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ANESTESIA DE CANÍDEOS DO CERRADO *IN SITU*: AVALIAÇÃO DE PROTOCOLOS A PARTIR DE DADOS COLETADOS A CAMPO

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À Giulia, a melhor coisa que eu já fiz na vida.
Que você possa ver muitos canídeos silvestres inseridos em seu habitat natural quando você crescer.

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"Aqueles que contemplam a beleza da Terra encontram reservas de força que durarão enquanto a vida durar."

Rachel Carson

RESUMO

A contenção química de canídeos em campo faz parte dos esforços de conservação, já que sua captura e manipulação para coleta de amostras e instalação de colares de telemetria são etapas essenciais nos estudos. Não há estudos que apresentem dados robustos comparando protocolos, especialmente sobre as espécies do Cerrado. Fundado em 2009, o Programa de Conservação de Mamíferos do Cerrado (PCMC) vem desenvolvendo pesquisas *in situ* nos estados de Minas Gerais e Goiás, acumulando notável experiência na captura e anestesia de carnívoros, com mais de 400 procedimentos realizados. Esses registros fundamentam o presente trabalho, que teve como objetivo avaliar os protocolos anestésicos utilizados na imobilização da raposinha-do-campo (*Lycalopex vetulus*), do cachorro-do-mato (*Cerdocyon thous*) e do lobo-guará (*Chrysocyon brachyurus*) capturados pelo PCMC entre 2008 e 2023. Constuímos uma base de dados a partir dos registros do PCMC. Planilhas foram desenvolvidas para cada espécie e exportadas para o software R Studio para análise de dose dos protocolos, tempo de decúbito, latência, imobilização, recuperação inicial, suplementação, tempo de recuperação com antagonistas, taxa de decúbito, frequência cardíaca, frequência respiratória, temperatura retal e profundidade anestésica. Totalizando 183 procedimentos com *C. thous*, 117 com *L. vetulus* e 21 com *C. brachyurus*, observamos que todos os protocolos promoveram profundidade. A associação cetamina-midazolam-butorfanol (KMB) foi a mais usada nas três espécies, apresentando tempos de indução, imobilização e recuperação compatíveis com procedimentos de campo, além de estabilidade comprovada dos sinais vitais e da profundidade anestésica. A necessidade de suplementação foi frequente, mas o conhecimento prévio das doses, tempo de ação e a obtenção de acesso venoso permitem uma preparação eficiente. Cetamina-midazolam-butorfanol-xilazina (KMBX) promoveu maior tempo de imobilização, melhor relaxamento muscular e menor necessidade de suplementação nos procedimentos com as raposas, porém a presença evidente de arritmias merece investigação. Cetamina-midazolam-butorfanol-dexmedetomidina (KMBD), embora tenha proporcionado maior tempo de imobilização, excelente relaxamento muscular, recuperação rápida e a possibilidade de usar antagonistas, apresentou arritmias marcantes, despertares repentinos e baixo fluxo na coleta sanguínea, exigindo preparo e atenção redobrados. Midazolam-butorfanol-dexmedetomidina (MBD) apresentou as mesmas desvantagens, potencializadas pela ausência da cetamina, resultando também em alterações na termorregulação. Tiletamina-zolazepam (Z) mostrou praticidade por possibilitar o uso de baixos volumes, o que é desejável em anestesia de animais silvestres. Porém seus efeitos colaterais de excitação e recuperação prolongada são amplamente documentados e foram demonstrados por meio dos elevados valores de frequência cardíaca e respiratória em cachorros-do-mato e raposinhas-do-campo, fazendo com que Z não seja a droga de escolha. Este estudo fornece uma robustez nos dados anestésicos ainda inexistente na literatura atual, contribuindo com informações valiosas para subsidiar os esforços de conservação dos canídeos do Cerrado.

Palavras-chave: Canídeos silvestres. Contenção química. Conservação. Raposinha-do-campo. Lobo-guará. Cachorro-do-mato.

ABSTRACT

Chemically restraining wild canids *in situ* is a part of conservation efforts for these species, since its capture and manipulation for biological sampling and telemetry collars setup are essential to the studies. No studies bring robust data comparing anesthetic protocols, specifically concerning the Cerrado species. Founded in 2009, the Cerrado Mammals Conservation Program (PCMC) has been developing *in situ* research at Minas Gerais and Goiás states and has been gathering notable experience in Cerrado carnivores capture and anesthesia, adding up to more than 400 chemical restraint procedures and these are the records that hold the foundation of the present work that aimed to access the chemical restraint protocols used in Crab-eating fox (*Cerdocyon thous*), Hoary fox (*Lycalopex vetulus*) and Maned wolf (*Chrysocyon brachyurus*) captured by the PCMC from 2008 to 2023. We built a database based on PCMC field anesthesia records. A spreadsheet was developed for each species and exporte do R Studio software to analyze protocol dosage, recumbency, latency, immobilization, initial recovery, supplementation duration, antagonism recovery, recumbency rate, heart rate, respiratory rate, rectal temperature and depth of anesthesia. The database comprised a total of 183 procedures with C. thous, 117 with L. vetulus and 21 with C. brachyurus. All the anesthetic protocols analyzed provided suitable depth of anesthesia for basic handling and sampling. Ketamine-midazolam-butorphanol (KMB) is the most numerous recorded protocol for the three species with times of induction, immobilization and recovery suitable for field procedures, and proven stability of vital signs and anesthesia depth. Anesthesia supplementation was very often, but knowing dosages, time of action and securing peripheral venous access makes it possible to prepare in advance. Ketamine-midazolam-butorphanol-xylazine (KMBX) produced longer immobilization time, better muscle relaxation and less need for supplementation in the foxes' procedures, but the evident presence of arrhythmia deserves further investigation. Ketamine-midazolam-butorphanol-dexmedetomidine (KMBD), despite providing longer immobilization time, great muscle relaxation, fast recovery and the possibility of using antagonists, the marked presence of arrhythmia, sudden awakening and low flow at blood collection brings the necessity of carefully thinking and preparing for this combination. Midazolam-butorphanol-dexmedetomidine (MBD) brings the same disadvantages, but is potentialized by the absence of ketamine, resulting also in temperature consequences. Tiletamine-zolazepam (Z) brings the practicality of usually resulting in low volumes, which is desirable for wild animals' anesthesia. But it is plainly registered in literature the excitation side effects, demonstrated in this study by the high values of heart and respiratory rates in both foxes. Its prolonged and agitated recovery is another currently known disadvantage, that takes Z off the place of drug of choice for wild canids. Our study provides a level of anesthetic data robustness not yet available in the current literature, hopefully contributing valuable information to support conservation efforts for Cerrado canids.

Key words: Wild canids. Chemical restraint. Conservation. Hoary fox. Maned wolf. Crab-eating fox

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INTRODUCTION

The maned wolf (*Chrysocyon brachyurus*), crab-eating fox (*Cerdocyon thous*), and hoary fox (*Lycalopex vetulus*) are three canid species that are sympatric in the Cerrado ecosystem (Juarez and Marinho-Filho, 2002; Jácomo *et al*., 2004; Lemos *et al*., 2011) with *L. vetulus* being an endemic species of this ecosystem (Dalponte, 2009).

Since the 1960s vast quantities of phosphorus and lime have been dispersed in order to support an exploding soybean and beef production at the Cerrado biome (Klink and Machado, 2005). As a result, habitat loss is currently the most significant threat to canid populations and other wildlife living in this ecosystem (IUCN, 2016).

As wild canids are at the top of the food chain, they hold a great ecological role because they can regulate natural prey populations and, therefore, influence their ecosystem dynamics. With the absence of predators, their natural prey, such as herbivore mammals, rodents, birds, reptiles and insects tend to multiply exponentially. This ecological unbalance, besides leading to biodiversity loss, may bring serious harm to agriculture and considerable financial loss (*Pitman et al*., 2002).

Carnivores in general are frequently persecuted as a perceived threat to human life, game species, and livestock (Creel and Creel, 1998). Wild canids are also at risk for being hit by vehicles and are sometimes chased, and even killed by domestic dogs (Lemos *et al*., 2011). The introduction and dispersal of exotic species and domestic animals is a major threat, being of great concern to many conservationists (Gurevitch and Padilla, 2004; Macdonald *et al*., 2007). For example, at the Brasília National Park, the maned wolf was recorded more frequently in sites without the presence of dogs (Lacerda *et al*., 2009; Lessa *et al*., 2016). In this context, domestic animals, wild fauna and humans are exposed to mutual exchange of parasites, microorganisms and diseases (Ramos *et al.*, 2020), and according to the National Plan of Action to the Conservation of Wild Canids, developed by Chico Mendes Institute for the Conservation of Biodiversity (ICMBio, 2025), diseases transmitted by domestic dogs are one of the main threats to Brazilian wild canids.

Maned wolves are currently listed as Near Threatened by the International Union for Conservation of Nature (IUCN) Red List of Threatened species (Paula and DeMatteo, 2016) and Vulnerable in Brazil (Paula *et al*., 2013), with approximately 17,000 maned wolves remaining in the country (Paula and DeMatteo, 2016). The crab-eating fox is listed as Least Concern by both

the IUCN (Lucherini, 2015) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) in Brazil (Beisiegel *et al*., 2013) because of its large distribution across South America and its ability to adapt to deforestation. The hoary fox is currently listed as Vulnerable (Lemos *et al*., 2013) in Brazil and is on the red list of species threatened by extinction in the state of São Paulo and Paraná (Chiarello *et al*., 2008).

Chemically restraining wild canids *in situ* is a part of conservation efforts for these species, since its capture and manipulation for biological sampling and telemetry collars setup are often an essential aspect to the studies (Mihoub *et al.*, 2017). As they possess anatomical and behavioral particularities which are adapted to tearing up tissues and breaking bones, wild canids manipulation, unlike the domestic ones, must be performed under anesthesia, both to ensure the animals' welfare and to provide safety to the staff (Horta *et al.*, 2012).

Several anesthetic drugs and their combinations have been used for the restraint of wild carnivores (Adams, 2001). One of the most used is ketamine, either as the sole anesthetic agent or in combination with other drugs. As a dissociative anesthetic, ketamine produces cataleptic anesthesia with moderate analgesia (Burroughs, 1993). It provides many advantages, such as large margin of safety in a wide range of species, little respiratory and cardiovascular effects, low cost, possibility of intramuscular administration, fast absorption and global availability. But ketamine also brings some side effects that include muscular contraction, excessive salivation, corneal or retinal damage by sun or strong light, mydriasis and rough inductions and recoveries (Adams, 2001).

In order to balance its side effects and to provide better analgesia, ketamine can be combined with alpha-2 adrenergic agonists such as xylazine and dexmedetomidine (Knight, 1980; Adams, 2001). Adrenergic agonists generally reduce the dose of anesthetic drugs required to obtain immobilization or general anesthesia, bringing sedation, analgesia, muscle relaxation and anxiolysis (Sinclair, 2003). Another great advantage is that they can be antagonized with yohimbine or atipamezole (Nagore *et al*., 2013.) The disadvantages of xylazine include regurgitation in carnivores, decrease in heart rate and blood pressure, cardiac arrhythmias (as atriventricular blocks and sinus arrest) and excessive salivation (Knight, 1980). xylazine may also induce abortion in late pregnancy and decrease in rectal temperature (Shindle and Tewes, 2000).

Medetomidine has been used worldwide as a sedative and analgesic in veterinary medicine (Cullen 1996). It is a racemic mixture, where only the D-isomer, dexmedetomidine, is

pharmacologically active in clinically relevant dosage (Virtanen et al. 1988; Savola & Virtanen 1991; Aantaa 1993). *Ansah et al.* (1998) showed that using half the dose of medetomidine, dexmedetomidine causes the same pharmacodynamic effects. Sanchez *et al.* (2008) demonstrated that the combination ketamine-dexmedetomidine induces less bradycardia and a shorter recovery time than ketamine-medetomidine.

Benzodiazepines are known to induce sedative effects and muscle relaxation without harming vital parameters (Muir *et al*., 2000). Midazolam is a benzodiazepine that induces sedative and anxiolytic effects and can be used as preanesthetic medication, adjuvant in anesthesia induction and maintenance and as a sedative for diagnoses and therapeutic procedures (Reves *et al.* 1985). Flumazenil antagonizes benzodiazepines' behavioral and neurologic actions, such as sedation and muscle relaxation (Sumida *et al.* 1995).

Butorphanol is a synthetically derived opioid agonist-antagonist analgesic, with low antagonist activity at μ-receptors and high affinity for κ-receptors. It provides minimal cardiopulmonary depression, and it can be antagonized with naloxone. In wild mammals, butorphanol is used for pain control and combined with sedatives to assist in minor manipulative procedures. When combined with α-2 adrenergic agonists, dissociative anesthetics and/or tranquilizers may produce safer anesthesia by minimizing many adverse effects (Bush, 2012).

The dissociative/tranquilizer association of tiletamine-zolazepam is another commonly used combination for immobilizing wild mammals (Burroughs, 1993). Tiletamine-zolazepam provides rapid induction, satisfactory muscle relaxation and maintenance of swallowing reflexes, with menial depression of physiological functions and wide safety margins, making it useful for immobilization when body weight must be estimated (Samelius *et al*., 2003). Disadvantages are similar to those with ketamine, including cardiopulmonary depression and low body temperature (Burroughs, 1993).

Associating α-2 agonists, benzodiazepines and opiates results in pain impediment without affecting certain areas of the brain that are blocked in orthodox anesthesia (Nilsson & Janssen 1961) and produces synergism of desirable effects (Ossipov *et al*. 1990). Additionally, all of these drugs can be antagonized, making them a great choice for wild canids.

Many combinations of drugs have been used for immobilizing free ranging canids (Acosta-Jamett *et al*., 2010; Kreeger *et al*., 1990; Aguirre *et al*., 2000) however, no studies bring robust data comparing anesthetic protocols, specifically concerning the Cerrado species.

Founded in 2009, the Cerrado Mammals Conservation Program (PCMC) has been developing *in situ* research at Minas Gerais and Goiás states, both regions with a high anthropization degree and still with a rich fauna. The program studies aspects about native mammal ecology and epidemiology, focusing on wild carnivores, and counts on the support of researchers of national and international institutions.

In 2013, PCMC consolidated a partnership with the São Paulo Zoological Foundation (FZSP), which gave start to an initial design of a canid anesthesia research project. The FZSP contributed between 2013 and 2020 with financial resources, equipment and with the support of the technical team at field work.

Since the beginning of their activities, PCMC has been gathering notable experience in Cerrado carnivores capture and anesthesia, adding up to more than 400 chemical restraint procedures and these are the records that hold the foundation of the present work, that aims to study these records in detail.

OBJECTIVES

The general objective of this study aimed to access the chemical restraint protocols used in the ecology and epidemiology studies carried out with the free-ranging Cerrado canids captured by the PCMC from 2008 to 2023.

The specific objectives are:

- Structure a database that was fed with all the *Chrysocyon brachyurus*, *Lycalopex vetulus* and *Cerdocyoun thous* captures data from 2008 to 2023;
- Describe and access the chemical restraint protocols regarding sedation effects, duration and stability of vital signs to each species;
- Compare the effects of different protocols regarding efficiency and safety, for the three species.

MATERIALS AND METHODS

This is a retrospective study based on procedures performed by the PCMC from 2008 to 2023. All captures and handlings were approved by Chico Mendes Institute for Biodiversity Conservation - ICMBio, under permit 65816-1. The study followed the rules issued by the National Council for Control of Animal Experimentation (CONCEA) and was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ; protocol number 1778211118).

Study Area

The captures took place at the Brazilian Cerrado, at agriculture and livestock farms located at the municipalities of Araguari (18°39′4″S, 48°11′7″W) - State of Minas Gerais, Cumari (18°15′46″S, 48°9′3″W), Corumbaíba (18°8′36″S, 48°33′32″W) and Água Limpa (18°4′18″S, 48°45′54″W) - State of Goiás, Brazil, from 2008 to 2023. The landscape is dominated by exotic pasture, with the remaining area being a mosaic of natural gallery and seasonal forests and open Cerrado *sensu stricto* (Lemos, 2016). These areas are characterized by an ecotone of intrusions of Atlantic Forest vegetation into the Cerrado domain.

The Cerrado is a vast ecoregion of tropical savanna in eastern Brazil. The main habitat types consist of forest savanna, wooded savanna, park savanna and gramineous-woody savanna. The Cerrado also includes savanna wetlands and gallery forests. This biome accounts for a full 21% of the country's land area and about 75% of the Cerrado's 2 million km² is privately owned.

The Cerrados's climate is typical of the wetter savanna regions, with a semi-humid tropical climate, and it is limited to two dominant seasons throughout the year: wet and dry. Annual temperatures average between 22 and 27 ºC and average precipitation is between 80 to 200 cm for over 90% of the area.

