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PROTOCOLO ANESTÉSICO PARA PREGUIÇAS-DE-COLEIRA (*Bradypus torquatus*) DE VIDA LIVRE - UMA ESPÉCIE NEGLIGENCIADA, AMEAÇADA E ENDÊMICA DA MATA ATLÂNTICA

ILHÉUS-BAHIA
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Orientadora: Flávia Regina Miranda.

Coorientador: Mária Sérgio Lima de Lavor.

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“Organize seu ódio.”

Paulo Roberto da Silva Lima (Galo de Luta)

PROTOCOLO ANESTÉSICO PARA PREGUIÇAS-DE-COLEIRA (*Bradypus torquatus*) DE VIDA LIVRE - UMA ESPÉCIE NEGLIGENCIADA, AMEAÇADA E ENDÊMICA DA MATA ATLÂNTICA

RESUMO

Os Xenarthra são uma superordem de mamíferos antiga, originária da América do Sul. O clado divide-se em três subclados, tamanduás (Vermilingua), tatus (Cingulata) e preguiças (Folivora). Existem 38 espécies vivas, das quais 26 encontram-se próximo ou em risco de extinção, ou com dados insuficientes. As populações de Xenarthras são impactadas por múltiplas pressões antrópicas, e dados biológicos são necessários para guiar ações conservacionistas. Para a coleta de dados, a captura de indivíduos é comum e, nesses casos, a anestesia pode proporcionar um procedimento mais seguro para o animal e para os pesquisadores. No entanto, o clado possui características anatômicas e fisiológicas que dificultam a sua anestesia. Inserido nesse contexto está a preguiça-de-coleira (*Bradypus torquatus*). A espécie é endêmica da Mata Atlântica, com ocorrência restrita aos estados do Sergipe e Bahia. Atualmente considerada como vulnerável à extinção, existem poucos estudos a seu respeito, e nenhum anestesiológico. Portanto, o objetivo geral deste estudo é determinar um protocolo anestésico seguro e eficaz para procedimentos a campo com preguiças-de-coleira (*B. torquatus*) de vida-livre. Especificamente objetivou-se (i) identificar e compilar quais protocolos anestésicos tem sido utilizados na anestesia de Xenarthras; (ii) identificar e compilar quais protocolos anestésicos foram estudados e considerados seguros e efetivos ou não para anestesia de Xenarthras; (iii) avaliar como os parâmetros fisiológicos dos Xenarthras responderam aos protocolos anestésicos estudados (ARTIGO 1); (iv) analisar um protocolo anestésico para a anestesia de preguiças-de-coleira (*B. torquatus*) de vida-livre (ARTIGO 2). Para contemplar os objetivos específicos do ARTIGO 1, realizou-se uma revisão sistemática dos protocolos anestésicos empregados para Xenarthras entre 1982 e 2022. Protocolos que incluíam associações de ciclohexaminas, agonistas alfa2-adrenérgicos, benzodiazepinos, opioides, e/ou anestésicos inalatórios foram considerados seguros e eficazes para anestesia de Xenarthras. Cetamina com um agonista alfa2-adrenérgico foi a associação mais fundamentada, mas depressão cardiovascular, respiratória e termoregulatória estavam presentes. Cetamina isolada, cetamina com acepromazina e butorfanol com isoflurano foram considerados inseguros e ineficazes. Nenhum estudo providenciou alta qualidade de evidência. Outros fármacos (alfaxalona, droperidol, fentanil, halotano, metadona, morfina, óxido nitroso, pentobarbital e propofol) foram implementados, mas seus efeitos anestésicos ainda não foram estudados ou publicados. Para contemplar o objetivo específico do ARTIGO 2, informações anestésicas foram registradas de 12 preguiças-de-coleira de vida livre, imobilizadas a campo para coleta de dados. Indivíduos foram anestesiados com uma associação de cetamina (4.0 mg/kg) e medetomidina (0.03 mg/kg), antagonizada com atipamezol (0.1 mg/kg). A indução foi rápida (3.21±0.76), mas a recuperação foi longa (113.3±18) quando comparada a outros estudos. Não houve diferença nos tempos de indução e recuperação em relação ao sexo e faixa etária. Frequência cardíaca, saturação de oxigênio e temperatura retal mantiveram-se estáveis durante o procedimento. A frequência respiratória diminuiu ao longo do tempo de 18.25±7.03 para 13.17±3.66

movimentos por minuto. Nossos resultados indicam que a associação descrita de cetamina e medetomidina é uma escolha segura e eficaz para a anestesia de preguiças-de-coleira.

