Evaluation of cardiopulmonary parameters and recovery from anesthesia in cougars (*Puma concolor*) anesthetized with detomidine/ketamine and isoflurane or sevoflurane[1]

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ABSTRACT.- Albuquerque V.B., Araújo M.A., Oliveira A.R., Cavalcanti G., Leal R.C., Santos E.B., Cavalvanti A.R. & Frazilio F.O. 2016. **Evaluation of cardiopulmonary parameters and recovery in cougars (*Puma concolor*) undergoing anesthesia with detomidine/ketamine and isoflurane or sevoflurane.** *Pesquisa Veterinária Brasileira 36(1):33-38.* Setor de Anestesiologia Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Av. Senador Filinto Müller 2443, Vila Ipiranga, Campo Grande, MS79074-460, Brazil. E-mail: fabrício.frazilio@ufms.br

The aim of this study was to assess the cardiopulmonary effects, the onset time after the administration of a detomidine/ketamine combination, and the recovery from anesthesia of cougars (*Puma concolor*) anesthetized with detomidine/ketamine and isoflurane or sevoflurane for abdominal ultrasound imaging. Fourteen animals were randomly allocated into two experimental groups: GISO (n=7) and GSEVO (n=7). Chemical restraint was performed using 0.15mg/kg detomidine combined with 5mg/kg ketamine intramuscularly; anesthesia induction was achieved using 2mg/kg propofol intravenously and maintenance with isoflurane (GISO) or sevoflurane (GSEVO). The following parameters were assessed: heart rate, respiratory rate, systolic and diastolic arterial blood pressure, mean arterial blood pressure, oxyhemoglobin saturation, rectal temperature, central venous pressure, and end-tidal carbon dioxide. The time to sternal recumbency (TSR) and time to standing position (TSP) were also determined. There was not statistically significant difference for the cardiopulmonary variables or TSP whereas TSR was significantly shorter in GSEVO. The time to onset of anesthesia was 11.1±1.2 minutes and 11.3±1.8 minutes for GISO and GSEVO, respectively. The anesthesia of cougars with detomidine/ketamine and isoflurane or sevoflurane was conducted with safety, cardiopulmonary stability, and increased time to sternal recumbency in the GISO group.

INDEX TERMS: Inhalation anesthesia, wild felids, sedation, alfa-2 agonists, dissociative anesthesia, chemical restraint.

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RESUMO.- [**Avaliação de parâmetros cardiorrespiratórios e recuperação da anestesia em pumas (*Puma concolor*) anestesiados com detomidina/cetamina e isoflurano ou sevoflurano.**] O objetivo do presente estudo foi avaliar os efeitos cardiorrespiratórios e a recuperação anestésica de onças-pardas (*Puma concolor*) submetidas à anestesia com detomidina/cetamina e isofluorano ou sevofluorano para avaliação ultrassonográfica abdominal. Para isso, foram utilizados 14 animais divididos alea-toriamente em dois grupos experimentais GISO (n=7) e GSEVO (n=7). Foram submetidos à contenção química com detomidina 0,15mg/kg associada à cetamina 5mg/kg pela via intramuscular, induzidos com propofol 2mg/kg pela via intravenosa e mantidos com isofluorano (GISO) ou sevofluorano. Foram avaliados os parâmetros: frequência cardíaca e

respiratória, pressão arterial sistólica, média e diastólica saturação de oxihemoglobina, temperatura retal, pressão venosa central e fração expirada de dióxido de carbono. O tempo para adoção de decúbito esternal e posição quadrupedal também foram avaliados. Não houve diferença estatística para as variáveis cardiorrespiratórias e no tempo para adoção da posição quadrupedal. O tempo para adoção de decúbito esternal foi significativamente menor no GSEVO em relação ao GISO. Concluiu-se que a anestesia de onças pardas com detomidina/cetamina e isoflurano ou sevoflurano foi realizada de maneira segura, com estabilidade cardiorrespiratória e com aumento no tempo para adoção de decúbito esternal no GISO.