Despite being a typical extensive cattle ranch landscape, it still shelters more than half of medium and large sized wild mammals known to occur in the native Cerrado, with one-third of them threatened (Lemos, 2016).

Captures and Chemical Restraint

The captures included in this study happened from 2008 to 2023, focusing on the following species: Crab-eating fox (*Cerdocyon thous*), Hoary fox (*Lycalopex vetulus*) and Maned wolf (*Chrysocyon brachyurus*). All the individuals were captured in tomahawk box traps using sardine and chicken meat as bait. The traps were set in the evenings and checked at dawn.

The drugs were administered by dart or by hand injection, using a squeeze panel adapted to the trap, making the animal physically restrained when triggered. The initial protocol was administered in first place, by intramuscular injection. This project aimed to produce dissociative anesthesia and initial protocol effects were classified as:

- Effect 1: the animal presents only light sedation with ataxia, partial loss of muscle tone but doesn't reach recumbency
- Effect 2: the animal could reach recumbency but still moves and reacts to stimuli.
- Effect 3: depth of chemical immobilization, where the protector reflexes are maintained, but the animal achieves lateral recumbency, immobilization, satisfactory level of muscle relaxation, with no response to sound, touch, light and discrete pain

  Considering the handling and sampling needed by the project, effect 3 was the goal.

After reaching the desired sedation effect, the individuals underwent the following procedures, generally: complete physical exam, blood draw, bone marrow collection, biometry, fur collection, microchip insertion, VHF and GPS collar installation and ear tag placement. Oral lesions due to trap biting were a common finding in foxes, so these individuals also received dental treatment when needed. Vital signs were monitored by Littmann® stethoscope, Myndray® oximeter and Omron® digital thermometer. Respiratory rate was determined with a stethoscope and visually by observing chest movements for 1-min periods. Rectal temperature was measured using a digital thermometer. To minimize stress during the procedure, noise levels were kept to a minimum and each animal's eyes were covered to avoid damage and undesired stimulus.

The different anesthetic protocols were identified and put into groups for later statistical analyses. Drug volumes (ml) were based on estimated weights by visual inspection and the animals were weighed after sedation. The real dosages (mg/kg) were calculated based on the administered volume, the drugs' concentration and the true weight.

Anesthesia supplementation was used when the animals started to move or show increase of reflexes before the end of the procedure.

The anesthesia project aimed to standardize dosage, including anesthesia supplementation. So, in some of the protocol groups, intravenous ketamine at half the initial protocol dose was administered as supplementation when needed. The final dosage of each protocol was established after a certain number of pilot procedures, when the desired sedation effect was achieved. The same is true for the supplementation doses. Alpha-2 agonists' doses (xylazine and dexmedetomidine) were chosen also based on the desired sedations effect, which means that equipotentcy between drugs was not specifically considered.

At the end of the procedure, the animals were placed back into the trap and released after full recovery, at the same spot that they were captured. Depending on the initial protocol used, antagonist drugs were administered at this step, using equivalent atipamezole dose to the dexmedetomidine dose. Animals' recovery was monitored, at least, until initial recovery to ensure safety. According to the staff schedule, recovery might or might not been monitored until animals were completely ready for release, presenting normal species behavior and neuromotor functions.

Database

The database was built based on the PCMC field anesthesia records, registered in paper sheets, that were then digitized and made available for this study (annex 1).

A spreadsheet was developed for each species, where all the information contained at the field anesthesia sheets were transcribed and organized in the following categories: procedure date, subject identification (species, name, ear tag, CENAP ID, gender, age class), prior health assessment (body condition, attitude and behavior, estimated and true weight), capture method, climate condition, initial sedation protocol (drug volumes, real dosages, time of administration, administration success, effect of sedation achieved), drug supplementations (volumes, real dosages, time of administration), antagonist protocol (volumes, real dosages, time of administration), recovery times (partial returns, head control, sternal recumbency, initial recovery and full recovery), vital signs monitoring every ten minutes (heart rate; respiratory rate; rectal temperature; capillary refill time; cardiac rhythm, presence of palpebral, anal and podal reflexes; eye rotation; and muscle relaxation), and findings of physical exams. Muscle relaxation was classified as absent, moderate or good. Palpebral, anal and podal reflexes and eye rotation were classified as present or absent. Capillary refill time was measured in seconds. Times of vital signs monitoring were classified as T1, T2, T3, T4, etc with 10 minutes within each other.

The spreadsheets were built in a way that could be analyzed by R Studio software.

According to the recorded information, we calculated the following intervals for later analysis:

- Recumbency: period between time of drug administration and time of recumbency.
- Latency: period between time of drug administration and time of effect 3 achievement.
- Immobilization: period between time of effect 3 achievement and time of first partial return (or time of initial recovery).
- Initial recovery: period between time of last partial return and time of initial recovery.
- Sup 1: period between time of supplement 1 and time of partial return 2.
- Sup 2: period between time od supplement 2 and time of partial return 3.
- Antagonism Recovery: period between time of antagonists' administration and time of initial recovery.
- Recumbency rate: percentage of animals who achieved effect 3 of sedation.

Please note that we considered partial returns as the recovery of some physiological reflexes or movement before the end of the procedure, demanding drug supplementation, and time of initial recovery was recorded when the animal was able to stand with discrete to severe ataxia, after the procedure was done.

Statistical analysis

This work was based on retrospective data of field sheets that were filled over the year, and for selecting the ones that would be included in the statistical analysis, we used the following criteria: real weight, time of drug administration e dosage of the initial protocol must be known. Anesthesia that needed IM supplementation after initial drug administration were not included. Physiological parameters were analyzed only during initial protocol effect, records after any kind of anesthesia supplementation were excluded from the analysis. Animals who received propofol or isoflurane as supplementations were excluded from supplementation and recovery analyses. The standardized intravenous ketamine supplementation was included. The recovery of animals who received antagonist administration were analyzed separately from the others.

Firstly we carried out the descriptive statistics of the initial protocols used (with a significant number of procedures) for each species, regarding weights, dosage, the intervals described in the database topic, and quantitative physiologic parameters (heart rate, respiratory

rate and rectal temperature) resulting in summaries with minimum (Min) and maximum (Max) values, mean, median and standard deviation (SD) of each variable.

In second place, after determining which protocols reached a significant number of procedures, we applied inferential statistics tests to compare time intervals, ketamine dosage and quantitative physiologic parameters among the anesthetic combinations, according to the following process: first, to determine if the variables obeyed a normal distribution, we applied the Shapiro-Wilk test, using alpha level of 0.05. For comparisons between more than two groups, ANOVA test was applied in variables with normal distribution (Gaussian), with a Tukey post-test when the global test p values <0.05; and Kruskal-Wallis's test was used in variables with non-gaussian distribution, with Dunn post-test (Bonferroni method) when the global test p value <0.05. In cases of comparisons between two groups, Student T test was used in Gaussian variables and Wilcoxon test in Non-Gaussian variables.

Linear regression analysis was performed in each protocol to evaluate changes in physiological parameters over time during anesthesia.

For the qualitative parameters (palpebral, anal and withdrawal reflexes, eye rotation, capillary refill time and muscle relaxation), we calculated the percentage of each observation, in each time, according to the classification described at database topic.

RESULTS

The database comprised a total of 183 procedures with *C. thous*, 117 with *L. vetulus* and 21 with *C. brachyurus*, between the years 2008 and 2023, including the field sheets that contained complete dosage information. Images of the database are exemplified at the appendix (figure 1). The database was then exported to R studio software for data analysis.

Table 1 summarizes the weights and doses of each protocol for the three species, considering the inclusion criteria described at materials and methods section.

Table 1. Target doses and summary of weight (kg) and dosage (mg/kg) in ketamine-midazolam-butorphanol (KMB), ketamine-midazolam-butorphanol-xylazine (KMBX), ketamine-midazolam-butorphanol-dexmedetomidine (KMBD), midazolam-butorphanol-dexmedetomidine (MBD) and tiletamine-zolazepam (Z) protocols used to capture free-ranging *Chrysocyon brachyurus*, *Lyclopex vetulus* and *Cerdocyon thous.*

Species	Protocol		N	Target dose	Min	Mean	Median	Max	SD
C. brachyurus	KMB	Weight	21	-	16.45	22.675	23.75	28.8	4.079
		K	12	8	6.667	8.396	8.157	12.105	1.632
		M	12	0.4	0.12	0.393	0.408	0.605	0.109
		B	12	0.15	0.05	0.15	0.15	0.21	0.03
L. vetulus	KMBD	Weight	10	-	3.19	3.797	3.94	4.19	0.371
		K	10	4	2.451	4.471	3.854	9.36	2.143
		M	10	0.5	0.418	0.471	0.461	0.559	0.044
		B	10	0.2	0.167	0.193	0.19	0.25	0.029

	D	10	0.008	0.005	0.007	0.007	0.013	0.002
KMBX	Weight	7	-	3.05	3.319	3.2	3.75	0.269
	K	7	13	11.2	13.866	12.579	18.75	2.775
	M	7	0.5	0.41	0.487	0.467	0.625	0.07
	B	7	0.2	0.164	0.19	0.187	0.25	0.029
	X	7	0.5	0.252	0.495	0.464	0.875	0.19
KMB	Weight	69	-	1.4	3.314	3.45	4.3	0.671
	K	69	15	6.197	15.092	15	25.714	2.869
	M	69	0.5	0.211	0.513	0.507	0.857	0.092
	B	69	0.2	0.085	0.206	0.201	0.357	0.041
Z	Weight	16	-	1.5	3.193	3.2	4.2	0.97
	Z	16	10	5	10.285	10	16.66	3.05
C. thous	KMBD	Weight	20	-	5.17	6.33	6.425	8.4	0.809
		K	20	4	2.675	4.341	4.148	8.85	1.448
		M	20	0.5	0.321	0.482	0.467	0.603	0.083
		B	20	0.2	0.162	0.218	0.21	0.476	0.067
		D	20	0.008	0.006	0.008	0.008	0.01	0.001
	KMBX	Weight	28	-	4.1	6.413	6.5	8.18	0.923
		K	28	13	4.038	13.503	13.87	20.417	4.111
		M	28	0.5	0.192	0.476	0.471	0.677	0.097

	B	28	0.2	0.146	0.202	0.2	0.312	0.055
	X	28	0.5	0.185	0.618	0.625	0.927	0.166
KMB	Weight	91	-	3.7	5.975	6	7.9	0.728
	K	91	15	0.177	15.631	15.556	22.432	2.43
	M	91	0.5	0.006	0.518	0.516	0.743	0.084
	B	91	0.2	0.002	0.21	0.207	0.297	0.036
MBD	Weight	5	-	5.34	5.708	5.57	6.3	0.393
	M	5	0.5	0.515	0.571	0.552	0.628	0.046
	B	5	0.2	0.22	0.235	0.237	0.251	0.013
	D	5	0.012	0.005	0.012	0.012	0.022	0.006
Z	Weight	18	-	4.2	6.277	6.45	7.6	0.903
	Z	18	8	4.918	7.816	7.977	12.244	1.922

We compared the Ketamine doses between protocols with this drug. There were significant differences in ketamine doses between protocols in hoary foxes ($p = 1.015x10^{-6}$). Ketamine doses in KMBD were significantly lower than KMB ($p = 0.0000$) and KMBX ($p = 0.017$). No significant differences were found between KMB and KMBX ($p = 0.29$). Figure 2 shows boxplots of ketamine doses of the three protocols.

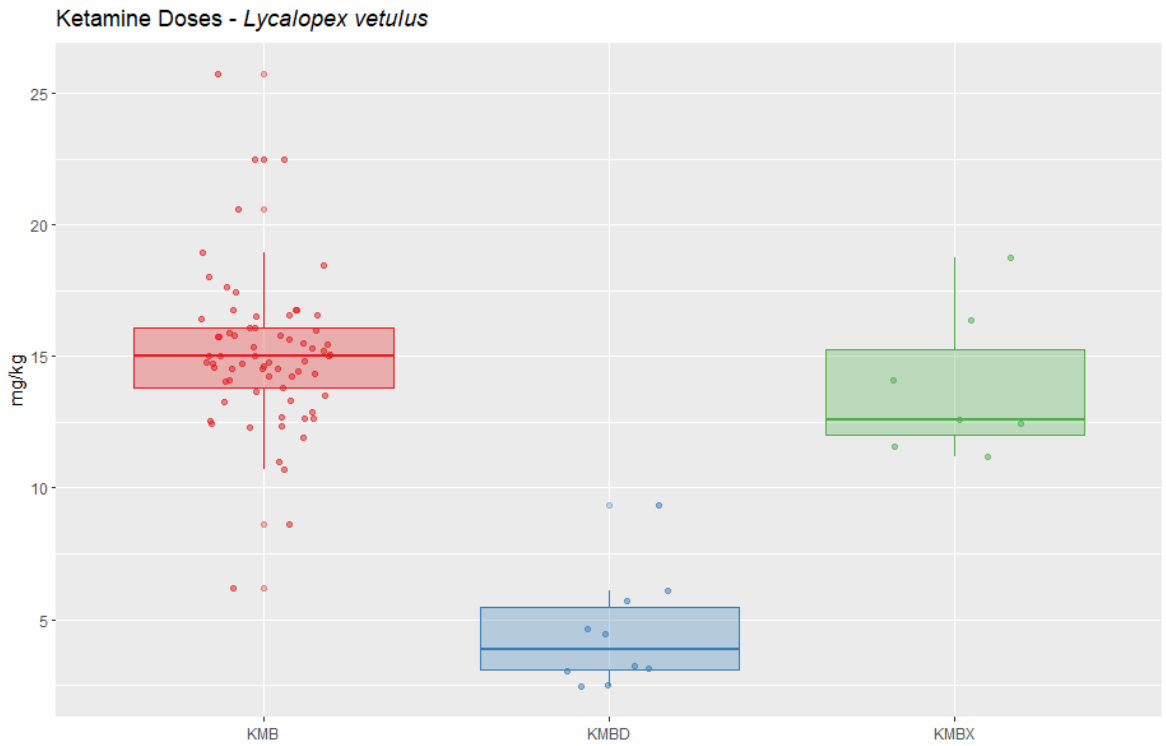

Figure 2. Ketamine doses (mg/kg) in the protocols ketamine-midazolam-butorphanol (KMB), ketamine-midazolam-butorphanol-dexmedetomidine (KMBD) and ketamine-midazolam-butorphanol-xylazine (KMBX), used to chemically restrain free-ranging *Lycalopex vetulus*.

Concerning crab-eating foxes, we found significant differences in ketamine dosage among all three of them, with ketamine dosage decreasing as the following: KMB > KMBX > KMBD. P value of the comparison of KMB x KMBD is 0.0000, KMB x KMBX is 0.0042 and KMBX x KMBD is 0.0001. Figure 3 shows boxplots of ketamine doses of the three protocols.

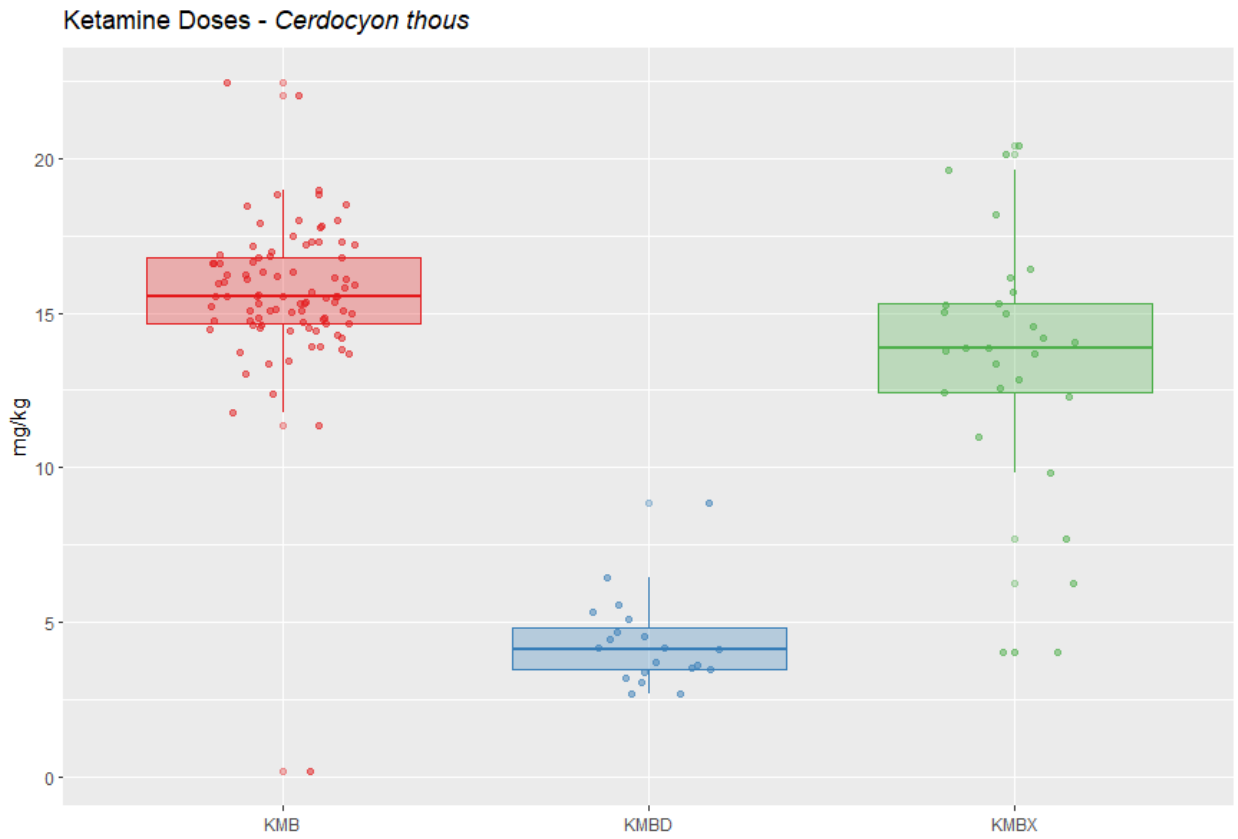

Figure 3. Ketamine doses (mg/kg) of ketamine-midazolam-butorphanol (KMB), ketamine-midazolam-butorphanol-dexmedetomidine (KMBD) and ketamine-midazolam-butorphanol-xylazine (KMBX), used do chemically restrain free-ranging *Cerdocyon thous*.