Palavras-chave: Xenarthras; Preguiça-de-coleira; Anestesia; Protocolos anestésicos.

ANESTHETIC PROTOCOL FOR FREE-RANGING MANED SLOTHS (*Bradypus torquatus*) – A NEGLECTED, THREATENED AND ENDEMIC SPECIES OF THE ATLANTIC FOREST

ABSTRACT

Xenarthras are an ancient mammal superorder, originated in South America. The clade is separated into three subclades, anteaters (Vermilingua), armadillos (Cingulata) and sloths (Folivora). There are 38 extant species, from which 26 are considered near or threatened, or have insufficient data. Xenarthras' population are impacted by multiple anthropic pressures, and biological data are necessary to guide conservation initiatives. The capture of individuals is often required, and, in such cases, anesthesia can provide a safer procedure for both animal and investigators. However, the clade has anatomical and physiological characteristics that can difficult their anesthesia. Within this context is the maned sloth (*Bradypus torquatus*). The species is endemic to the Atlantic Forest, occurring only in Sergipe and Bahia states. Currently assessed as vulnerable to extinction, there are many knowledge gaps regarding the species, including its anesthesia. Therefore, the general objective of this study is to determinate a safe and effective anesthetic protocol for field procedure with free-ranging maned sloths (*B. torquatus*). Specific objectives were to (i) identify and compile which anesthetic protocols have been used for Xenarthras' anesthesia; (ii) identify and compile which anesthetic protocols have been studied and considered safe and effective or not for Xenarthras' anesthesia; (iii) assess how Xenarthras' physiological parameters responded to studied anesthetic protocols (MANUSCRIPT 1); (iv) analyze an anesthetic protocol for free-ranging maned sloths (*B. torquatus*) (MANUSCRIPT 2). To achieve MANUSCRIPT 1 specific objectives, a systematic review was performed for anesthetic protocols implemented for Xenarthras' anesthesia from 1982 to 2022. Protocols that included combinations of cyclohexamines, alpha2-agonists, benzodiazepines, opioids, and/or inhalant anesthetics were considered safe and effective for Xenarthra's anesthesia. Ketamine with an alpha2-agonists was the most substantiated combination, but cardiovascular, respiratory, and thermoregulatory depression was presented. Ketamine alone, ketamine with acepromazine, and butorphanol with isoflurane were considered unsafe and ineffective. No study provided high quality of evidence. Other drugs (alfaxalone, droperidol, fentanyl, halothane, methadone, morphine, nitrous oxide, pentobarbital, and propofol) have been implemented but their anesthetic effects have not yet been evaluated or published. To achieve MANUSCRIPT 2 specific objective, anesthetic data was collected from 12 free-ranging maned sloths that were immobilized for a field examination. Individuals were anesthetized using a combination of ketamine (4.0 mg/kg) and medetomidine (0.03 mg/kg) and antagonized with atipamezole (0.1 mg/kg). Induction was fast (3.21±0.76), but recovery was longer (113.3±18) when compared to other studies. Induction and recovery times were not different across sex or age classes. Rectal temperature, heart rate, and oxygen saturation remained stable throughout the procedure. Respiratory rate significantly decreased over time, from 18.25±7.03 to 13.17±3.66 movements per minute. Our results indicate that the described combination of ketamine and medetomidine is a safe and effective choice for anesthesia of maned sloths.

Keywords: Xenarthra; Maned sloth; Anesthesia; Anesthetic protocols.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