TERMOS DE INDEXAÇÃO: Anestesia inalatória, felinos selvagens, sedação, agonistas alfa-2, anestesia dissociativa, contenção química.

INTRODUCTION

The cougar (*Puma concolor*) is Brazil's second largest felid. This species is found in a wide variety of habitats -from forests to savannas - and groups are occasionally seen in altered environments such as farmlands and pasturelands; in fact, cougars may be present in every Brazilian biome (Mazzolli 1993, Quadros et al. 2009).

The effects of most anesthetics routinely used in veterinary medicine on cougars are still unclear. However, studies involving these animals require chemical restraint, given their unruly behavior in the presence of humans. Therefore, the various chemical immobilization techniques of wild mammals are an invaluable tool in the search of these animals (Hime 1974, Logan et al. 1986, Barone et al. 1994, Tomizawa et al. 1997, Miller et al. 2003, Fahlman et al. 2005, Jacquier et al. 2006, Carpenter & Brunson 2013).

Ideally, drugs that we should use in these animals, whether free ranging or captive, should enable fully reversible, rapid, and reliable anesthesia induction and immobilization with cardiorespiratory stability (Wenger et al. 2010). Combinations of alpha-2 adrenergic agonists and dissociative agents have long been reported in the immobilization of large carnivores (Logan et al. 1986, Tomizawa et al. 1997, Jacquier et al. 2006, Carpenter & Brunson 2013) as an available and safe option - requiring, however, supplemental doses or induction of general anesthesia in procedures lasting longer than 5-20 minutes (Carpenter & Brunson 2013).

Another safe and available option for induction and maintenance of general anesthesia are inhalant anesthetic agents such as isoflurane and sevoflurane. Whenever feasible, these are the anesthetics of choice for lengthy procedures, since the control of anesthetic depth is more precise and recovery time is considered short (Tomizawa et al. 1997, Carpenter & Brunson 2013).

Given the scarcity of data in the literature on safe and flexible anesthesia protocols for cougars, the aim of the present study was to evaluate the cardiopulmonary effects, the onset time of anesthesia after the administration of detomidine/ketamine to allow handling by the team, and the recovery time of cougars (*Puma concolor*) anesthetized with detomidine/ketamine and isoflurane or sevoflurane for abdominal ultrasound imaging. The study hypothesis was that the anesthesia protocol would not cause clinically relevant cardiorespiratory alterations in either group and the time of recovery from anesthesia would be shorter in the sevoflurane group.

MATERIALS AND METHODS

The study has been approved by the local Committee of Ethics in Animal Use under the registration number-628/2014 and by Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), under the registration number-05188761 and was conducted in compliance with good clinical practice and animal welfare guidelines. Fourteen cougars classified as ASA I obtained from the *Centro de Reabilitação de Animais Silvestres* (Wild Animal Rehabilitation Center) in Campo Grande, MS, Brazil and from the Green Farm CO_2 Free in Itaquiraí, MS, Brazil were included. The animals were anesthetized for abdominal ultrasound imaging academic studies.

All the study animals were maintained under the same experimental conditions, underwent the same fasting period (no food or water), and were handled by the same team using the same methods to prevent the influence of extraneous variables on the results.

Given the unruly nature of this species, the cougars underwent -without a baseline assessment-chemical restraint with detomidine hydrochloride at 0.15mg/kg (10mg/mL, Dormium V, Agener União Saúde Animal, Pouso Alegre, MG, Brazil) combined with ketamine at 5mg/kg (100mg/mL, Vetaset, Fort Dodge Saúde Animal, Campinas/SP, Brazil) intramuscularly (IM) by remote darting (Dist-Inject nylon easy model 5mL, 11mm, Switzerland) using an air rifle (Syringer projector Dist-Inject model 50N, Switzerland).