Chrysocyon brachyurus

Fourteen males and 7 females of 22.67 kg (± 4.09) were captured, using 6 types of anesthetic combinations: one with ketamine-midazolam (KM), ketamine-midazolam-butorphanol (KMB) with 14 records, one using ketamine-midazolam-butorphanol-dexmedetomidine (KMBD), two using ketamine-midazolam-butorphanol-xylazine (KMBX), one using ketamine-midazolam-dexmedetomidine (KMD) and one with tiletamine-zolazepam (Z). One of the field sheets did not contain information about the protocol.

Since only the KMB protocol was recorded in a significant number of anesthesias, it was the only one statistically analyzed. The procedures that were included in the analysis are the ones that meet our inclusion criteria, besides reaching enough numbers. Recumbency rate (percentage of animals who reached recumbency with initial protocol) was 83%.

According to the necessity of duration of procedures 50% of the procedures required drug supplementation: intravenous ketamine (6 animals), intravenous propofol (2 animals) and

isoflurane via inhalation route (1 animal). IV ketamine was administered as supplementation up to four times.

Table 2 summarizes recumbency time, latency, time of immobilization, initial recovery time, dosage and duration of ketamine IV supplementation. Figure 4 shows boxplots of the measured intervals.

Table 2. Summary of recumbency time, latency, time of immobilization, initial recovery time, dosage and duration of ketamine IV supplementation of the Ketamine-Midazolam-Butorphanol (KMB) protocol used in *C. brachyurus*

		N	**Min**	**Mean**	**Median**	**Max**	**SD**
Recumbency (min)		10	3	5.2	4.5	13	2.898
Latency (min)		7	5	6	6	7	0.577
Immobilization (min)		7	9	35.571	34	57	17.047
Initial Recovery time (min)		3	25	54.333	53	85	30.022
Sup 1	Dose (mg/kg)	8	3.333	4.431	4.167	6.053	1.007
	Duration (min)	5	18	35.8	39	60	17.655
Sup 2	Dose (mg/kg)	7	0.952	3.723	4.122	5.422	1.543
	Duration (min)	3	13	29.666	20	56	23.072
Sup 3	Dose (mg/kg)	4	4.077	4.686	4.559	5.422	0.681
	Duration (min)	1	21	21	21	21	-
Sup 4	Dose (mg/kg)	2	4.077	4.077	4.077	4.077	-

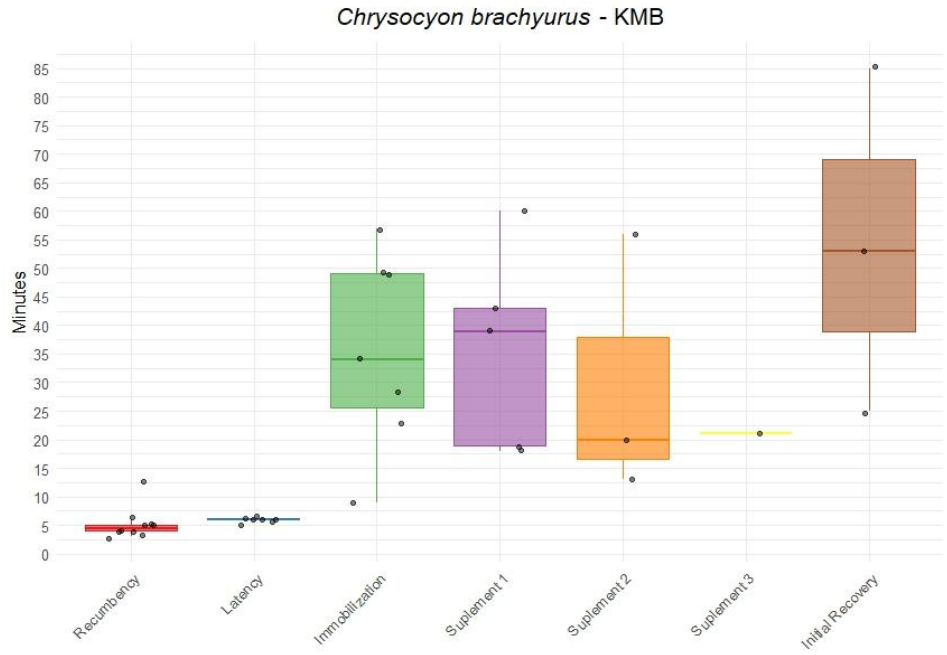

Figure 4. Times of recumbency, latency, immobilization, IV ketamine supplements 1, 2 and 3 durations and initial recovery of free ranging maned wolves (*C. brachyurus*) anesthetized with ketamine-midazolam-butorphanol combination.

Figure 5 demonstrates values over time of the physiological parameters heart rate, respiratory rate and rectal temperature produced by KMB.

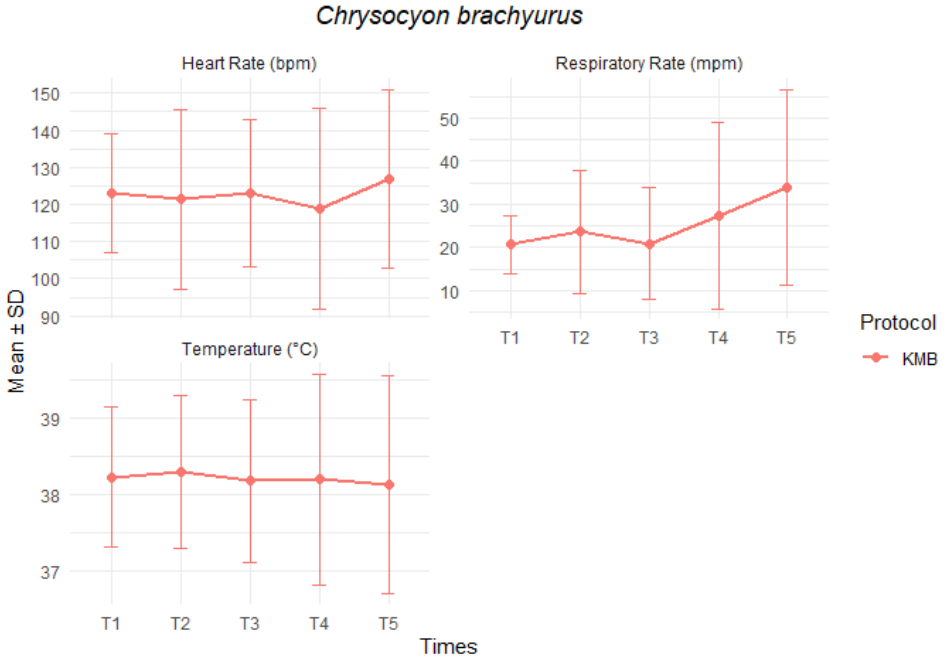

Figure 5. Mean and standard deviation of Heart rate (beats per minute), Respiratory rate (movements per minute), temperature (in Celsius degrees) of free-ranging *Chrysocyon brachyurus* anesthetized with ketamine-midazolam-butorphanol (KMB) from T1 to T5.

Linear regression analysis showed no significant difference throughout the time in heart rate, respiratory rate and temperature for KMB (p values of 0.9, 0.2 and 0.86, respectively).

Regarding qualitative parameters, figure 6 shows the percentage of each one over time.

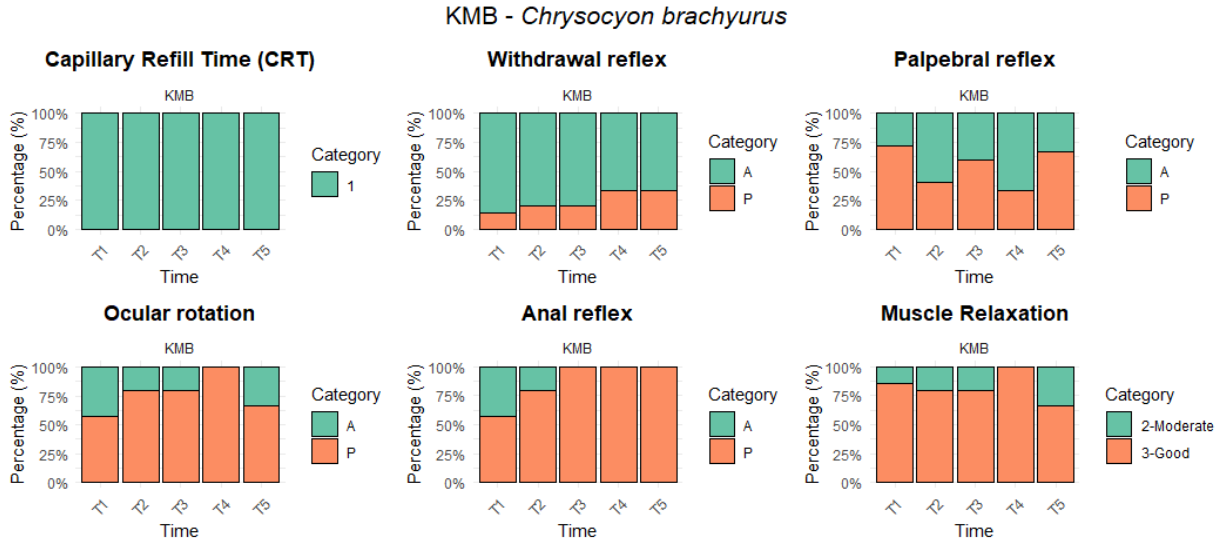

Figure 6. Percentage of presence (P) or absence (A) of withdrawal, palpebral and anal reflexes and ocular rotation; capillary refill time (1, 2 or 3 seconds) and muscle relaxation (absent, moderate or good) in free-ranging *Chrysocyon brachyurus* anesthetized with ketamine-midazolam-butorphanol (KMB), from T1 to T5.

Figure 7 represents parameters over time of animals with and without IV ketamine supplementation in the KMB protocol.

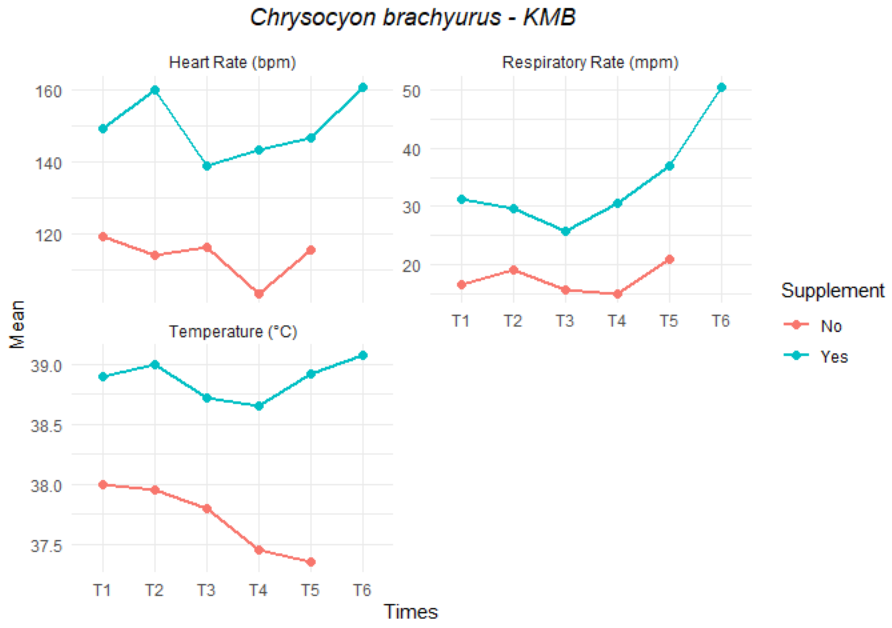

Figure 7. Heart rate (beats per minute), Respiratory rate (movements per minute), temperature (in Celsius degrees) of free-ranging *Chrysocyon brachyurus* anesthetized with ketamine-midazolam-butorphanol (KMB), with or without intravenous ketamine supplementation, from T1 to T6.

As side effects, 7.14% (n = 1) of the animals presented cardiac sinus arrhythmia in KMB, 100% (n = 1) in KMBD and 100% (n = 2) in KMBX.

Lycalopex vetulus

Sixty males and 57 females were captured with five different anesthetic protocols. Using the described inclusion criteria, these are the final number of each protocol: Z = 15, KM = 2, KMD = 3, KMBD = 10, KMBX = 7 e KMB = 69. The protocols analyzed are the ones that meet our inclusion criteria and reach enough records.

KMBD recumbency rate was 90%. Anesthesia supplementation was administered according to the procedures' necessities in 7 animals (2 IV ketamine, and 5 IV propofol). According to the number of records of each previously established interval, at table 3, we describe induction, immobilization, recovery and ketamine supplementation times.

Table 3. Recumbency, latency, immobilization, initial recovery, ketamine supplementation and antagonism recovery times of *Lycalopex vetulus* anesthetized with ketamine-midazolam-butorphanol-dexmedetomidine (KMBD).

		N	Min	Mean	Median	Max	SD
Recumbency (min)		9	2	4	3	11	2.915
Latency (min)		9	3	5.444	4	12	2.743
Immobilization (min)		9	31	48.333	51	63	11.926
Initial Recovery time (min)		4	5	8.75	8	14	4.5
Sup 1	Dose (mg/kg)	2	6.107	10.648	10.648	15.19	6.423
	Duration (min)	1	15	15	15	15	-
Antagonism recovery		3	5	8	7	12	3.605

At the end of the procedures, all 10 animals were antagonized with a combination of atipamezole, flumazenil and naloxone, but only the ones that did not receive IM ketamine and IV

propofol supplementation were considered in the period analysis. The antagonists' dosages are detailed in table 4.

Table 4. Summary of antagonists administered for anesthesia recovery of free-ranging *Lycalopex vetulus* chemically restrained with ketamine-midazolam-butorphanol-dexmedetomidine (KMBD).

Drugs	**Min**	**Mean**	**Median**	**Max**	**SD**
Atipamezole (mg/kg)	0.03	0.115	0.121	0.231	0.072
Flumazenil (mg/kg)	0.008	0.009	0.009	0.011	0.0009
Naloxone (mg/kg)	0.034	0.038	0.037	0.044	0.003

KMBX recumbency rate was 100% and 4 animals needed IV ketamine supplementation. Anesthesia times are described at table 5.

Table 5. Recumbency, latency, immobilization, initial recovery and ketamine supplementation times of *Lycalopex vetulus* anesthetized with ketamine-midazolam-butorphanol-xylazine (KMBX).

		N	**Min**	**Mean**	**Median**	**Max**	**SD**
Recumbency (min)		6	3	3.333	3	4	0.5163
Latency (min)		4	3	6.25	6	10	2.986
Immobilization (min)		4	27	37.75	36	52	11.5
Initial Recovery time (min)		4	35	38.75	38	44	3.774
Sup 1	Dose (mg/kg)	4	4.918	5.651	5.699	6.289	0.568
	Duration (min)	3	15	17	18	18	1.732

KMB recumbency rate was 94%, 27 animals needed IV ketamine supplementation and one of them needed additional isoflurane, and 9 needed propofol. Details of the times measured in this protocol are at table 6.