SUMÁRIO

1 INTRODUÇÃO

A superodem Xenarthra é considerada um dos quatro maiores clados de mamíferos placentários no mundo e o único originário da América do Sul, com sua história evolutiva intrinsicamente ligada ao continente (BORGES *et al.*, 2023; DELSUC; VIZCAÍNO; DOUZERY, 2004; MAGNUS; MACHADO; CÁCERES, 2018; SOIBELZON, 2019; VIZCAÍNO; BARGO, 2014). O clado teve seu surgimento e diversificação entre 100 e 58 milhões de anos atrás, possuindo ao longo de sua história pelo menos 218 gêneros descritos (DELSUC; VIZCAÍNO; DOUZERY, 2004; MORAES-BARROS; ARTEAGA, 2015; VIZCAÍNO; BARGO, 2014). Os Xenarthra dividem-se em duas ordens, Pilosa e Cingulata, categorizadas também em três subclados, tamanduás (Vermilingua), tatus (Cingulata) e preguiças (Folivora) (MORAES-BARROS; ARTEAGA, 2015). Atualmente existe apenas uma pequena fração de representantes da sua diversidade original (BORGES *et al.*, 2023; DELSUC; VIZCAÍNO; DOUZERY, 2004; MORAES-BARROS; ARTEAGA, 2015), consistindo em 14 gêneros com 38 espécies (FEIJÓ *et al.*, 2022), das quais 18 ocorrem no Brasil (ICMBIO, 2015).

Ainda que muito da diversidade biológica do clado tenha-se perdido (DELSUC; VIZCAÍNO; DOUZERY, 2004; TOLEDO *et al.*, 2015), as características gerais que o definem mantiveram-se representadas ao longo do tempo nas espécies sobreviventes (GAUDIN; CROFT, 2015). Dentre as principais peculiaridades anatômicas e fisiológicas, os Xenarthras possuem uma articulação adicional lombar (*xenarthrous*, que dá o nome ao grupo); ossificação das costelas vertebroesternais; dentição ausente (tamanduás) ou reduzida (tatus e preguiças), homodonte, hipsodonte e não esmaltada; taxa metabólica basal baixa; e homeotermia imperfeita (ATTIAS *et al.*, 2018; CLIFFE *et al.*, 2018; FEIJÓ *et al.*, 2022; GAUDIN; CROFT, 2015; MILLER; LAMBERSKY; CALLE, 2019; SUPERINA; ABBA, 2020; VIZCAÍNO; BARGO, 2014).

O fato do clado possuir baixo metabolismo e serem homeotermos imperfeitos pode ter sido importante fator de vulnerabilidade na extinção de grande parte das espécies, especialmente as de maior porte, no contexto das flutuações climáticas e geológicas históricas (MAGNUS; MACHADO; CÁCERES, 2018; MORAES-BARROS; ARTEAGA, 2015; SOIBELZON, 2019). Da mesma forma, essas características são um fator conservacionista importante para as espécies sobreviventes

considerando as mudanças climáticas de origem antrópica que potencialmente acarretarão múltiplos impactos negativos, diretos e indiretos (BORGES *et al.*, 2023; GALLO, 2023; SANTOS *et al.*, 2022; TOURINHO *et al.*, 2022). Além disso, os Xenarthras são impactados também por outras pressões. Das 38 espécies existentes, 26 encontram-se próximo ou em risco de extinção, ou com dados insuficientes (FEIJÓ *et al*., 2022; ICMBIO, 2015). As principais pressões que o clado sofre são a caça, atropelamentos, exposição a patógenos, incêndios florestais, e perda e fragmentação do habitat (BORGES *et al*., 2023; FEIJÓ *et al*., 2022; ICMBIO, 2015; SUPERINA; ABBA, 2020)

A Mata Atlântica, considerada como um dos hotspots da biodiversidade do planeta, apresenta apenas 11,4% a 28% da sua área original, considerando dados coletados até 2013. O restante da sua extensão teve a paisagem convertida para uso de atividades antrópicas (REZENDE *et al.*, 2018; RIBEIRO *et al.*, 2009, 2011). No estado da Bahia, segundo dados da associação SOS Mata Atlântica e do Instituto Nacional de Pesquisas Espaciais - INPE (2021), a estimativa de área natural remanescente é de 11,7% em 2020.

Dentro deste contexto está a preguiça-de-coleira (*Bradypus torquatus*), espécie endêmica do bioma e com ocorrência limitada a dois estados brasileiros, sendo a principal população abrigada na Bahia (HRISCH; CHIARELLO, 2012; LARA-RUIZ; CHIARELLO; SANTOS, 2008; MIRANDA *et al.*, 2022). É atualmente considerada vulnerável à extinção (B2ab(ii,iii))[1] e, apesar dos estudos sobre a espécie terem avançado nas últimas décadas, ainda existem informações biológicas desconhecidas que são necessárias para direcionar ações de conservação (CHIARELLO *et al.*, 2022; HRISCH; CHIARELLO, 2012).