After 30 minutes, the animals were moved to the operating department, where they were manipulated for collection of data regarding the parameters for the T0 time point: respiratory rate (RR), heart rate (HR), invasive systolic arterial pressure (SAP), mean arterial pressure (MAP), and diastolic arterial pressure (DAP) measurements by arterial catheterization (20 G Insyte; BD, Juiz de For a/MG, Brazil) of the right metatarsal artery, oxyhemoglobin saturation (SpO_2), and rectal temperature (RT). Those parameters were recorded with the aid of a multiparametric monitor (DX 2010, Dixtal, Manaus/AM, Brazil). An intravenous catheter (20 G Insyte; BD, Juiz de Fora/MG, Brazil) was placed into the right cephalic vein for fluid therapy with Ringer lactate (Glicolabor, Ribeirão Preto/SP, Brazil) at 5mL/kg/h.

Subsequently, induction of anesthesia was achieved with propofol at a dosage of 2mg/kg (10mg/mL, Propovan, Cristália, São Paulo/SP, Brazil) intravenously (IV), followed by tracheal intubation with a 9.5 endotracheal tube. The animals were maintained on 100% oxygen and anesthesia was maintained with isoflurane or sevoflurane.

The cougars were allocated into two experimental groups of seven animal each: GISO, animals anesthetized with isoflurane (Isoforine, Cristália, São Paulo/SP, Brazil) and GSEVO, animals anesthetized with sevoflurane (Sevocris, Cristália, São Paulo/SP, Brazil). Parameters were assessed at the following time points: T0-30 minutes after the administration of detomidine/ketamine; T1-10minutes after induction with propofol, and T2, T3, T4,and T5-45, 60, 75, and 90 minutes of anesthesia, respectively.

At each time point (T0 to T5); HR, RR and SpO_2 were assessed using a multiparametric monitor (DX 2010, Dixtal, Manaus/AM, Brazil). RT was measured using a clinical digital thermometer (Incoterm Donotherm, Porto Alegre/RS, Brazil), and SAP, MAP and DAP were determined by means of an arterial catheter (20G Insyte;

BD, Juiz de For a/MG, Brazil) placed into the right metatarsal artery and connected to a calibrated pressure transducer placed at the level of the sternum and zeroed prior to the recordings.

Central venous catheterization was performed by placing a catheter into the right jugular vein (16G Intracath; BD, Juiz de Fora/MG, Brazil) to gauge central venous pressure (CVP). End-tidal carbon dioxide ($EtCO_2$) and end-tidal sevoflurane (ETsev) and isoflurane (ETiso) were determined using a modular gas analyzer (DX 2010, Dixtal, Manaus/AM, Brazil). All these variables were determined at the time points T1 to T5.

Further, the onset time of sedation was investigated for both groups, considering the time span between the administration of detomidine/ketamine IM and the moment when the animal could be handled by the team. The recovery time from anesthesia was also assessed, considering the time of inhalant supply cessation to sternal recumbency (TSR) and the time required for the animal to standup (TSP). The animals were also monitored for adverse effects or complications of anesthesia during the first 24 hours following the procedure.

The data were expressed as means and standard deviations (M±SD). The Shapiro-Wilk test was used to evaluate normality. Normally distributed data (parametric) were examined with repeated measures analysis of variance (ANOVA), and Tukey's post-hoc test for multiple comparisons was used when statistical significance was found. Data that failed the normality test (nonparametric) were analyzed using the Friedman test followed by Dunn's post-hoc test. The level of significance was $p<0.05$. Tests were performed using computer software (Graphpad Prism 6.01; Graphpad Software Inc., CA, USA).

RESULTS

The mean weight of the animals was 50±16 kg and 47±9 kg for the GISO and GSEVO, respectively. The values of ETiso and ETsev consistently averaged 1.4 V% and 2.0 V% for isoflurane and sevoflurane, respectively (Table 1). No statistical difference was found regarding the variables HR, RR, SAP, DAP, MAP, SpO_2, $EtCO_2$, RT, and CVP when T0 was compared with the other time points in each group. Likewise, there was no statistical difference when the values for each time point were compared between groups (Table 2).