Table 6. Recumbency, latency, immobilization, initial recovery and ketamine supplementation times of *Lycalopex vetulus* anesthetized with ketamine-midazolam-butorphanol (KMB).

		N	Min	Mean	Median	Max	SD
Recumbency (min)		65	1	3.0615	3	10	1.618
Latency (min)		39	2	5.743	5	13	2.592
Immobilization (min)		37	6	35.432	37	55	10.46
Initial Recovery time (min)		10	9	30.1	21.5	74	22.233
Sup 1	Dose (mg/kg)	27	5.357	7.108	7.335	8.671	0.867
	Duration (min)	18	1	23.666	25	36	7.522
Sup 2	Dose (mg/kg)	7	6.173	6.936	7.18	7.519	0.591
	Duration (min)	3	11	16	15	22	5.567
Sup 3	Dose (mg/kg)	3	6.173	6.763	6.763	7.353	0.834
	Duration (min)	1	12	12	12	12	-

Comparing KMB, KMBX and KMBD, Recumbency and latency did not present significant statistical differences by Kruskal-Wallis's test (p values of 0.282 and 0.8146, respectively). Figure 8 shows boxplots that represent all the intervals measured of the 3 protocols.

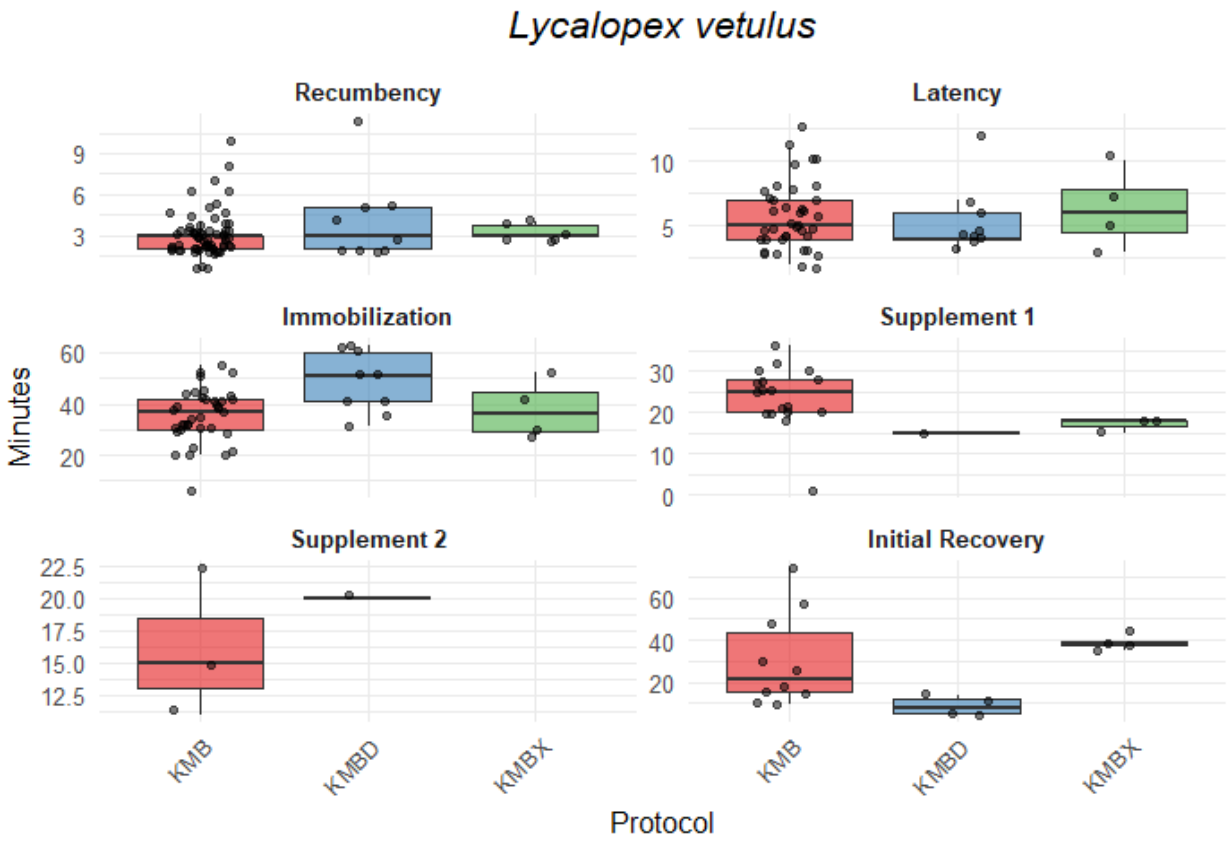

Figure 8. Times of recumbency, latency, immobilization, IV ketamine supplements 1 and 2 and initial recovery of free ranging *Lycalopex vetulus* captured with combinations of ketamine-midazolam-butorphanol (KMB), ketamine-midazolam-butorphanol-dexmedetomidine (KMBX) and ketamine-midazolam-butorphanol-xylazine (KMBX).

Immobilization showed significant difference among the groups using ANOVA test (p = 0.0093) and it was significantly lower in KMB compared to KMBD (p = 0.006). There was no difference between KMB and KMBX (p= 0.91) and ZMBD and KMBX (p=0.24).

There were significant differences in initial recovery times between groups (p = 0.023). KMBD had significantly lower initial recovery timer compared to KMB (p = 0.046) and KMBX (p=0.012). There were no differences between KMB and KMBX (p = 0.46).

Figure 9 demonstrates values over time of physiological parameters heart rate, respiratory rate and rectal temperature of the anesthetic combinations that reached sufficient monitoring records.

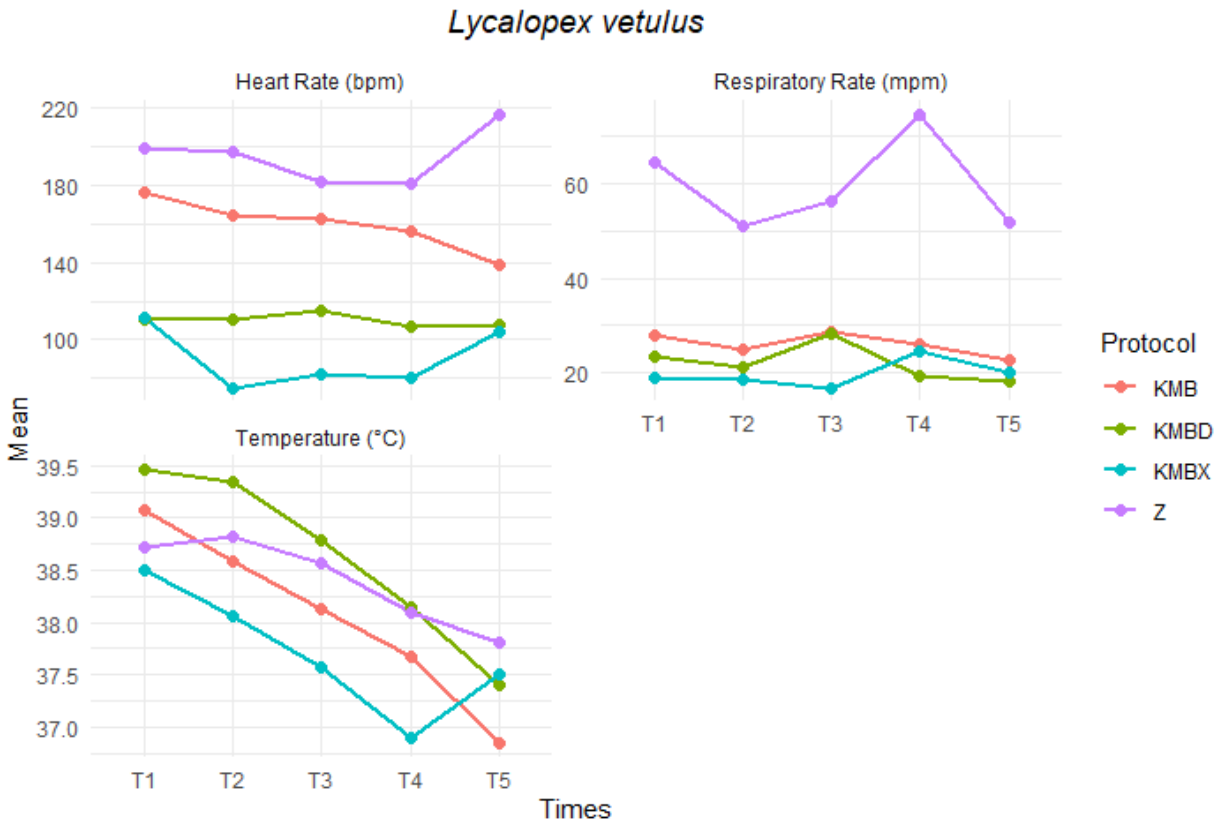

Figure 9. Heart rate (beats per minute), Respiratory rate (movements per minute), temperature (in Celsius degrees) of free-ranging *Lycalopex vetulus* anesthetized with ketamine-midazolam-butorphanol (KMB), ketamine-midazolam-butorphanol-dexmedetomidine (KMBD), ketamine-midazolam-butorphanol-xylazine (KMBX), and tiletamine-zolazepam (Z) from T1 to T5.

Comparing parameters among the protocols, we found significant differences in heart rate from T1 to T4, and in respiratory rate from T1 to T4. P values of each pair of protocol obtained in post-tests are detailed in table 7. Detailed global and Shapiro-Will tests are at table 8 in appendix.

Table 7. P-values of physiological parameters (heart rate, respiratory rate and temperature) in relation to time of monitoring, that indicate significant difference between pairs of protocols used to anesthetize free-ranging *Lycalopex vetulus*

Parameter	**T**	**Protocols**	**P value**
Heart Rate	T1	KMB x KMBD	0.0001
		KMB x KMBX	0.0002
		Z x KMBD	8.24×10^{-6}
		Z x KMBX	1.08×10^{-5}
	T2	KMB x KMBD	0.004

		KMB x KMBX	$9.48x10^{-5}$
		Z x KMBD	0.0001
		Z x KMBX	$3.03x10^{-6}$
	T3	KMB x KMBD	0.013
		KMB x KMBX	0.001
		Z x KMBD	0.001
		Z x KMBX	0.0001
	T4	KMB x KMBX	0.002
		Z x KMBD	0.008
		Z x KMBX	0.0005
Respiratory Rate	T1	Z x KMB	$3.91x10^{-6}$
		Z x KMBD	0.0005
		Z x KMBX	$1.02x10^{-5}$
	T2	Z x KMB	0.0002
		Z x KMBD	0.002
		Z x KMBX	0.001
	T3	Z x KMB	0.001
		Z x KMBD	0.024
		Z x KMBX	0.0002
	T4	Z x KMB	0,003
		Z x KMBD	0.015

Linear regression analysis showed significant difference throughout the time in heart rate for KMB ($p = 0.002$), and in temperature for KMB, KMBD and KMBX (p values of $4.64x10^{-10}$, 0.001 and 0.03, respectively).

Figure 10 represents parameters over time of animals with and without IV ketamine supplementation in the KMB protocol

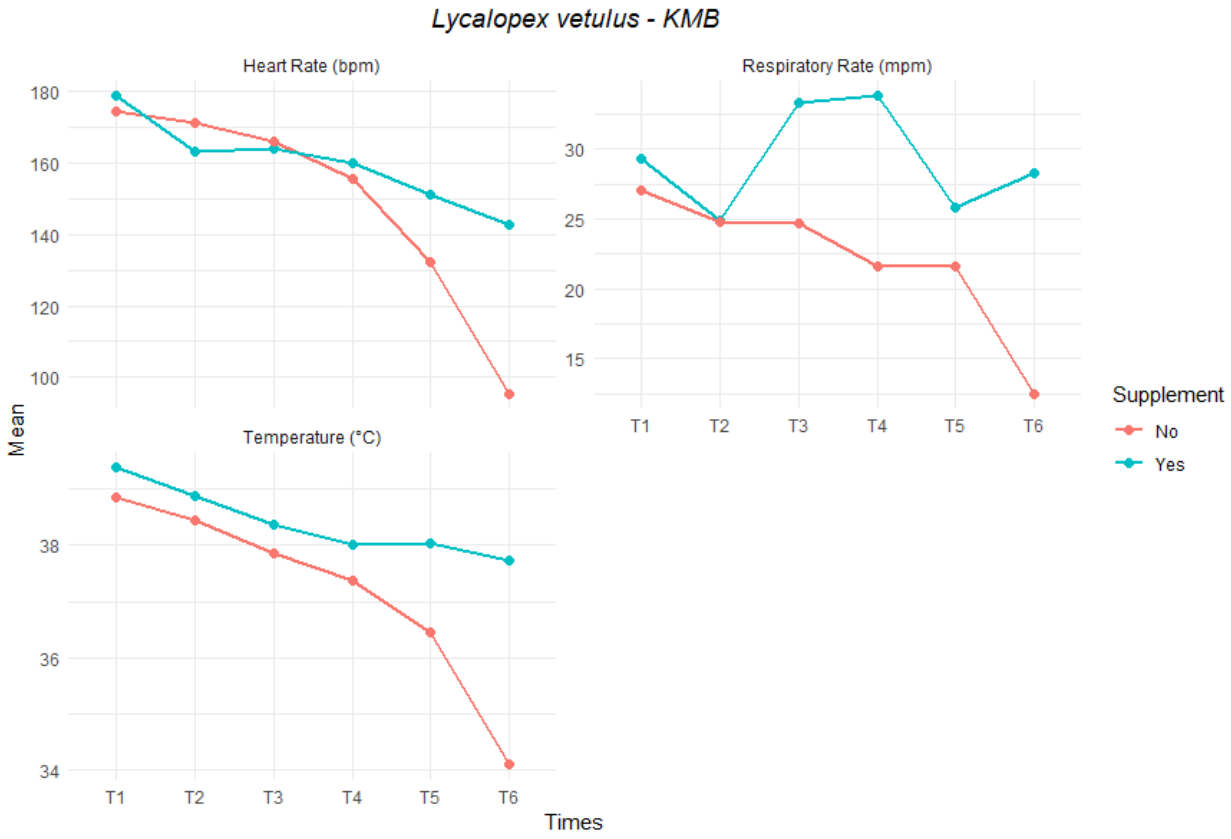

Figure 10. Heart rate (beats per minute), Respiratory rate (movements per minute), temperature (in Celsius degrees) of free-ranging *Lycalopex vetulus* anesthetized with ketamine-midazolam-butorphanol (KMB), with or without intravenous ketamine supplementation, from T1 to T6.

Regarding qualitative parameters, figure 11 shows the percentage of each one over time of the protocols that reached sufficient records. Please note that there are protocols which the parameters monitoring did not reach enough records to be included in the analysis, therefore, are not shown at figure 11.