Para o desenvolvimento de pesquisa e ações de conservação, comumente é necessária a captura de indivíduos de vida livre para coleta de dados. Especialmente em procedimentos para coleta de amostras biológicas, marcação individual ou colocação de equipamentos radiotransmissores. Nestes casos, o uso de contenção química deve ser considerado, visto que pode permitir maior segurança tanto para os manipuladores quanto para o animal, além de maior eficácia para as coletas (CHINNADURAI *et al.*, 2016; HAWKINS; GUZMAN; PAUL-MURPHY, 2020). De modo geral, estudos envolvendo a preguiça-de-coleira em que a captura de indivíduos foi realizada não

[1]Área de ocupação severamente fragmentada, com declínio contínuo da área de ocupação e extensão do habitat. Ver mais em: https://portals.iucn.org/library/sites/library/files/documents/RL-2001-001-2nd.pdf

relatam o método de contenção (*e.g.*: CASSANO; KIERULFF; CHIARELLO, 2011; GINÉ *et al*., 2015; SNOECK *et al*., 2011) ou utilizam somente a contenção física, mas não abordam seus potenciais efeitos estressores ao animal (*e.g.*: CATENACCI *et al*., 2018; LARA-RUIZ; CHIARELLO, 2005; LARA-RUIZ; CHIARELLO; SANTOS, 2008). Existem apenas dois estudos na literatura científica relatando a contenção química de preguiça-de-coleira (BERNARDES *et al*., 2022; PINDER, 1993), mas que não discutem seus efeitos farmacológicos. Este cenário difere-se de outras espécies de preguiças, as quais, apesar de somente a contenção física também ser utilizada, possuem estudos anestésicos (DUARTE *et al*., 1989; HANLEY *et al*., 2008; KINNEY *et al*., 2013; VOGEL; DE THOISY; VIÉ, 1998). Dentre os estudos citados, a maioria dos protocolos anestésicos testados envolvem a associação de cetamina com um agonista alfa2-adrenérgico, sugeridos como seguros e eficazes. A cetamina é um anestésico dissociativo e os fármacos alfa2 agonistas são sedativos-analgésicos que, quando usados em conjunto, apresentam efeitos sinérgicos benéficos e amplamente conhecidos. No entanto, efeitos deletérios também podem estar presentes, como relatado pelos autores. As particularidades fisiológicas dos Xenarthras, citadas anteriormente, podem influenciar no processo anestésico (DEEM; FIORELLO, 2002; MORENO, 2018; WEST; CARTER; SHAW, 2014). Portanto, a preguiça-de-coleira carece de um protocolo anestésico que garanta segurança ao animal, considerando suas características, e eficácia para execução dos procedimentos a campo.

2 OBJETIVO GERAL

O objetivo geral deste estudo é determinar um protocolo anestésico seguro e eficaz para procedimentos a campo com preguiças-de-coleira (*Bradypus torquatus*) de vida-livre.

3 OBJETIVOS ESPECÍFICOS

- Identificar e compilar os protocolos anestésicos que têm sido utilizados na anestesia de Xenarthras, avaliando quais foram estudados e considerados seguros e eficazes, bem como aqueles que não atenderam a esses critérios.

- Avaliar a resposta dos parâmetros fisiológicos dos Xenarthras aos protocolos anestésicos estudados;

- Analisar a associação de cetamina e medetomidina como protocolo anestésico para a anestesia de preguiças-de-coleira (*B. torquatus*) de vida-livre.

4 REVISÃO DE LITERATURA

4.1 Preguiça-de-coleira

A preguiça-de-coleira passou recentemente por uma revisão taxonômica. Anteriormente considerada uma única espécie, foi separada em duas espécies distintas: *Bradypus torquatus* (Illiger, 1811) e *Bradypus crinitus* (Gray, 1850) (MIRANDA *et al.*, 2022). Ambas as espécies são endêmicas da Mata Atlântica, originalmente presentes em quatros estados brasileiros (HRISCH; CHIARELLO, 2012; LARA-RUIZ; CHIARELLO; SANTOS, 2008; MOREIRA *et al.*, 2014). Com a revisão taxonômica, compreendeu-se que a *B. torquatus* ocorre nos estados do Sergipe e Bahia, enquanto a *B. crinitus* ocorre nos estados do Espírito Santo e Rio de Janeiro (MIRANDA *et al.*, 2022). A Bahia abriga a principal população da *B. torquatus* (HRISCH; CHIARELLO, 2012; LARA-RUIZ; CHIARELLO; SANTOS, 2008), estabelecendo assim o estado e a espécie como foco do segundo artigo desta dissertação.

