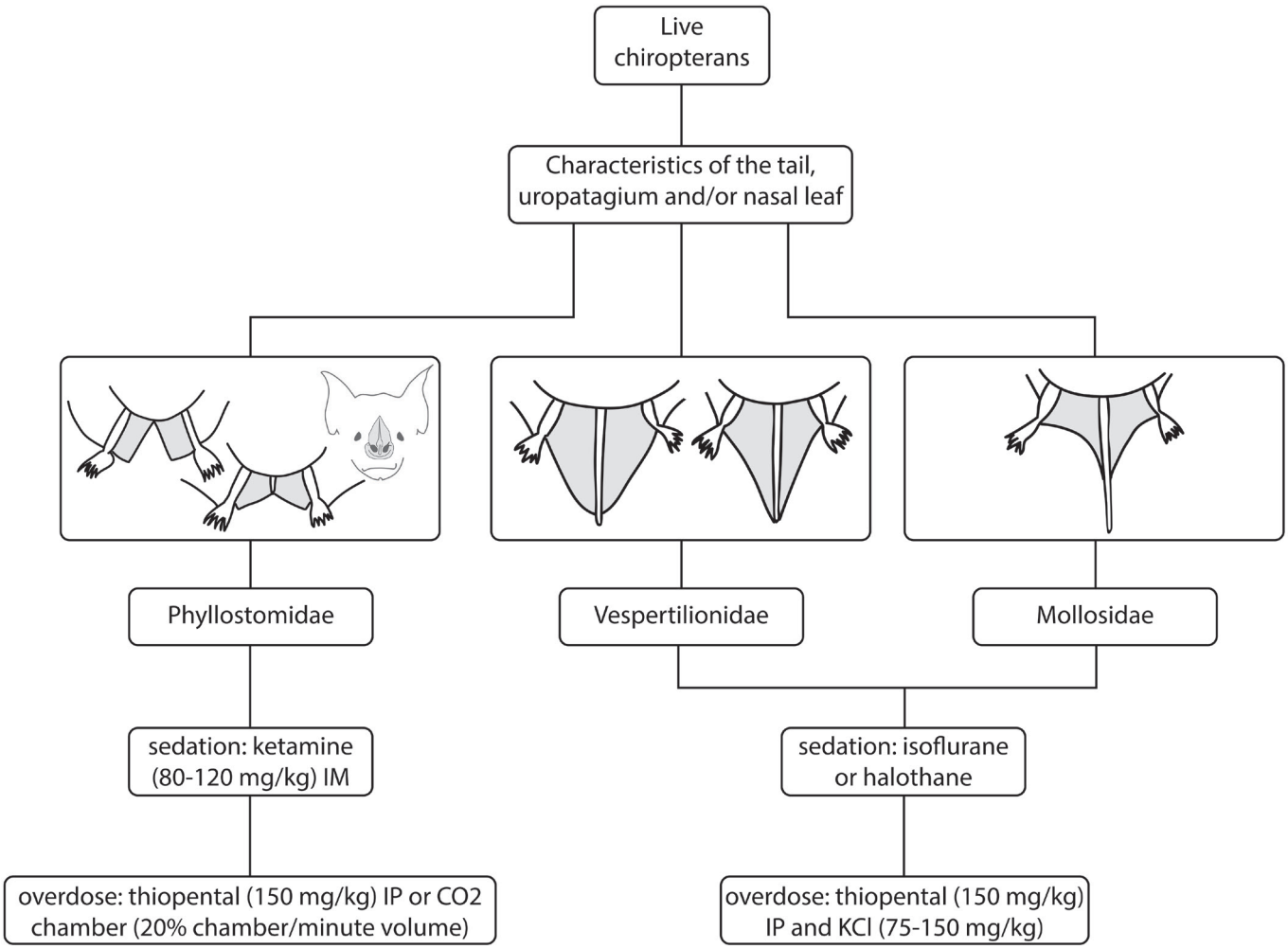

Figure 3 – Algorithm for choosing the method of euthanasia of bats. This algorithm is based on the identification of neotropical bat families based on the morphological characteristics of tail, uropatagium and nasal leaf. Source: Lauri (2019).

these agents: a) direct or indirect hypoxia, b) neuronal depression, and/or c) activity interruption of the brain with neuronal annihilation (Brasil, 2018). These mechanisms of action can be interfered by physiology, such as the feeding habits of these animals (Lollar, 2010). In this sense, the identification of the family, even if in a more general way, can help to determine the most common eating habits and, consequently, help in choosing the methods of euthanasia, minimizing unexpected outside effects.

The identification of the bat family can be based on the shape and length of the tail, the presence or absence of a nasal leaf, as well as on the characteristics of the interfemoral membrane (Miranda et al., 2011). Thus, we believe that the proposed method (Figure 3) can help professionals who perform the euthanasia of bats in identifying the taxonomic family based on simple morphological criteria. Once the family is identified by the ZSD technicians, the choice of method can be simplified. Those ZSDs that receive more than one family of bats (Table 1) will be able to adapt and standardize the euthanasia methods through the following protocols: 1. Use of ketamine and thiopental association or carbon dioxide trap as alternatives for Phyllostomidae bats; and 2. general inhalational anesthesia using isoflurane or halothane for sedation and also for overdose for a long period of exposition or sedation followed by application IP of thiopental and KCl for bats of the Molossidae and Vespertilionidae families (Figure 3). Additionally, there is an indication of periodic training and qualifications to continuously standardize the proposed protocols, being carried out according to each local reality.

Conclusions

The study showed that the euthanasia methods applied by most ZSDs in the State of São Paulo are acceptable chemical methods according to technical and bioethical recommendations. However, the methods are not standardized and differ from the methods used in small rodents. Application of the proposed decision algorithm will facilitate standardization and could allow, based on the recognition of bat families and prevailing eating habits, the

choice of euthanasia method to respect technical, bioethical, and animal welfare guidelines.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Statement

The study was approved by the Ethic Committee in the Use of Animals of School of Veterinary Medicine and Animal Science (CEUA - FMVZ/USP) under protocol 9836240517.

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