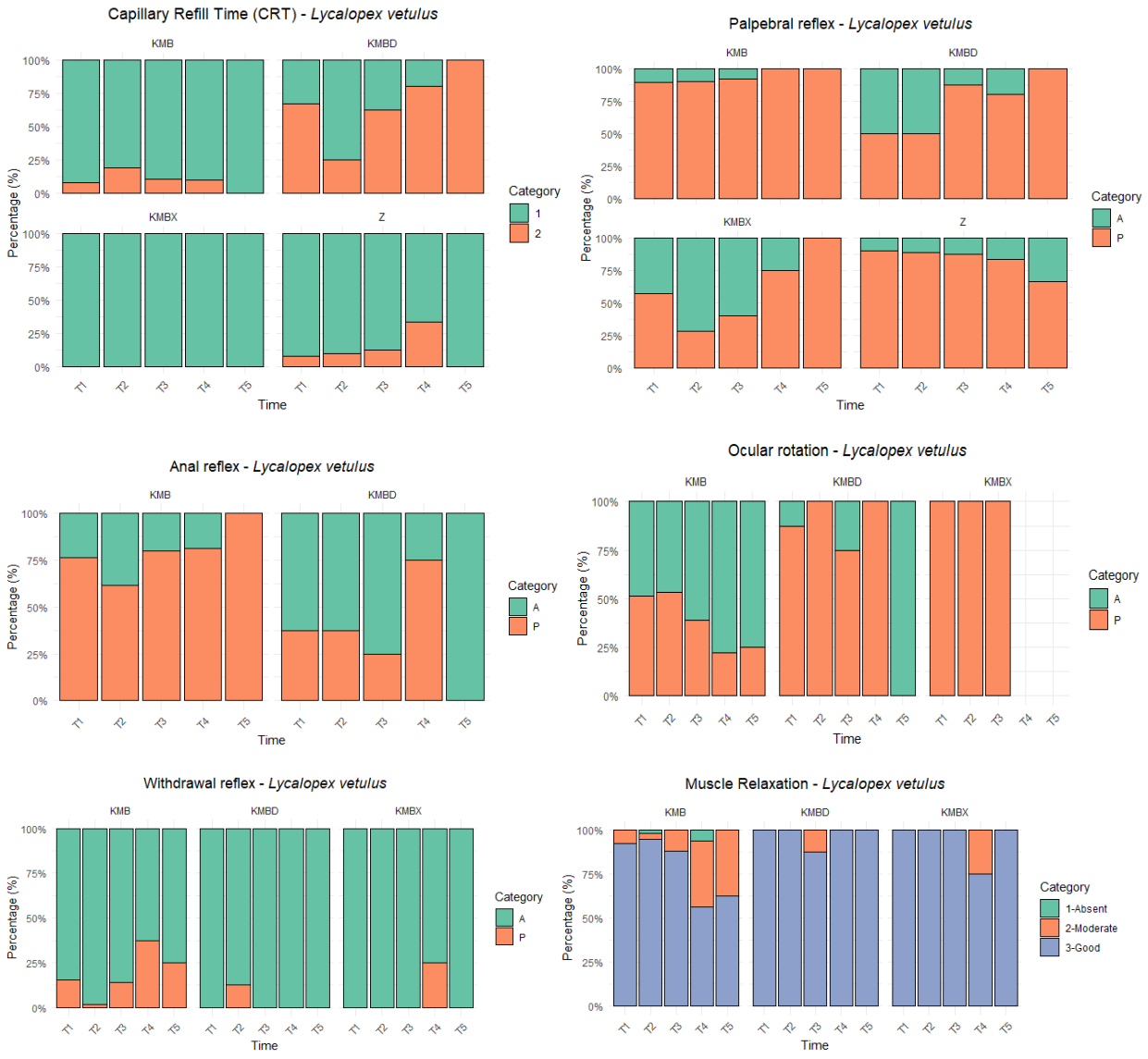

Figure 11. Presence (P) or absence (A) of withdrawal, palpebral and anal reflexes and ocular rotation; capillary refill time (1, 2 or 3 seconds) and muscle relaxation (absent, moderate or good) in free-ranging *Lycalopex vetulus* anesthetized with ketamine-midazolam-butorphanol (KMB), ketamine-midazolam-butorphanol-dexmedetomidine (KMBD), ketamine-midazolam-butorphanol-xylazine (KMBX), and tiletamine-zolazepam (Z) from T1 to T5.

Regarding side effects, sialorrhea was observed at 1.4% (n = 1) of animals anesthetized with KMB, 50% (n = 1) with KM and 26.66% (n = 4) with Z. Sinus arrhythmia was recorded in 18.3% (n = 13) of KMB, 50% (n = 5) of KMBD, 71.42% (n = 5) of KMBX and 33.33% (n = 1) of KMD. Sudden awakening was seen in 1.4% (n = 1) of animals immobilized with KMB, 50% (n = 5) with KMBD and 33.33% (n = 1) with KMD. Low flow at blood collection was seen in 20% (n = 2) of KMBD.

Cerdocyon thous

94 males and 89 females were captured, weighing 6,49 kg (± 3,99). Nine different anesthetic combinations were accounted for: Z = 24, KM = 1, KMBX = 28, KMBD = 20, KMB = 91, midazolam-butorphanol-dexmedetomidine (MBD) = 5, ketamine-midazolam-tramadol (KMT) = 1, KMD = 1 e ketamine-midazolam-xylazine (KMX) = 2. The procedures included in the analyses are the ones that reached enough records and meet our inclusion criteria.

For KMBD, according to the individual response and procedures necessities, the following drug supplementation was used: IV ketamine (15 animals), IV propofol (6 animals) and isoflurane (1 animal). The recumbency rate was 100%. Intervals' details are at table 9.

Table 9. Recumbency, latency, immobilization, initial recovery, ketamine supplementation and antagonism recovery times of *Cerdocyon thous* anesthetized with ketamine-midazolam-butorphanol-dexmedetomidine (KMBD).

		N	**Min**	**Mean**	**Median**	**Max**	**SD**
Recumbency (min)		20	2	3.4	3	6	1.313
Latency (min)		20	3	5.75	5.5	10	2.124
Immobilization (min)		19	24	47	52	66	11.435
Initial Recovery time (min)		10	1	6.4	5	17	5.891
Sup 1	Dose (mg/kg)	2	1.527	1.865	1.865	2.203	0.478
	Duration (min)	2	8	23.5	23.5	39	21.920
Antagonism recovery		7	0	4.571	2	15	5.028

At the end of the procedures, from 20 procedures, 17 anesthesias were antagonized with a combination of atipamezole, flumazenil and naloxone, and the same criteria of supplementation was considered for the interval analysis. The antagonists' dosages are detailed at table 10.

Table 10. Summary of antagonists administered for anesthesia recovery of free-ranging *Cerdocyon thous* chemically restrained with ketamine-midazolam-butorphanol-dexmedetomidine (KMBD).

Drugs	**Min**	**Mean**	**Median**	**Max**	**SD**
Atipamezole (mg/kg)	0.037	0.102	0.073	0.296	0.088
Flumazenil (mg/kg)	0.0081	0.009	0.0097	0.012	0.0032
Naloxone (mg/kg)	0.0324	0.036	0.0389	0.048	0.013

KMBX recumbency rate was 96,55% and IV ketamine supplementation was administered to 17 animals. Only two received isoflurane. Intervals are described at table 11.

Table 11. Recumbency, latency, immobilization and ketamine supplementation times of *Cerdocyon thous* anesthetized with ketamine-midazolam-butorphanol-xylazine (KMBX).

		N	**Min**	**Mean**	**Median**	**Max**	**SD**
Recumbency (min)		26	1	3.615	3	10	2.210
Latency (min)		13	0	3.923	4	7	2.059
Immobilization (min)		10	34	54.5	52.5	93	17.238
Sup 1	Dose (mg/kg)	15	2.703	6.157	6.935	10	2.291
	Duration (min)	9	10	16.888	18	27	5.254

KMB recumbency rate was 94,5%, 34 animals received ketamine supplementations (one of them received additional propofol), and one received propofol. Intervals are detailed at table 12.

Table 12. Recumbency, latency, immobilization, initial recovery and ketamine supplementation times of *Cerdocyon thous* anesthetized with ketamine-midazolam-butorphanol (KMB).

		N	Min	Mean	Median	Max	SD
Recumbency (min)		83	1	2.879	3	9	1.291
Latency (min)		45	2	5.933	4	19	3.725
Immobilization (min)		42	5	38.59	40.5	115	16.829
Initial Recovery time (min)		9	7	24	20	45	14.387
Sup 1	Dose (mg/kg)	33	2.904	7.552	7.636	11.236	1.392
	Duration (min)	24	4	26.625	23.5	66	16.425
Sup 2	Dose (mg/kg)	7	5.696	7.693	7.778	9.074	1.24
	Duration (min)	4	4	14.75	17.5	20	7.274

Comparing KMB, KMBX and KMBD, Recumbency and latency did not present significant statistical differences by Kruskal-Wallis's test (p values of 0.15 and 0.11, respectively). Figure 12 shows boxplots that represent all the intervals measured of the 3 protocols.

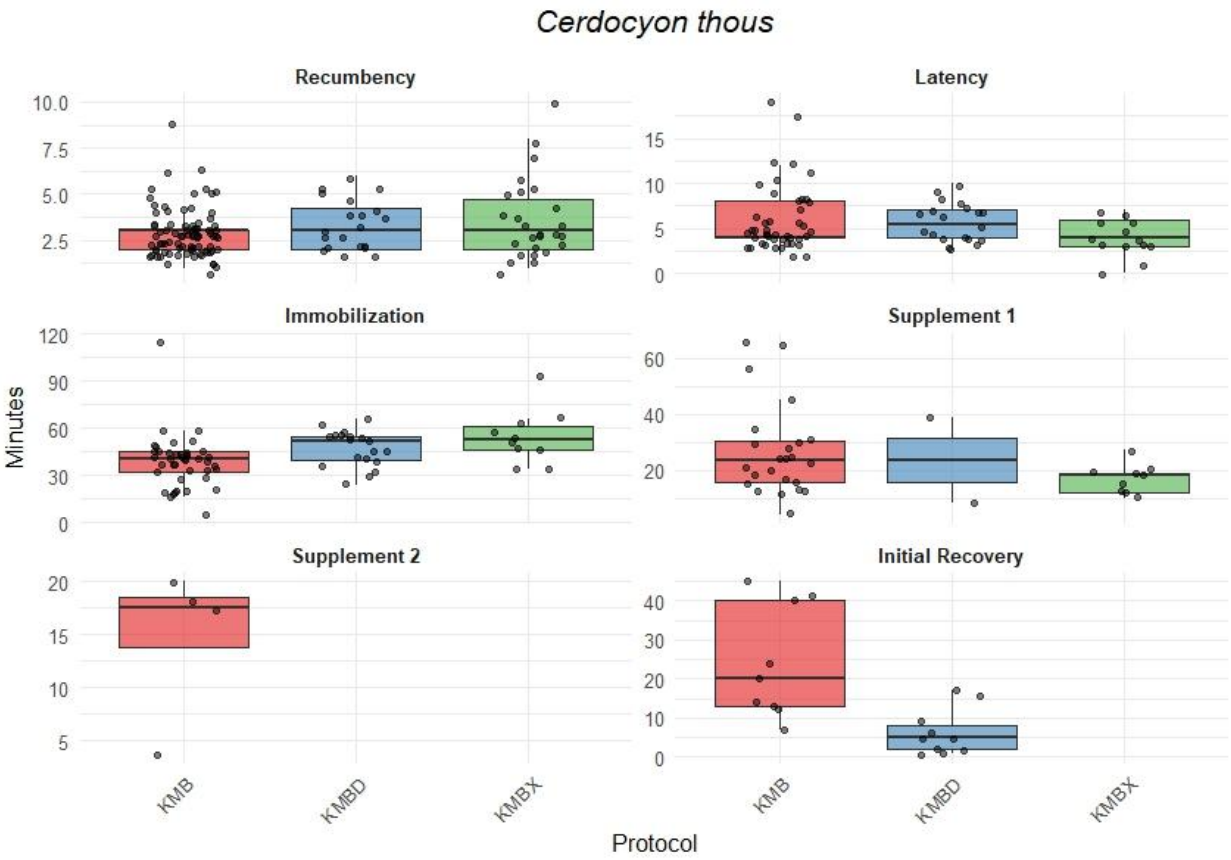

Figure 12. Times of recumbency, latency, immobilization, IV ketamine supplements 1 and 2 and initial recovery of free ranging *Cerdocyon thous* captured with combinations of ketamine-midazolam-butorphanol (KMB), ketamine-midazolam-butorphanol-dexmedetomidine (KMBX) and ketamine-midazolam-butorphanol-xylazine (KMBX).

There were significant differences in immobilization times between groups (p = 0.0017). KMB had significantly lower immobilization time compared to KMBD (p = 0.014) and KMBX (p=0.004). There were no differences between KMBD and KMBX (p = 0.566).

Duration of supplement 1 did not show significant difference between KMB and KMBX by Wilcoxon text. Using the same test, we observed that KMBD has Initial Recovery time significantly lower than KMB (p = 0.0037).

Figure 13 demonstrates values over time of physiological parameters heart rate, respiratory rate and rectal temperature of the anesthetic combinations that reached sufficient monitoring records.

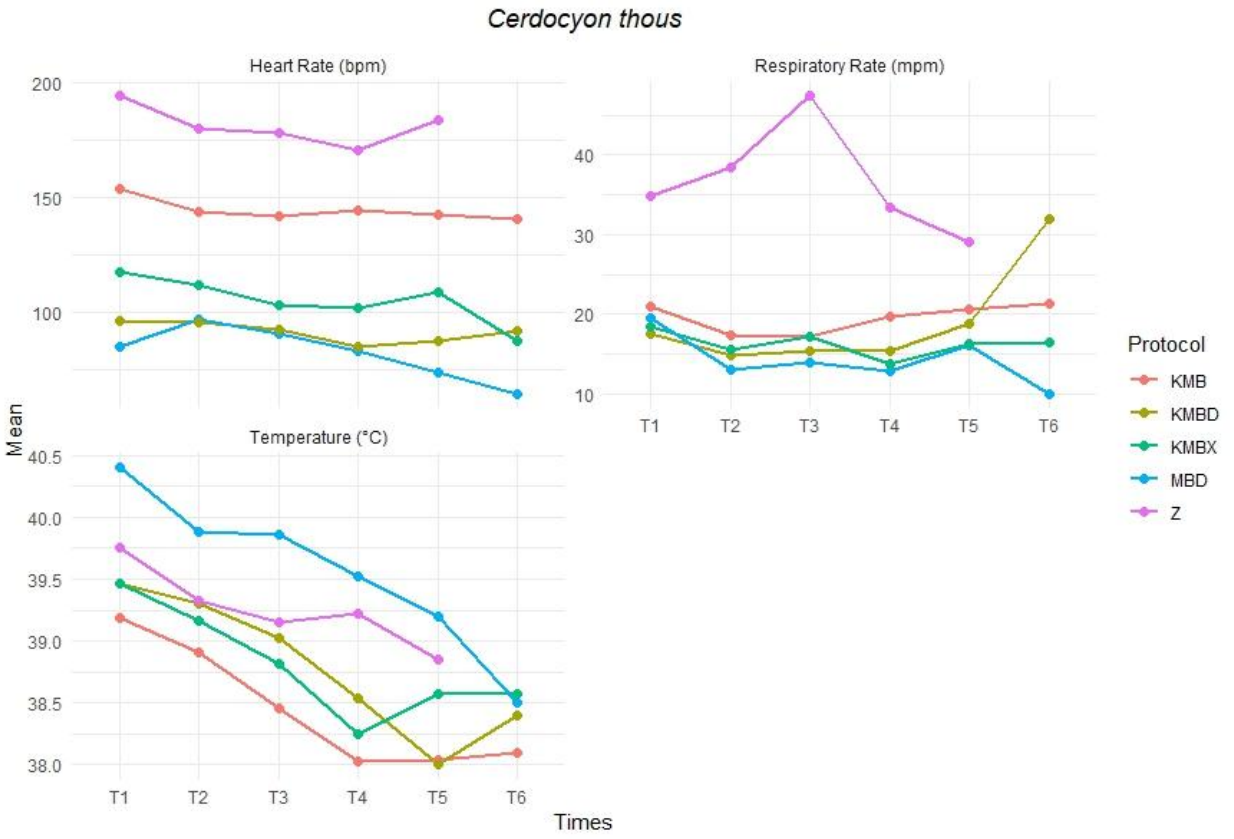

Figure 13. Heart rate (beats per minute), Respiratory rate (movements per minute), temperature (in Celsius degrees) of free-ranging *Cerdocyon thous* anesthetized with ketamine-midazolam-butorphanol (KMB), ketamine-midazolam-butorphanol-dexmedetomidine (KMBD), ketamine-midazolam-butorphanol-xylazine (KMBX), midazolam-butorphanol-dexmedetomidine (MBD) and tiletamine-zolazepam (Z) from T1 to T6.

Comparing parameters among the protocols, we found significant differences in heart rate T1 to T5, in respiratory rate from T1 to T3 and in temperature, in T1. P values of each pair of protocol obtained in post-tests are detailed in table 13. Detailed global and Shapiro-Will tests are at table 14 in appendix.

Table 13. P-values of physiological parameters (heart rate, respiratory rate and temperature) in relation to time of monitoring, that indicate significant difference between pairs of protocols used to anesthetize free-ranging *Cerdocyon thous*.