As preguiças-de-coleira possuem a pelagem de coloração marrom com uma "crina" preta que se desenvolve com idade e distingue a espécie visualmente (Figura 1) (LARA-RUIZ; CHIARELLO, 2005; PINDER, 1993). Indivíduos adultos pesam em média 6,5 kg e medem em média da cabeça à cauda 66,5 cm. As fêmeas pesam mais e são potencialmente maiores que os machos, enquanto os machos possuem crinas mais volumosas (LARA-RUIZ; CHIARELLO, 2005).

A espécie habita principalmente florestas ombrófilas em baixa altitude (HRISCH; CHIARELLO, 2012) e, ainda que a espécie seja plástica, parece preferir áreas com vegetação abundante, árvores altas e bem conectadas. Apesar de possuir pouca mobilidade e área de vida relativamente pequena, de fato, a espécie esta intrinsicamente associada a floresta. O táxon é arborícola e herbívoro, utilizando assim as árvores para locomoção, abrigo (CASSANO; KIERULFF; CHIARELLO, 2011; CHIARELLO *et al.*, 2004; FALCONI *et al.*, 2015; SANTOS *et al.*, 2016) e alimentação (CHIARELLO, 1998a; GINÉ; MUREB; CASSANO, 2022), com preferências alimentares de acordo com a diversidade vegetal (MUREB *et al.*, 2023).

Majoritariamente diurna, a espécie varia seus padrões de atividade de acordo com a temperatura ambiental (CHIARELLO, 1998b; GINÉ *et al.*, 2015), utilizando as árvores também como parte integrante da sua termorregulação (LOPES *et al.*, 2023; MURAMATSU *et al.*, 2022; SANTOS *et al.*, 2022; TOURINHO *et al.*, 2022). No entanto, a espécie é globalmente considerada vulnerável à extinção, justamente devido a perda e fragmentação da sua pequena área de ocorrência (B2ab(ii,iii)) (CHIARELLO *et al.*, 2022).

Figura 1 – Indivíduo adulto, macho, de preguiça-de-coleira (*Bradypus torquatus*). Arquivo pessoal.

Segundo a última avaliação do seu estado de conservação, “dados sobre dispersão, proporção sexual, sistema de acasalamento e densidade populacional” foram considerados desconhecidos, mas necessários para ações conservacionistas (CHIARELLO *et al.*, 2022). Ainda que não presente na avaliação, existem apenas

estudos iniciais sobre aspectos sanitários (BERNARDES et al., 2022; CATENACCI et al., 2017), sendo que a avaliação da saúde populacional de espécies silvestres é igualmente importante para fins conservacionistas (KOPHAMEL *et al.*, 2022). A espécie, assim como outras preguiças, ainda pode ser um reservatório no ciclo de arboviroses (CATENACCI *et al.*, 2018; DE THOISY; DUSSART; KAZANJI, 2004; MEDLIN *et al.*, 2016). Além disso, possuem uma gama pouco estudada de micro e macroorganismos em sua pelagem que pode desempenhar importante papel eco-epidemiológico (BERNARDES et al., 2022; KAUP; TRULL; HOM, 2021; PINDER, 1993).

4.2 Estresse e contenção física

Desde os estudos iniciais sobre o *stress* (estresse) durante o século XX, diferentes conceitos foram atribuídos ao termo e, ainda hoje, não há consenso na sua definição ou emprego dentro da biologia. No entanto, os conceitos passam pelo entendimento geral de que estresse é um processo de adaptação fisiológica para manutenção da homeostase frente a um agente estressor (CHROUSOS, 2009; KARAER; ČEBULJ-KADUNC; SNOJ, 2023; KOOLHAAS *et al*., 2011; LU; WEI; LI, 2021; ORSINI; BONDAN, 2014; REEDER; KRAMER, 2005).