Adequate sedation was achieved within an average of 11.1±1.2 minutes and 11.3±1.8 minutes for the GISO and GSEVO, respectively. The time required for sternal recumbency was significantly shorter in GSEVO (22±2.4 minutes) in relation to GISO (26±2.5 minutes). The mean time for the animals to stand was 62±3.7 minutes and 57±5.1 minutes, respectively, in GISO and GSEVO; however, with no statistical difference (Table 3).

No adverse effects or complications of anesthesia were noted during the first 24hours following the procedure in any of the cougars in this study.

Table 1. Mean and standard deviation of weights and end-tidal isoflurane or sevoflurane of fourteen cougars anesthetized with detomidine/ketamine and isoflurane (GISO, n=7) or sevoflurane (GSEVO, n=7)

	Groups	
	GISO	GSEVO
Weight (Kg)	49,9±16	47,4±8,8
V (%)	1,4±0,12	2,1±0,12

Table 2. Mean and standard deviation of heart rate (HR), systolic arterial pressure (SAP), mean arterial pressure (MAP), diastolic arterial pressure (DAP), respiratory rate (RR), oxyhemoglobin saturation (SpO2), end-tidal carbon dioxide (EtCO2), central venous pressure (CVP) and rectal temperature (RT) of fourteen cougars anesthetized with detomidine/ketamine and isoflurane (GISO, n=7) or sevoflurane (GSEVO, n=7)

	Physiological* values	Groups	Time points					
			T0	T1	T2	T3	T4	T5
HR (per min)	70-140	GISO	87±10	96±11	95±12	89±9	88±11	83±5
		GSEVO	98±23	98±18	93±20	90±19	90±19	90±17
SAP (mmHg)	Nf	GISO	124±10	112±4	113±7	124±14	125±5	106±5
		GSEVO	112±15	106±24	108±23	107±23	114±20	109±15
MAP (mmHg)	Nf	GISO	106±9	97±7	99±5	102±15	105±13	92±7
		GSEVO	92±16	93±25	91±18	88±20	91±18	87±14
DAP (mmHg)	Nf	GISO	85±6	84±15	81±3	85±15	79±6	85±5
		GSEVO	71±23	72±29	72±21	68±21	71±23	67±16
RR (per min)	8-24	GISO	15±6	10±5	12±4	14±3	14±4	14±4
		GSEVO	14±6	16±7	16±5	14±5	16±6	15±7
SPO2 (%)	Nf	GISO	96±3	96±2	98±2	97±2	97±2	98±1
		GSEVO	95±3	97±2	99±1	99±1	97±2	97±1
EtCO2 (mmHg)	Nf	GISO	nd	43±10	43±10	43±7	43±7	42±6
		GSEVO	nd	43±7	42±2	43±6	44±7	41±4
CVP (mmHg)	Nf	GISO	nd	3±1	5±1	4±1	5±1	4±1
		GSEVO	nd	4±1	5±1	5±0,5	4±0,4	4±1
RT (°C)	37.0-39.5	GISO	38,2±0,4	38,6±0,6	38,8±0,9	38,7±0,9	38,4±0,8	38,2±0,9
		GSEVO	38,4±0,5	38,3±0,6	38,4±0,9	38,2±1,1	38,1±1	37,8±0,9

*Nd = not done, Nf = not found.

Table 3. Mean and standard deviation of time to sternal recumbency (TSR) and time to standing position (TSP) of fourteen cougars anesthetized with detomidine/ketamine and isoflurane (GISO, n=7) or sevoflurane (GSEVO, n=7)

	Groups	
	GISO	GSEVO
TSR (minutes)	26±2,5*	22±2,4
TSP (minutes)	62±3,7	57±5,1

* p<0.05 GISO different of GSEVO.

DISCUSSION

The combinations of drugs used in this study to achieve chemical restraint in wild felid species during long non-invasive examinations should provide fast induction and recovery from anesthesia, muscle relaxation and hemodynamic stability. Similarly, chemical immobilization in those animals with anesthetic agents allows safe manipulation with the level of stress reduced to a minimum (Rockhill et al. 2011). However, routine pre-anesthetic evaluations are not possible, which corroborates the few data in regard to physiological variables of wild felids.