Parameter	**T**	**Protocols**	**P value**
Heart Rate	T1	KMB x MBD	0.002
		KMB x KMBD	$7.27x10^{-08}$
		KMB x KMBX	0.0002
		Z x MBD	$5.13x10^{-06}$

		Z x KMB	0.005
		Z x KMBD	$2.76x10^{-11}$
		Z x KMBX	$5.63x10^{-08}$
	T2	KMB x MBD	0.004
		KMB x KMBD	$1.21x10^{-08}$
		KMB x KMBX	$6.82x10^{-05}$
		KMB x Z	0.0001
		Z x MBD	$8.85x10^{-7}$
		Z x KMBD	$2.96x10^{-13}$
		Z x KMBX	$4.27x10^{-10}$
	T3	KMB x MBD	0.0003
		KMB x KMBD	$2.05x10^{-10}$
		KMB x KMBX	$6.10x10^{-07}$
		KMB x Z	0.002
		Z x MBD	$2.60x10^{-07}$
		Z x KMBD	$1.76x10^{-11}$
		Z x KMBX	$2.99x10^{-09}$
	T4	KMB x MBD	$9.77x10^{-06}$
		KMB x KMBD	$2.05x10^{-10}$
		KMB x KMBX	$9.43x10^{-07}$
		Z x MBD	$1.06x10^{-5}$
		Z x KMBD	$3.50x10^{-07}$
		Z x KMBX	$4.03x10^{-05}$
	T5	KMB x MBD	0.016
		KMB x KMBD	0.003
		Z x MBD	0.014
		Z x KMBD	0.013
Respiratory Rate	T1	KMB x Z	0.001
		Z x KMBD	0.0003
		Z x KMBX	0.0004

	T2	Z x MBD	0.0012
		Z x KMB	$4.11x10^{-06}$
		Z x KMBD	$1.17x10^{-05}$
		Z x KMBX	$1.12x10^{-05}$
	T3	Z x MBD	0.01
		Z x KMB	0.002
		Z x KMBD	0.001
		Z x KMBX	0.001
Temperature	T1	KMB x MBD	0.041

Linear regression analysis showed significant difference throughout the time in heart rate for KMB ($p = 0.03$) and KMBX ($p = 0.048$), and in temperature for MBD, KMB, KMBD, KMBX and Z (p values of 0.007, $3.82x10^{-12}$, 0.0001, 0.022, 0.033, respectively).

Figure 14 represents parameters over time of animals with and without IV ketamine supplementation in the KMB protocol.

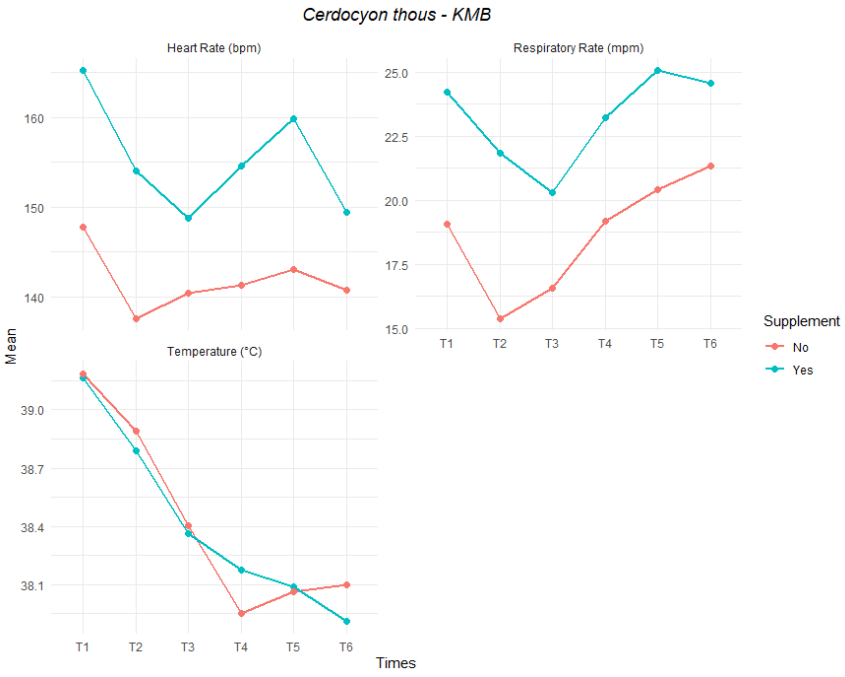

Figure 14. Heart rate (beats per minute), Respiratory rate (movements per minute), temperature (in Celsius degrees) of free-ranging *Cerdocyon thous* anesthetized with ketamine-midazolam-butorphanol (KMB), with or without intravenous ketamine supplementation, from T1 to T6.

Regarding qualitative parameters, figure 15 shows the percentage of each one overtime of the protocols that reached sufficient records.

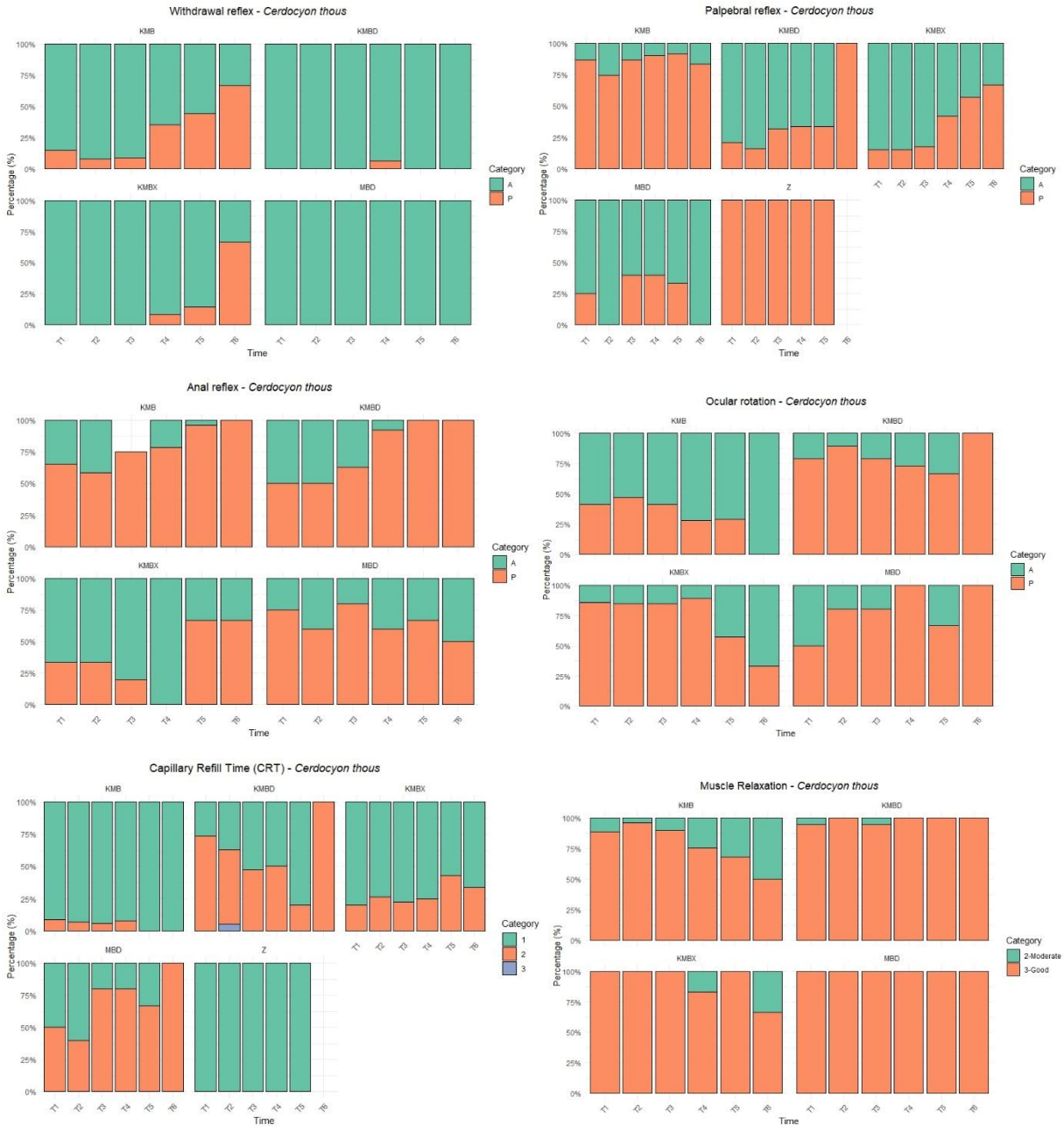

Figure 15. Presence (P) or absence (A) of withdrawal, palpebral and anal reflexes and ocular rotation; capillary refill time (1, 2 or 3 seconds) and muscle relaxation (absent, moderate or good) in free-ranging *Cerdocyon thous* anesthetized with ketamine-midazolam-butorphanol (KMB), ketamine-midazolam-butorphanol-dexmedetomidine (KMBD), ketamine-midazolam-butorphanol-xylazine (KMBX), midazolam-butorphanol-dexmedetomidine (MBD) and tiletamine-zolazepam (Z) from T1 to T6.

Side effects were observed in 5 protocols. KMB caused sialorrhea in 2.19% (n = 2) of the animals, arrhythmia in 29.67% (n = 27), sudden awakening in 1.09% (n = 1) and bradypnea in 1.09% (n = 1). KMBD produced arrhythmia in 95% (n = 19) of the animals, sudden awakening in 35% (n = 7) and low flow at blood collection in 5% (n = 1). Sinus arrhythmia was observed in 100% (n = 5) of the animals anesthetized with MBD, sudden awakening in 60% (n = 3) and low flow at blood collection in 40% (n = 2). KMBX caused arrhythmia in 35% (n = 10) and sudden awakening in 3.57% (n = 1). Z produced sialorrhea in 4.16% (n = 1) of the animals.

DISCUSSION

All the anesthetic protocols that reached sufficient numbers and were statistically analyzed provided suitable depth of anesthesia for basic handling and sampling, but each regimen affected physiological parameters and anesthesia depth differently, also providing diverse intervals of immobilization and recovery, and differences on side effects.

Our findings have proven the effectiveness of ketamine-midazolam-butorphanol (KMB) protocol in the chemical immobilization of free-ranging maned wolves, mainly considering its recumbency rate, immobilization time and recovery. Ketamine-midazolam based protocols have been reported as useful and safe many times in wild canids and other carnivores (Riberio *et al*., 2022; Lima *et al*., 2016; Shilo *et al*., 2010). The additional butorphanol may have contributed to lowering doses, bringing analgesia and balancing possible side effects.

The current published studies with maned wolf anesthesia include mainly protocols with tiletamine-zolazepam (Bronson *et al*., 2021; Furtado *et al*., 2006) and ketamine-xylazine (Curi & Talamoni, 2006). Ketamine-midazolam is not a combination that has been extensively investigated for wild canids, probably because of the availability of tiletamine-zolazepam, a relatively inexpensive, high concentration dissociative-benzodiazepine alternative (Larsen and Kreeger, 2014). Additionally, many authors seem to prefer to combine ketamine with α-2 agonists.

The recumbency rate observed in this study was 83%, indicating that KMB was effective in inducing the desirable level of immobilization (effect 3) in most animals. This success rate is comparable to previous studies using ketamine-based combinations in wild canids, such as Acosta-Jamett *et al*. (2010). Differences in handling, species behavior, or environmental conditions may explain variations in success rates across studies. Another point to be considered is that weight was estimated before drug administration, which could imply sub dosage. Additionally, increasing ketamine dosage could also increase recumbency rate.

The mean latency time was 6.0 ± 0.57 minutes, which falls within the expected range for intramuscular ketamine combinations in similar species (Telesco & Sovada, 2002; Justo *et al*., 2019). Short latency is advantageous in field conditions, minimizing stress and the risk of escape or injury. Comparable latencies were reported in *C. brachyurus* by Curi & Talamoni (2006), who observed 6.7 minutes with ketamine-xylazine. Slight differences may reflect species-specific responses or drug and dosage variations.

The average time of immobilization was 35.57 ± 17.04 minutes, which is sufficient to perform routine field procedures such as physical exams and sample collection. This duration is consistent with previous reports using similar drug combinations in wild carnivores (Larsen *et al*., 2002). Prolonged or shortened immobilization times in literature may be influenced by drug choice and dosage, animal condition, or environmental temperature. Curi & Talamoni recorded a working time of 66 minutes using ketamine-xylazine (8 and 2 mg/kg, respectively), but it is to be noticed that these authors do not specify the intervals measured and they may not be comparable to ours. In 50% of the animals, IV ketamine supplementation was required to maintain adequate anesthesia depth or to extend anesthesia time according to the procedure's necessities; and supplements were administered up to four times. Curi & Talamoni (2006) also reported the need for ketamine supplementation in 28.57% of the maned wolves immobilized with ketamine-xylazine. Anesthesia supplementation could be interpreted as a disadvantage in a field immobilization protocol, but choosing the IV route for ketamine supplementation allows us to use smaller doses than the IM route, and smaller doses in the initial protocol, contributing also to a softer recovery. High doses of IM ketamine tend to prolong time of recovery without extending immobilization duration (Larsen & Kreeger, 2014). Supplementing anesthesia with IV ketamine instead of the commonly used propofol is also the safest choice in the field, since propofol may induce apnea, a severe side effect when ventilation equipment is not available. Knowing the estimate duration of the immobilization protocol and of each supplementation allows the field veterinarian to predict the needs of supplementation, assuring safer procedures.

Initial recovery was observed at 54.33 ± 30.02 minutes after the last partial return. Although KMB lacks specific reversal agents to all the drugs, the recovery length was acceptable, aligning with findings from other studies using ketamine-based protocols in canids (Kreeger *et al*., 1990). Tiletamine-zolazepam protocols usually provide longer initial recoveries, as registered by Bronson et al. (2021), who observe a mean of 70 minutes for maned wolves lift their heads after the end of the procedure.

Multiple side effects were observed by Furtado *et al*. (2006) with Tiletamine-Zolazepam, most notably compulsive licking, hypersalivation, muscle tremors, and muscle twitching. Curi & Talamoni (2006) observed vocalization during recovery with ketamine-xylazine. We did not see these effects in our study, what demonstrates a superiority of KMB over Z, but we did register 7.14% of sinus arrhythmia. Respiratory arrhythmia, also referred to as respiratory sinus arrhythmia

(RSA), is a normal and physiological modulation of heart rhythm that occurs in synchrony with the respiratory cycle. It is characterized by a fluctuation in heart rate, increasing during inspiration and decreasing during expiration (Shykoff *et al*., 1991; Ribeiro *et al*., 2022). Yet, as this side effect was diagnosed with a stethoscope and the use of a proper electrocardiograph would better elucidate the nature of these arrhythmias.

Heart rate (median range from 107 to 129 bpm), produced by KMB is slightly lower than the mean of 129 bpm found by Bronson *et al.* (2021), in maned wolves anesthetized with tiletamine-zolazepam (mean dose of 4.6 mg/kg). Furtado *et al*. (2006) recorded significantly higher heart rate (mean range from 146 to 151), that can be attributed to the smaller Z dosage (mean of 2.77 mg/kg) and its consequent more superficial anesthesia. Larsen *et al*. (2002) using medetomidine instead of midazolam, recorded heart rates below 100 bpm in *Canis rufus* (a wild canid with similar weight to *C. brachyurus*), probably because of the expected cardiovascular depressing effect caused by the addition of the α-2 agonist. Curi & Talamoni (2006) using a high dose of xylazine (2 mg/kg), observed heart rates of 75 ± 13.2 bpm. The respiratory rate (18 to 24 mpm) found in this study is also lower than Furtado *et al*. (2006) findings, but similar to Larsen's. Temperature (37.8 to 38.2°C) falls within the expected, according to the same studies. KMB induced physiological parameters represent a middle-ground between tiletamine-zolazepam and α-2 agonists combined protocols. This fact added to the stability over time indicates the safety and consistency of this protocol.

The capillary refill time of 1 second registered throughout the entire time of monitoring indicates adequate blood perfusion and blood pressure. Percentages of withdrawal, palpebral and anal reflex, ocular rotation and muscle relaxation suggest that the anesthesia depth is suitable for non-invasive with minimum pain procedures. KMB is not suitable for surgery.

When comparing parameters of animals that received IV ketamine supplementation to the ones who did not (Figure 7), it is interesting to observe that the supplemented animals already started the procedures with higher values of heart and respiratory rates, even before any supplementary IV dose. This may suggest that these individuals presented higher levels of stress at the beginning of the procedures, probably by individual response or difficulties in handling prior to anesthesia, what could justify the need for supplementation during the procedure. The increasing tendency in both parameters during the procedure may be attributed both to IV ketamine administration and anesthesia metabolization overtime.

The following discussion will concern both *C. thous* and *L. vetulus* results to each protocol, as they received very similar dosage.

Regarding KMB protocol, around 94% of hoary foxes and 94.5% of crab-eating foxes achieved effect 3 with the initial protocol, latency was 5.74 ± 2.59 for *L. vetulus* and 5.93 ± 3.72 for *C. thous*, with a mean immobilization time of 35.43 ± 10.46 and 38.59 ± 16.8 minutes, respectively. This demonstrates that KMB protocol is suitable for chemically restraining this species at field conditions, providing adequate immobilization time for brief procedures.