Um agente estressor pode ser qualquer fator biótico ou abiótico, exógeno ou endógeno, físico ou psicológico em que o organismo compreende como nocivo, gerando as respostas neuro-hormonais e comportamentais do estresse. Imediatamente ao estímulo ocorre a fase de alerta. Nesta fase, considerada aguda, há a estimulação do sistema nervoso simpático, com aumento da liberação de catecolaminas e a resposta de luta, fuga ou paralização. Caso o agente estressor seja mantido, o organismo entra na fase de adaptação, considerada crônica. Ocorre o efeito predominante do eixo hipotalâmico-hipofisário-adrenal, com aumento da liberação de glicocorticoides para maior disponibilidade energética. O estresse é um mecanismo evolutivo, de sobrevivência, que caminha junto com a capacidade de adaptação das espécies. Quando o estresse desencadeado se encontra dentro da capacidade de adaptação do organismo, portanto não apresentando risco à saúde, ele é potencialmente benéfico (*eustress*) gerando plasticidade à homeostase. Por outro lado, o organismo tem uma capacidade finita de adaptação. Se a fase de adaptação for ultrapassada (*distress*), seja por

esgotamento temporal ou intensidade do estímulo estressor, leva-se o organismo à exaustão (CHROUSOS, 2009; KARAER; ČEBULJ-KADUNC; SNOJ, 2023; KOOLHAAS *et al*., 2011; LU; WEI; LI, 2021; ORSINI; BONDAN, 2014; REEDER; KRAMER, 2005).

A captura é potencialmente um dos eventos mais estressantes na vida de um animal silvestre. Os efeitos deletérios deste evento podem correr muito tempo após a soltura do indivíduo, sem que o pesquisador tenha conhecimento. A miopatia de captura é uma doença não contagiosa, de ordem metabólica, com etiologia intrinsicamente relacionada a falha de adaptação ao estresse e à atividade muscular do processo de captura (contenção física, manipulação e translocação) de animais silvestres. Os sinais clínicos podem manifestar-se de forma hiperaguda (imediatamente ou em até 6 horas), subaguda (entre um e dois dias), aguda (entre poucas horas ou dias) ou crônica (indefinidamente). Esta síndrome é caracterizada pela associação de múltiplos mecanismos que tem menor ou maior influência conforme apresentação clínica. O aumento nos níveis de catecolaminas e glicocorticoides, bem como a lesão das fibras musculares levam à hipertermia, acidose metabólica, isquemia tecidual, e falência renal e cardíaca. O prognóstico é desfavorável e a síndrome é comumente fatal (BREED *et al.*, 2019; ORSINI; BONDAN, 2014; WEST; HEARD; CAULKETT, 2014). Ainda que a miopatia de captura seja evitada, a imunossupressão causada pelo aumento de glicocorticoides do estresse da captura coloca em risco não somente a saúde do indivíduo, mas a própria conservação das populações intra- e interespecíficas (HING *et al.*, 2016).

Para coleta de dados de animais de vida-livre, a captura de indivíduos é comumente necessária. É inevitável que a captura e o procedimento decorrente dela sejam estressantes, mas a intensidade do estresse pode ser mitigada com conhecimento, planejamento e experiência. Ainda assim, a contenção física prolongada, em conjunto com a percepção dos estímulos da manipulação (coleta de dados), oferece alto potencial estressor para o animal. Nesses casos, a anestesia, quando igualmente bem executada, pode ser uma alternativa mais segura e eficaz (BREED *et al.*, 2019; CHINNADURAI *et al.*, 2016; HAWKINS; GUZMAN; PAUL-MURPHY, 2020; WEST; HEARD; CAULKETT, 2014).

4.3 Anestesia de Xenarthras

Anestesia é um termo abrangente que se refere a perda de sensação local ou sistêmica, sendo os anestésicos os fármacos responsáveis por este efeito. Dentro da anestesia existem ainda outros conceitos que evoluíram de acordo com o desenvolvimento da anestesiologia e farmacologia. A anestesia geral se refere a depressão reversível do sistema nervoso central (SNC), produzindo inconsciência e perda de sensibilidade, a qual foi classificada em quatro estágios anestésicos originalmente com base no uso de anestésico inalatório em humanos. No terceiro estágio encontra-se o plano cirúrgico (anestesia geral cirúrgica), em que o anestésico inalatório deprime o SNC suficientemente para promover também relaxamento muscular e analgesia para um procedimento cirúrgico, ainda com manutenção da vida. No entanto, atualmente existem fármacos com maior especificidade de efeito, ou seja, em que a depressão do SNC, o relaxamento muscular e a analgesia não necessariamente ocorrem em conjunto. Portanto, o conceito de anestesia vem sendo amplamente utilizado como a associação farmacológica capaz de produzir graus de inconsciência, relaxamento muscular e analgesia suficientes para o determinado procedimento. Outros efeitos desejáveis também podem estar incluídos no conceito, como a tranquilização (ansiólise) e amnésia (CHINNADURAI *et al.*, 2016; GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020; WEST; HEARD; CAULKETT, 2014). Ainda, é importante diferenciar a anestesia da contenção química ou farmacológica, a qual se refere somente a imobilização do paciente, suficientemente para execução de um determinado procedimento. A imobilização pode ser atingida de diferentes formas (*e.g.:* anestesia dissociativa; sedação), mas não necessariamente inclui inconsciência, relaxamento muscular ou analgesia (KREEGER; ARNEMO, 2018).