According to Caulkett & Arnemo (2013), in agitated and ferocious animals such as wild felids the use of alpha-2 agonists alone is not sufficient for immobilization and therefore combinations with opioids or dissociative agents are recommended, which supports the use of ketamine in this study.

Based on the research done for this study there has not been a defined dose of detomidine for cougars (*Puma concolor*). For this reason the dose used in this study was based on a pilot study and was chosen from references of medetomidine and ketamine in jaguars (*Panthera onca*), which range from 0.07 to 0.09mg/kg and 2.5 to 10mg/kg, respectively (Jalanka & Roeken 1990, Hoogesteijn et al. 1996, Kreeger 1996, Harrison et al. 2011). However, during the pilot study unsatisfactory sedation was observed, which would hinder manipulation. In order to achieve adequate sedation with minimum cardiovascular interference, detomidine and ketamine were thusly adjusted to 0.15 and 5mg/kg, respectively.

Although the baseline variables were obtained after chemical restraint in this study, it is possible to observe that cardiovascular and respiratory variables, as well as temperature, were within predictable values for physiological conditions of the wild felid species (Deem & Karesh 2005).

Most of the variables analyzed in the present study (HR, RR, SAP, DAP, MAP, SpO_2, $EtCO_2$, RT, and CVP) showed no statistically significant differences between time points nor between groups. Such findings are in line with those of Wenger et al. (2010), who also reported stability in the cardiopulmonary parameters of African lions anesthetized with butorphanol, medetomidine, and midazolam. Similarly, Juvenal et al. (2008) also noted cardiovascular stability in tigrinas (*Leopardus tigrinus)* managed by the xylazine and tiletamine-zolazepam protocol. In line with those studies, Hikasa et al. (1997), when anesthetizing domestic cats with atropine, ketamine, and nitrous oxide, and isoflurane or sevoflurane, found no alterations in CVP nor in any other cardiovascular variables in the isoflurane and sevoflurane groups. However, Pypendop et al. (2011) have reported increased CVP and ascribed this finding to the decreased cardiac output observed in their study in domestic felines who received a combination of an alpha-2 agonist and isoflurane.

Body temperature was not significantly decreased and was maintained within physiological ranges for the species, albeit the known thermoregulatory depression caused by alpha-2 adrenergic agonists. According to Caulkett & Arnemo (2013) these drugs can produce hypothermia or hyperthermia as well as opioides due to their effect on the thermoregulatory mechanisms, which explains the hyperthermia observed in two lions by Wenger et al. (2010). These authors accredited the increase in temperature to the alpha-2 agonist or to the high room temperature. Tomizawa et al. (1997) found a significant decrease in RT, HR, and RR in African lions after the onset of anesthesia with medetomidine-ketamine and Jaquier et al. (2006) reported a decrease of 60 to 85% in RR during immobilization of African lions. Similarly, Monteiro et al. (2008) observed reduced RR, HR, and RT in cats under xylazine or xylazine/methadone anesthesia. In another study, Selmi et al. (2005) noted a significant increase in HR 5 minutes after premedication with xylazine and ketamine in domestic felids and a decrease in the mean values of SAP, DAP, and MAP. Bradycardia, defined as heart rate lower than 50 beats/minute, was found by Wenger et al. (2010) in half of the lions anesthetized with alpha-2 agonists.

Isoflurane anesthesia is known to potentiate some effects of alpha-2 agonists such as hypotension and according to Pypendop et al. (2011) there might be a significant influence on cardiac output, since the effects of isoflurane are reportedly dose-related. When studying domestic felines, Pypendop et al. (2011) have reported a decrease in the minimum alveolar concentration of isoflurane after dexmedetomidine sedation and thus recommend the association of these drugs in felines rather than their use alone. Therefore, the low concentrations of inhalant anesthetics used for both groups in this study can be explained by the need to keep the animals unconscious only, so they would allow handling - however, with no painful stimuli. Another reason is the use of anesthetic adjuvants such as alpha-2 agonists. This finding is similar to that of Hikasa et al. (1997), who obtained, in the anesthesia of domestic cats, mean values of 1.7 V% and 2.7 V% for isoflurane and sevoflurane, respectively, also in the absence of a painful stimuli.