These findings are very similar to the ones observed in *C. brachyurus* anesthetized with the same protocol, but here a much higher initial ketamine dose (15.09 ± 2.86 mg/kg for *L. vetulus* and 15.63 ± 2.43 for *C. thous*) is to be noticed. This may be attributed to the smaller weight (3.79 ± 0.37 kg and 5.97 ± 0.72, respectively) compared with 22.67 ± 4.09 kg of the maned wolves, and consequent faster metabolism of both species. Kreeger *et al*. (1990) used 30.8 mg/kg of ketamine and 0.6 mg/kg of midazolam to immobilize red foxes (*Vulpes vulpes*), whose weight is similar to *C. thous*, producing latency of 4.9 and immobilization of 18.6 minutes. This information suggests that doubling the ketamine dosage shortens latency but does not extend immobilization time, which makes it possible to conclude that the addition of butorphanol to the protocol may have contributed to adjust anesthesia depth and time. Nevertheless, 15 mg/kg of ketamine (and 7.5 mg/kg for IV supplementations) is notably higher than the usual dosage for animals in captivity (Nunes & Cortopassi, 2007), indicating that stress levels of free-ranging animals enduring human contact makes ir necessary to significantly increase dissociative doses.

Considering that procedures duration to fulfill all the activities needed in this project was about 45-60 minutes, 39.13% of *L. vetulus* and 38.46% of *C. thous* required IV ketamine supplementation, what may not be a disadvantage, considering safety and quality of induction and recovery under field conditions. Shorter procedures might not need supplementation. As mentioned before, ketamine-midazolam combinations do not seem to be enough studied in wild canids, as most literature presents tiletamine-zolazepam or α-2 agonists combinations. Like *C. brachyurus*, the crab-eating foxes who needed ketamine supplementation also presented higher heart and respiratory rates at the beginning of the procedures (figure 14), suggesting a particular stress response or handling issue, justifying the need for supplementation in these individuals. The same cannot be affirmed about the hoary foxes, as heart and respiratory rate values are very similar

with or without ketamine supplementation. But a marked increase in respiratory rates in T3 and T4 may be caused by IV ketamine administration (figure 10).

Initial recovery happened 30.1 ± 22.23 minutes after the last partial return for *L.vetulus* and 24 ± 14.38 for *C. thous*. These intervals may be considered adequate when compared to longer recovery periods provided by tiletamine-zolazepam.

As observed in maned wolves, the capillary refill time of 1 second registered during most of the time of monitoring indicates adequate blood perfusion and blood pressure for both foxes. Percentages of withdrawal, palpebral and anal reflex, ocular rotation and muscle relaxation suggest that the anesthesia depth is suitable for non-invasive with minimum pain procedures, as well.

Perhaps the only noticeable side effect recorded to both foxes was respiratory arrhythmia (18.3% in hoary-foxes and 28.42% in crab-eating foxes). As previously discussed, an electrocardiograph would better elucidate the nature of them, as well as blood pressure monitoring.

Moving on to KMBX, recumbency rate was 100% for hoary foxes and 96.55% for crab-eating foxes, demonstrating KMBX as an efficient choice for chemical restraint of both species. Latency of 6.25 ± 2.96 min and immobilization of 37.75 ± 11.5 min for *L. vetulus.* Latency of *C. thous* (3.92 ± 2.05) were similar to KMB, but we found significant difference in time of immobilization (54.5 ± 17.23) for *C. thous* in relation to KMB, demonstrating the adding xylazine made anesthesia last longer. Curi & Talamoni (2006) found a similar immobilization time of 61.8 ± 22.6 min for *C. thous* using ketamine-xylazine. This may be an important advantage, mainly considering that we also found significant difference in ketamine dosage in KMBX (13.5 mg/kg) in relation to KMB (15.63 mg/kg). Decreasing dissociative drugs dosage may contribute to a faster and softer recovery, besides turning the protocol prone to the use of antagonists.

Regarding the qualitative parameters that indicate anesthesia depth, they can be considered very alike to the ones in KMB, but in KMBX we start to perceive a better muscle relaxation in both species and a higher frequency of capillary refill time of 2 seconds. Both effects are expected when adding α-2 agonists to the protocol, because of their known action in muscle relaxation and peripheral vasoconstriction.

KMBX presents a higher percentage of respiratory arrythmia in *L. vetulus* (71.42%) and in *C. thous* (32.25%) than KMB. This frequency is not something to pass unnoticed and deserves further investigation. Arrhythmia is to be expected when using α-2 agonists, therefore this class of anesthetic should be avoided in knowingly cardiopaths.

The combination ketamine-midazolam-butorphanol-dexmedetomidine (KMBD) has shown itself to be effective to field procedures in free ranging *L. vetulus* and *C. thous*, according to the analyzed data. 90% of the hoary foxes and 100% of the crab-eating foxes achieved effect 3 of sedation. We found statistical evidence that immobilization time for both species (48.33 ± 11.92 minutes for *L. vetulus* and 47 ± 11.43 for *C. thous*) was longer than KMB, meaning that the addition of dexmedetomidine brings an advantage to field procedures. Another advantage of KMBD protocol in relation to KMB and KMBX regarding hoary foxes is the statistically proven shorter initial recovery time (8.75 ± 4.5 min). For the crab-eating foxes, the same is true in relation to KMB. Adding dexmedetomidine, a very efficient and specific α-2 agonist, made it possible to significantly reduce the ketamine dosage to about 30% of the KMB dosage (mean of 4.47 mg/kg for *L. vetulus* and 4.34 for *C. thous*), resulting in shorter recovery.

Another observed great advantage of KMBD with low ketamine doses is the possibility of using antagonists, making the animals ready to release even sooner, a very desired characteristic in a field anesthesia protocol.

Even so, 70% of hoary foxes needed anesthesia supplementation and sudden awakening was seen in 50 % of hoary foxes and 33.33% of crab-eating foxes. These facts suggest that the low ketamine dosage produces a lack of stability in anesthesia depth, what neeed to be considered a point of attention when handling carnivores and probably making the use of KMBD with these ketamine doses advisable only to experienced professionals. Another drawback is the remarkable presence of respiratory arrhythmia (50% in *L.vetulus* and 90.47% in *C. thous*) and low flow at blood collection (20 % in *L. vetulus* and 4.76% in *C. thous*). Depending on the line of research, it is necessary to collect large volumes of blood, which can be compromised using this specific α-2 agonist.

Analyzing the capillary refill time (Figures 11 and 15), KMBD clearly produces a longer response time, including records of 3 seconds in *C. thous*, which relates to the peripheral vasoconstriction caused by dexmedetomidine (that also is probably related to the low flow at blood collection). Further studies are needed to investigate the presence of hypertension. On the other hand, KMBD produces great muscle relaxation when compared to KMB and KMBX.

For the tiletamine-zolazepam procedures, sialorrhea was recorded in 22.22% of hoary foxes and 4.16% of crab-eating foxes. The capillary refill time lasted mostly up to 1 second in both foxes, similarly to KMB, and palpebral reflex exhibited the higher frequency among the protocols

in *C. thous*. These findings are expected to observe using Z and compatible with what Bronson *et al*. (2021) and Furtado *et al*. (2006) observed. Regarding Z records, it is important to highlight that this protocol was used in the first field procedures carried on by the PCMC staff, when the anesthesia project was not yet defined and records of induction and recovery were not consistent in the files.

Midazolam-butorphanol-dexmedetomidine was used in 5 *C. thous*, and because of its absence of ketamine, this protocol deserves to be analyzed. As expected, the stability assured by ketamine is not seen, proven by the presence of 100% respiratory arrhythmia, 60% sudden awakening and 40% low flow at blood collection. Also as expected, MBD presents the lowest frequency of reflexes and the best muscle relaxation. Nevertheless, the small number of MBD records makes it difficult to recommend or not the use of this protocol. MBD was used as an alternative of total reversible protocol by the PCMC staff, and its instability and high occurrence of sudden awakening made it necessary to add low doses of ketamine, replacing it with the KMBD protocol.

In *L. vetulus'* heart rate monitoring there is no significant difference between Z and KMB, but both are significantly higher than the protocols that include α-2 agonists, from T1 to T3, clearly demonstrating the difference between dissociative drugs and α-2 agonists' actions to the cardiovascular system, as the first stimulate it and, the latter, depress it. The same effect can be observed in respiratory rate monitoring, but only with Z being significantly higher than the others, showing the commonly known potency of tiletamine.

C. thous' heart and respiratory rate monitoring exhibits the same previously explained actions of dissociative versus α-2 agonists, but in this species, we observe an evident contrast of Z over the other protocols, as Z also produces significantly higher values than KMB, mainly at the beginning if the procedures. Heart rate's means over 175 bpm may result in hypertension, and a mean respiratory rate above 45 mpm deserves to be further investigated, concerning its consequences to blood gas analyses.

About temperature, there is a noticeable detail at T1 in *C. thous* monitoring. Besides existing significant lower values of KMB in relation to MBD, even considering the few records of the latter protocol, MBD is the only protocol with mean values over 40°C. This could be elucidated by the remarkable vasoconstriction caused by MBD, as seen in CRT and low flow at blood

collection. The peripheral vasoconstriction makes it harder for the animals to lose heat, which could result in higher temperature values in a warm environment.

C. thous and *L. vetulus* were the species where a statistically significant decrease overtime was seen in heart rate (in KMB and KMBX) and in temperature (KMB, KMBX, KMBD, MBD and Z). The decrease in heart rate may be explained by a higher rate in the beginning of the procedures, expected by commonly observed high levels of stress in this species during handling, and by the high numbers of records of both protocols. The decrease in temperature registered in all protocols is something expected because anesthesia procedures in small carnivores are commonly known to cause temperature drop.

The 321 anesthesia sheets recorded by PCMC over more than 10 years of field efforts served as a rich base for this work, bringing plenty of information of various drug combinations to chemically restrain wild canids, and there are no published studies with this level of robustness.

It is not by chance that KMB is the most numerous recorded protocol for the three species, demonstrating an evident preference for this combination by the PCMC staff. Times of induction, immobilization and recovery are suitable for their field procedures, and the stability of vital signs and anesthesia depth prove the safety of this specific drugs. Anesthesia supplementation was very often, but knowing dosages, time of action and securing peripheral venous access makes it possible for the field team to organize and prepare for anesthesia supplementation in advance. IV ketamine supplementation has been proven to be a secure and cheap option, besides providing a smooth recovery. These findings support the use of KMB as a viable anesthetic option for free-ranging maned wolves, hoary foxes and crab-eating foxes, offering adequate immobilization with manageable recovery profiles. Studies with measurements of blood pressure, blood oxygen saturation and electrocardiogram monitoring are important to further investigation of KMB in these canids.

KMBX produced longer immobilization time, better muscle relaxation and less need for supplementation in the foxes' procedures, but the evident presence of arrhythmia deserves further investigation.

Concerning KMBD, despite providing longer immobilization time, great muscle relaxation, fast recovery and the possibility of using antagonists, the marked presence of arrhythmia, sudden awakening and low flow at blood collection brings the necessity of carefully thinking and preparing for this drug combination, mainly considering the cardiovascular effects in

the animals and the hazards that carnivore's sudden awakening may bring to the staff and equipment. However, it could be a good choice for procedures with experienced teams that need to do less invasive procedures requiring a reversible protocol, allowing a fast recovery in the field.

MBD protocol brings the same disadvantages, but potentialized by the absence of ketamine, resulting also in temperature consequences. The few records of the protocol highlight the need for more robust research.

Tiletamine-zolazepam brings the practicality of already offering two drugs previously combined in the same bottle, besides usually resulting in low volumes, which is desirable for wild animals' anesthesia in general. But it is plainly registered in literature the excitation side effects, demonstrated in this study by the high values of heart and respiratory rates in both foxes. Its prolonged and agitated recovery is another currently known disadvantage, that takes Z off the place of drug of choice for wild canids.

It is important to note that field procedures are known to happen in a less controlled environment, many times with difficult access and, therefore, with limited equipment and financial resources. These factors imply in limitations of monitoring and ventilation support but does not preclude that the field team works prepared for emergencies with cheap and simple equipment such as tracheotubes, ambu bags and vasoactive drugs.

CONCLUSION

The present work has succeeded in fulfilling its general and specific objectives. The built database based on the PCMC field sheets was adequate to all descriptive and inferential statistical analyses, which allowed us to access and compare five different anesthetic combinations used to chemically immobilize the free-ranging Cerrado canids *Chrysocyon brachyurus*, *Lycalopex vetulus* and *Cerdocyon thous*.

Ketamine-midazolam-butorphanol has proven itself to be a safe and efficient protocol for minimum invasive field procedures for the three species, bringing adequate intervals of induction, immobilization and recovery. The often need for intravenous ketamine supplementation is not to be considered a disadvantage, as it has proven to be a safe and cheap option, making anesthesia time and depth manageable, besides showing no interference with recovery. The values and stability of physiological parameter overtime is another aspect of why this combination brings the desirable security, both to the animals and the staff.

Adding α-2 agonists to the protocols, improves muscle relaxation, extends anesthesia time and shortens recovery. But its cardiovascular side effects need further research to assure the protocols' safety. Sudden awakening is also a point of attention.

This information added to the extensive literature about tiletamine-zolazepam side effects, makes it clear that there are more efficient and more safe combinations that can be used to chemically restrain wild canids, highlighting the importance of knowing the physiological implications of each drug to vital sings and anesthesia depth.

Inconsistencies in field sheets' notes and few numbers of some protocols are the limitations of the study, and a more complete monitoring of cardiovascular and respiratory functions will better elucidate the protocols' safety.

Well-designed and safe anesthetic protocols that meet the practical needs of field research are essential for the conservation of wild carnivores. Our study provides a level of anesthetic data robustness not yet available in the current literature, hopefully contributing valuable information to support conservation efforts for Cerrado canids.

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APPENDIX

Figure 1. Images that exemplify the database built based on PCMC field records.

	A	B	C	D	E	F	G	H
1	data	especie	nome	id	brinco	sexo	peso_est	peso_real
2	3/23/2019	L. vetulus	Emma	bPve431	267 amarel	F	3,3	3,19
3	3/25/2019	L. vetulus	Constance	bPve373	250	F	3,5	3,77
4	3/25/2019	L. vetulus	Marsh	bPve430	78 verde	M	2,8	3,15
5	3/24/2019	L. vetulus	Janine	bPve429	389 amarel	F	2,8	3,42
6	3/22/2019	L. vetulus	Schaller	bPve400	165 azul	M	3,5	3,4
7	3/21/2019	L. vetulus	Peary	bPve411	275 vermel	F	3,5	3,1
8	3/20/2019	L. vetulus	Cope	bPve428	274 vermel	M	3	2,9

zoletil_DR	quetamina_DR	midazolam_DR	butorfanol_DR	xilazina_DR	dexmedeto_01_DR	dexmedeto_05_DR	tramadol_DR
NA	3,134796238	0,517241375	0,219435737	NA	NA	0,007836991	NA
NA	13,79310345	0,464190983	0,185676393	NA	NA	NA	NA
NA	13,33333333	0,444444444	0,19047619	NA	NA	NA	NA
NA	12,28070175	0,409356725	0,175438596	NA	NA	NA	NA
NA	15,29411765	0,514705883	0,205882353	NA	NA	NA	NA
NA	16,77419355	0,564516125	0,225806452	NA	NA	NA	NA
NA	15,51724138	0,517241375	0,206896552	NA	NA	NA	NA
NA	14,51612903	0,483870968	0,193548387	NA	NA	NA	NA

tempos_par	hora	fr	fc	mperatu	tpc	ref_podal	ref_pap	ref_anal	relax	rot_oc
T2	10:47	30	204	39,2	1	A	A	P	B	P
T3	10:57	30		38,4	1	A	P	P	B	A
T1	13:35	30	192	39	1	A	A	P	B	P
T2	13:45	28	186	38,9	1	A	A	A	B	P
T3	13:55	24	204	38,9	2	A	P	P	B	A
T1	11:52	42	180	38,7	1	P	P	P	R	A
T2	12:02	30	180	38	1	P	P	P	R	A
T3	12:22	24	168	37,5	1	P	P	P	R	P
T1	10:07	32	168	39,4	1	A	P	P	B	A
T2	10:17	18	168	38,9	2	A	P	A	B	P
T3	10:27	16	156	38,7	1	A	P	A	B	A
T4	10:37	42	162	38,5		P	P	P	R	

Table 8. P-values of physiological parameters (heart rate, respiratory rate and temperature) in relation to time of monitoring, that indicate significant difference between pairs of protocols used to anesthetize free-ranging *Lycalopex vetulus*