Evidentemente, a escolha do fármaco é essencial na prática anestésica. O fármaco ideal deve ser potente, eficaz, previsível, produzir efeito rápido e suave, com alta margem de segurança, não provocar efeitos colaterais e nem alterações fisiológicas, não produzir metabólicos tóxicos, ser reversível de forma rápida e suave, estável sob variações ambientais, e seguro de manipular. No entanto, não existe fármaco que possua todas essas características (GRIMM *et al.*, 2015; KREEGER; ARNEMO, 2018). Sabendo-se disso, a anestesia balanceada é o conceito de se utilizar múltiplos fármacos sinérgicos, produzindo-se o efeito desejado, com as qualidades necessárias e o mínimo

de desvantagens possível (CHINNADURAI *et al.*, 2016; GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020).

Existem poucos estudos sobre o estresse de Xenarthras, e a miopatia de captura ainda não foi relatada. No entanto, as evidências demonstram que o estresse é um fator preponderante para o clado (AMAYA *et al.*, 2019; CARDER *et al.*, 2018; DE ABREU REZENDE *et al.*, 2023; DUARTE *et al.*, 1987, 2007; DUARTE; DA COSTA; HUGGINS, 1982; EGUIZÁBAL *et al.*, 2022; GILMORE; DA-COSTA; DUARTE, 2000; RIDEOUT *et al.*, 1985). Além disso, são animais que podem se tornar agressivos quando manipulados, com potencial de causar danos severos utilizando as garras (MORENO, 2018; WEST; CARTER; SHAW, 2014). Dessa forma, a anestesia pode trazer múltiplas vantagens ao bem-estar do animal e eficiência do procedimento. O animal propriamente anestesia não sofrerá o potencial estresse e exaustão relacionados à contenção física prolongada e a manipulação, e o risco do animal se lesionar, ser lesionado ou lesionar os manipuladores é reduzido, sem que haja detrimento do tempo de procedimento. A inconsciência e o relaxamento muscular podem permitir uma coleta de amostras mais tranquila e segura. No caso de coletas invasivas, a anestesia balanceada poderá garantir a analgesia necessária. Ainda, a inconsciência, preferencialmente associada com amnésia e ansiólise, pode tornar o evento psicologicamente menos traumático para o animal (CHINNADURAI *et al.*, 2016; HAWKINS; GUZMAN; PAUL-MURPHY, 2020).

Por outro lado, existem muitos desafios na anestesia de Xenarthras, especialmente devido as suas particularidades anatômicas e fisiológicas. O clado possui uma grande gama de pesos e tamanhos entre as diferentes espécies, com valores normais de parâmetros fisiológicos pouco estabelecidos. Fármacos, dosagens e efeitos farmacológicos são pouco conhecidos para o clado, e os resultados esperados para a anestesia podem ser diferentes devido ao baixo metabolismo. A homeotermia imperfeita e a baixa temperatura corporal são fatores de risco, especialmente no uso de fármacos que afetam a termorregulação (*e.g.*: agonistas alfa2-adrenérgicos; opioides). O clado é conhecido por ser suscetível a apresentar apneia e, apesar de serem considerados resistentes a hipoxemia, caso a intervenção seja necessária, a entubação é pouco viável. A mensuração não-invasiva da saturação de oxigênio também pode ser difícil devido à falta de sensores apropriados. Dessa forma, todos esses fatores devem ser levados em

consideração para o planejamento e execução adequados da anestesia (BRAINARD *et al.*, 2008; DEEM; FIORELLO, 2002; FARO *et al.*, 2015; MORENO, 2018; WEST; CARTER; SHAW, 2014).