The onset time from which the animals would tolerate handling, which is a sign of adequate chemical restraint (means, 11.1±1.2 minutes and 11.3±1.8 minutes for GISO and GSEVO, respectively), is consistent with most time values found in the literature on wild animals restrained with alpha-2 agonists combined or not with a dissociative (Tomizawa et al. 1997, Carvalho et al. 2006, Sadanand et al. 2009, Wenger et al. 2010).

Sadanand et al. (2009) have anesthetized captive wild felids (Asiatic lions, tigers, and leopards) with xylazine and ketamine and have observed rapid induction and deep anesthesia in all the animals, with good muscle relaxation and satisfactory analgesia within 15-25 minutes after anesthetic administration by dart. Tomizawa et al. (1997), when sedating African lions with medetomidine and atropine, have observed deep sedation within an average of 5.7 minutes after IM administration of the drugs. Wenger et al. (2010) recorded 7.25±2.3 minutes with the use of butorphanol, medetomidine, and midazolam in African lions. These means are lower than those found in the present study; this discrepancy is likely due to the fact that they did not include

ketamine, as this drug increases cardiac output and blood pressure as a result of its sympathomimetic action (Carvalho et al. 2006). In the study of Lescano et al. (2014) the time to achieve sternal recumbency was 6.9±4.8 minutes and is the lowest onset found in literature. The association with two other pharmacological classes of drugs produced adequate muscle relaxation with rapid onset, although the use of dissociative anesthetics. Results found by Selmi et al. (2003) showed that adding butorphanol to romifidine and tiletamine-zolazepam reduced significantly the time for onset of anesthesia and extended the anesthetic time without an impact on recovery time in domestic cats.

In the present study sternal recumbency lasted for 22±2.4 and 26±2.5 minutes in GSEVO and GISO, respectively. These are relatively shorter periods compared to those observed by Lescano et al. (2014), who have reported sternal recumbency that lasted for 32.5±17.7 minutes. Similarly, the recovery period until animals were able to stand was shorter (62±3.7 and 57±5.1 minutes in GISO and GSEVO, respectively) in both groups compared to the 83.3±35.1 minutes reported by Lescano et al. (2014). These findings can be accredited to the use of inhalation anesthesia in this study. According to Carpenter & Brunson (2013), anesthesia with isoflurane or sevoflurane should be the method of choice with large wild felids whenever available, since they allow the monitoring of anesthetic depth and provide faster recovery. The animals in the GISO took longer to recover than those in the GSEVO, which corroborates most studies addressing the recovery of animals anesthetized with those drugs (Hikasa et al. 1997, Johnson et al. 1998, Oliva et al. 2000).

Sevoflurane is an inhalation anesthetic agent that provides cardiovascular stability and myocardial protection against catecholamines (Hisaka et al. 1997), which explains the stability in heart rate within acceptable ranges during the entire procedure. In one study using sevoflurane in domestic felines by Souza et al. (2005) a decrease in heart rate has been reported compared to baseline measures. However this difference was ascribed to the stress during baseline evaluation and the hypothesis of the anesthetic agent reducing heart rate was discarded.

During recovery vomiting can be a common side effect after the administration of alpha-2 agonists such as medetomidine (Jaquier et al. 2006). Some authors have reported this effect on 50% of domestic cats given medetomidine (Vaha-Vahe 1989). Wenger et al. (2010) have observed vomiting two minutes after an animal was able to stand; however, this cannot be due to detomidine since no animals presented this effect in the present study.

Because cougars are unruly animals that will not accept handling without chemical restraint, one limitation to the present study was that the baseline (T0) was recorded 30 minutes after detomidine/ketamine anesthesia.

The results obtained in the present study allowed the authors to conclude that the anesthesia of cougars with detomidine/ketamine combined to either isoflurane or sevoflurane was conducted with safety, cardiovascular stability and a slight increase in recovery time in the isoflurane group.

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