Parameters	**T**	**Protocol**	**N**	**P value**	**Normalty**	**Test**	**P value**
Respiratory Rate	T1	KMB	64	0.0002	Non normal	Kruskal-Wallis	$7.7x10^{-07}$
	T1	KMBD	8	0.31	normal		
	T1	KMBX	7	0.5	normal		
	T1	Z	15	0.0003	Non normal		
	T2	KMB	59	$2.62x10^{-06}$	Non normal	Kruskal-Wallis	0.0001
	T2	KMBD	8	0.04	Non normal		
	T2	KMBX	6	0.58	normal		
	T2	Z	13	0.02	Non normal		
	T3	KMB	51	$3.60x10^{-08}$	Non normal	Kruskal-Wallis	0.0004
	T3	KMBD	7	0.06	normal		
	T3	KMBX	5	0.11	normal		
	T3	Z	8	0.85	normal		
	T4	KMB	29	$1.94x10^{-07}$	Non normal	Kruskal-Wallis	0.01
	T4	KMBD	5	0.004	Non normal		
	T4	KMBX	3	0.89	normal		
	T4	Z	5	0.002	Non normal		
	T5	KMB	8	0.06	normal	Kruskal-Wallis	0.18
	T5	KMBD	1	NA	Non normal		
	T5	KMBX	1	NA	Non normal		
	T5	Z	2	NA	Non normal		
Heart Rate	T1	KMB	63	0.08	normal	ANOVA	$4.73x10^{-08}$
	T1	KMBD	7	0.79	normal		
	T1	KMBX	7	0.72	normal		
	T1	Z	15	0.29	normal		
	T2	KMB	56	0.23	normal	ANOVA	$2.13x10^{-07}$
	T2	KMBD	8	0.06	normal		

	T2	KMBX	5	0.2	Normal		
	T2	Z	12	0.46	Normal		
	T3	KMB	50	0.006	Non normal	Kruskal-Wallis	$2.11x10^{-05}$
	T3	KMBD	7	0.65	normal		
	T3	KMBX	5	0.007	Non normal		
	T3	Z	9	0.47	normal		
	T4	KMB	31	0.63	normal	ANOVA	0.0001
	T4	KMBD	5	0.11	normal		
	T4	KMBX	4	0.12	normal		
	T4	Z	8	0.27	normal		
	T5	KMB	8	0.41	normal	Kruskal-Wallis	0.17
	T5	KMBD	1	NA	Non normal		
	T5	KMBX	1	NA	Non normal		
	T5	Z	3	0.42	normal		
Temperature	T1	KMB	59	0.34	normal	Kruskal-Wallis	0.59
	T1	KMBD	5	0.47	normal		
	T1	KMBX	2	NA	Non normal		
	T1	Z	16	0.01	Non normal		
	T2	KMB	56	0.34	normal	ANOVA	0.18
	T2	KMBD	8	0.44	normal		
	T2	KMBX	6	0.84	normal		
	T2	Z	15	0.66	normal		
	T3	KMB	49	0.56	normal	Kruskal-Wallis	0.16
	T3	KMBD	8	0.98	normal		
	T3	KMBX	4	0.64	normal		
	T3	Z	9	0.02	Non normal		
	T4	KMB	32	0.63	normal	Kruskal-Wallis	0.65
	T4	KMBD	5	0.52	normal		
	T4	KMBX	2	NA	Non normal		

	T4	Z	8	0.21	Normal		
	T5	KMB	7	0.83	normal	Kruskal-Wallis	0.81
	T5	KMBD	1	NA	Non normal		
	T5	KMBX	1	NA	Non normal		
	T5	Z	2	NA	Non normal		

Table 14. P-values of physiological parameters (heart rate, respiratory rate and temperature) in relation to time of monitoring, that indicate significant difference between pairs of protocols used to anesthetize free-ranging *Cerdocyon thous*

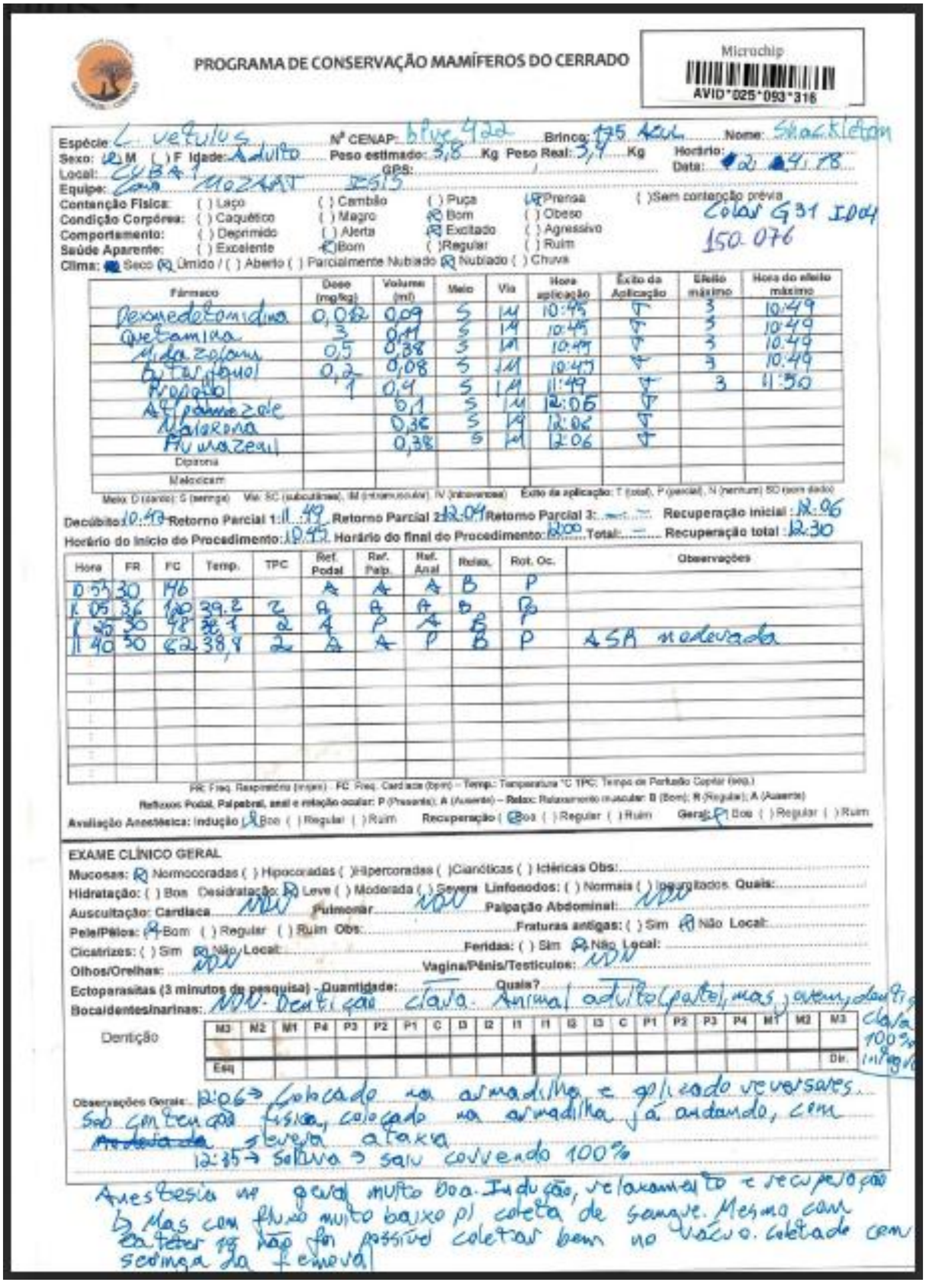
Parameters	**T**	**Protocolos**	**N**	**P value**	**Normalty**	**Test**	**P value**
Respiratory Rate	T1	MBD	4	0.79	normal	Kruskal-Wallis	0.0005
	T1	KMB	84	0.034	non normal		
	T1	KMBD	19	0.22	normal		
	T1	KMBX	25	0.08	normal		
	T1	Z	15	0.52	normal		
	T2	MBD	5	0.63	normal	Kruskal-Wallis	$2.93x10^{-06}$
	T2	KMB	77	$2.12x10^{-06}$	non normal		
	T2	KMBD	19	0.17	normal		
	T2	KMBX	21	0.06	normal		
	T2	Z	14	0.36	normal		
	T3	MBD	5	0.1	normal	Kruskal-Wallis	0.003
	T3	KMB	69	$4.73x10^{-05}$	non normal		
	T3	KMBD	19	0.17	normal		
	T3	KMBX	20	$8.67x10^{-05}$	non normal		
	T3	Z	7	0.22	normal		
	T4	MBD	5	0.68	normal	Kruskal-Wallis	0.026
	T4	KMB	50	$2.54x10^{-06}$	non normal		
	T4	KMBD	14	0.03	non normal		
	T4	KMBX	15	0.81	normal		
	T4	Z	3	0.86	normal		

	T5	MBD	3	0.21	normal	Kruskal-Wallis	0.23
	T5	KMB	25	0.891	normal		
	T5	KMBD	6	0.087	normal		
	T5	KMBX	9	0.51	normal		
	T5	Z	2	NA	non normal		
	T6	MBD	2	NA	non normal	Kruskal-Wallis	0.24
	T6	KMB	6	0.75	normal		
	T6	KMBD	1	NA	non normal		
	T6	KMBX	3	0.14	normal		
Heart Rate	T1	MBD	5	0.64	normal	Kruskal-Wallis	$3.87x10^{-15}$
	T1	KMB	83	0.16	normal		
	T1	KMBD	19	0.17	normal		
	T1	KMBX	25	0.056	normal		
	T1	Z	16	0.009	non normal		
	T2	MBD	5	0.17	normal	ANOVA	$6.29x10^{-16}$
	T2	KMB	75	0.38	normal		
	T2	KMBD	19	0.35	normal		
	T2	KMBX	23	0.51	normal		
	T2	Z	15	0.99	normal		
	T3	MBD	5	0.51	normal	ANOVA	$1.42x10^{-16}$
	T3	KMB	69	0.44	normal		
	T3	KMBD	19	0.22	normal		
	T3	KMBX	19	0.51	normal		
	T3	Z	8	0.64	normal		
	T4	MBD	5	0.95	normal	ANOVA	$2.32x10^{-14}$
	T4	KMB	50	0.45	normal		
	T4	KMBD	14	0.506	normal		
	T4	KMBX	15	0.13	normal		
	T4	Z	4	0.91	normal		

	T5	MBD	3	0.701	normal	Kruskal-Wallis	0.0001
	T5	KMB	25	0.904	normal		
	T5	KMBD	6	0.27	normal		
	T5	KMBX	9	0.38	normal		
	T5	Z	2	NA	non normal		
	T6	MBD	2	NA	non normal	Kruskal-Wallis	0.12
	T6	KMB	5	0.809	normal		
	T6	KMBD	1	NA	non normal		
	T6	KMBX	4	0.96	normal		
Temperature	T1	MBD	4	0.68	normal	Kruskal-Wallis	0.029
	T1	KMB	83	0.35	normal		
	T1	KMBD	14	0.03	non normal		
	T1	KMBX	24	0.81	normal		
	T1	Z	14	0.57	normal		
	T2	MBD	5	0.011	non normal	Kruskal-Wallis	0.083
	T2	KMB	76	0.12	normal		
	T2	KMBD	19	0.17	normal		
	T2	KMBX	21	0.81	normal		
	T2	Z	13	0.53	normal		
	T3	MBD	5	0.24	normal	ANOVA	0.02
	T3	KMB	70	0.39	normal		
	T3	KMBD	19	0.14	normal		
	T3	KMBX	20	0.21	normal		
	T3	Z	8	0.99	normal		
	T4	MBD	5	0.72	normal	Kruskal-Wallis	0.069
	T4	KMB	51	0.0007	não normal		
	T4	KMBD	13	0.13	normal		
	T4	KMBX	15	0.47	normal		
	T4	Z	4	0.58	normal		

	T5	MBD	3	0.85	normal	ANOVA	0.304
	T5	KMB	25	0.81	normal		
	T5	KMBD	5	0.37	normal		
	T5	KMBX	9	0.507	normal		
	T5	Z	4	0.42	normal		
	T6	MBD	2	NA	não normal	Kruskal-Wallis	0.98
	T6	KMB	5	0.99	normal		
	T6	KMBD	1	NA	não normal		
	T6	KMBX	4	0.27	normal		

ANNEX


PROGRAMA DE CONSERVAÇÃO MAMÍFEROS DO CERRADO

Microchip
AVID*025*093*316

Espécie: L. vetulus Nº CENAP: blue 422 Brinco: 175 Azul Nome: Shackleton
Sexo: (X) M ( ) F Idade: Adulto Peso estimado: 3,8 Kg Peso Real: 3,4 Kg Horário:
Local: CUBA 1 GPS: Data: 02/04/18
Equipe: Caio, Mozart, Lisis
Contenção Física: ( ) Laço ( ) Cambão ( ) Puça (X) Prensa ( ) Sem contenção prévia
Condição Corpórea: ( ) Caquético ( ) Magro (X) Bom ( ) Obeso
Comportamento: ( ) Deprimido ( ) Alerta (X) Excitado ( ) Agressivo
Saúde Aparente: ( ) Excelente (X) Bom ( ) Regular ( ) Ruim
Clima: (X) Seco (X) Úmido / ( ) Aberto ( ) Parcialmente Nublado (X) Nublado ( ) Chuva
Colar G31 ID04 150.076

Fármaco	Dose (mg/kg)	Volume (ml)	Meio	Via	Hora aplicação	Êxito da Aplicação	Efeito máximo	Hora do efeito máximo
Dexmedetomidina	0,02	0,09	S	IM	10:45	T	3	10:49
Quetamina	3	0,11	S	IM	10:45	T	3	10:49
Midazolam	0,5	0,38	S	IM	10:45	T	3	10:49
Butorfanol	0,2	0,08	S	IM	10:45	T	3	10:49
Propofol	1	0,4	S	IV	11:49	T	3	11:50
Atipamezole		0,1	S	IM	12:06	T		
Naloxona		0,38	S	IM	12:06	T		
Flumazenil		0,38	S	IM	12:06	T		
Dipirona								
Meloxicam								

Meio: D (dardo); S (seringa) Via: SC (subcutânea), IM (intramuscular), IV (intravenosa) Êxito da aplicação: T (total), P (parcial), N (nenhum) SD (sem dado)

Decúbito: 10:49 Retorno Parcial 1: 11:49 Retorno Parcial 2: 12:04 Retorno Parcial 3: — Recuperação inicial: 12:06
Horário do início do Procedimento: 10:45 Horário do final do Procedimento: 12:00 Total: — Recuperação total: 12:30

Hora	FR	FC	Temp.	TPC	Ref. Podal	Ref. Palp.	Ref. Anal	Relax.	Rot. Oc.	Observações
10:55	30	146			A	A	A	B	P	
11:05	36	120	39,2	2	A	A	A	B	P	
11:25	30	98	38,7	2	A	P	A	B	P	
11:40	30	62	38,8	2	A	A	P	B	P	ASA moderada

FR: Freq. Respiratória (mpm) - FC: Freq. Cardíaca (bpm) - Temp.: Temperatura °C TPC: Tempo de Perfusão Capilar (seg.)
Reflexos: Podal, Palpebral, anal e rotação ocular: P (Presente); A (Ausente) – Relax: Relaxamento muscular: B (Bom); R (Regular); A (Ausente)
Avaliação Anestésica: Indução (X) Bom ( ) Regular ( ) Ruim Recuperação (X) Boa ( ) Regular ( ) Ruim Geral: (X) Boa ( ) Regular ( ) Ruim

EXAME CLÍNICO GERAL
Mucosas: (X) Normocoradas ( ) Hipocoradas ( ) Hipercoradas ( ) Cianóticas ( ) Ictéricas Obs:
Hidratação: ( ) Boa Desidratação: (X) Leve ( ) Moderada ( ) Severa Linfonodos: ( ) Normais ( ) Ingurgitados Quais:
Auscultação: Cardíaca NDN Pulmonar NDN Palpação Abdominal: NDN
Pele/Pêlos: (X) Bom ( ) Regular ( ) Ruim Obs: Fraturas antigas: ( ) Sim (X) Não Local:
Cicatrizes: ( ) Sim (X) Não Local: Feridas: ( ) Sim (X) Não Local:
Olhos/Orelhas: NDN Vagina/Pênis/Testículos: NDN
Ectoparasitas (3 minutos de pesquisa) - Quantidade: Quais?
Boca/dentes/narinas: NDN. Dentição clara. Animal adulto (parte), mas jovem, dentição clara 100%

Dentição: M3 M2 M1 P4 P3 P2 P1 C I3 I2 I1 I1 I2 I3 C P1 P2 P3 P4 M1 M2 M3 (Esq / Dir)

Observações Gerais: 12:06 → Colocado na armadilha e aplicado reversores. Sob contenção física, colocado na armadilha já andando, com moderada severa ataxia
12:35 → Soltura → saiu correndo 100%
Anestesia no geral muito boa. Indução, relaxamento e recuperação boa. Mas com fluxo muito baixo p/ coleta de sangue. Mesmo com cateter 19 não foi possível coletar bem no vácuo. Coletado com seringa da femoral

Annex1. Example of field-sheet used by PCMC staff to record anesthesia data, that was used to structure and fill the database.