5 ARTIGO 1

ANESTHETIC PROTOCOLS USED FOR XENARTHRA'S ANESTHESIA FROM 1982 TO 2022: A SYSTEMATIC REVIEW

(À submeter: *Veterinary Anaesthesia and Analgesia*)

ANESTHETIC PROTOCOLS USED FOR XENARTHRA'S ANESTHESIA FROM 1982 TO 2022: A SYSTEMATIC REVIEW

RESUMO

Os objetivos desta revisão sistemática foram de (i) investigar quais protocolos anestésicos vem sendo utilizados para anestesia de Xenarthras, (ii) quais foram avaliados e considerados seguros e efetivos ou não, e (iii) como os parâmetros fisiológicos dos Xenarthras respondem a tais protocolos anestésicos. Uma busca sistemática foi conduzida de março à outubro de 2023. As bases de dados virtuais utilizadas foram *PubMed* (março e abril), *Web of Science* (maio e junho), *Scopus* (julho e agosto) e *Google Scholar* (setembro e outubro). O intervalo de tempo foi arbitrariamente escolhido entre 1982 e 2022. Os termos de busca utilizados foram (*"xenarthra" OR "sloth" OR "bradypus" OR "choloepus" OR "anteater" OR "cyclopes" OR "myrmecophaga" OR "tamandua" OR "armadillo" OR "cabassous" OR "calyptophractus" OR "chaetophractus" OR "chlamyphorus" OR "dasypus" OR "euphractus" OR "priodontes" OR "tolypeutes" OR "zaedyus"*) *AND* (*immobilization OR anesthesia OR restraint OR sedation OR immobilized OR anesthetized OR restrained OR sedated*). Foram excluídos estudos que: não continham espécies de Xenarthras e protocolos anestésicos no seu título, resumo ou metodologia; avaliaram ou implementaram protocolos anestésicos para eutanásia; avaliaram outros táxons além de Xenarthras; não eram artigos revisados por pares (literatura cinzenta). Protocolos que incluíam associações de ciclohexaminas, agonistas alfa2-adrenérgicos, benzodiazepinos, opioides, e/ou anestésicos inalatórios foram considerados seguros e eficazes para anestesia de Xenarthras. Cetamina com um agonista alfa2-adrenérgico foi a associação mais fundamentada, mas depressão cardiovascular, respiratória e termoregulatória estavam presentes. Cetamina isolada, cetamina com acepromazina e butorfanol com isoflurano foram considerados inseguros e ineficazes. Nenhum estudo providenciou alta qualidade de evidência. Outros fármacos (alfaxalona, droperidol, fentanil, halotano, metadona, morfina, óxido nitroso, pentobarbital e propofol) foram implementados, mas seus efeitos anestésicos ainda não foram estudados ou publicados.

Palavras-chave: anestesia; protocolos anestésicos; revisão; xenarthras.

Abstract

Objective This systematic review aimed to investigate (i) which anesthetic protocols have been used to anesthetize Xenarthrans, (ii) which ones have been evaluated and considered safe and effective or not, and (iii) how Xenarthrans' physiological parameters responded to such anesthetic protocols.
Database used Systematic search was conducted from March to October of 2023. Online databases used were PubMed (March and April), Web of Science (May and June), Scopus (July and August), and Google Scholar (September and October). The timespan used for the systematic search was arbitrarily chosen from 1982 to 2022. Used search string was ("xenarthra" OR "sloth" OR "bradypus" OR "choloepus" OR "anteater" OR "cyclopes" OR "myrmecophaga" OR "tamandua" OR "armadillo" OR "cabassous" OR "calyptophractus" OR "chaetophractus" OR "chlamyphorus" OR "dasypus" OR "euphractus" OR "priodontes" OR "tolypeutes" OR "zaedyus") AND (immobilization OR anesthesia OR restraint OR sedation OR immobilized OR anesthetized OR restrained OR sedated). Were excluded reports that: did not contain Xenarthra species and anesthetic protocols in their title, abstract, and/or methodology; evaluated or implemented anesthetic protocols for euthanasia purposes; evaluated more taxons in addition to Xenarthrans; non-peer-reviewed reports (gray literature).
Conclusion Protocols that included combinations of cyclohexamines, alpha2-adrenoreceptor agonists, benzodiazepines, opioids, and/or inhalant anesthetics were considered safe and effective for Xenarthra's anesthesia. Ketamine with an alpha2-adrenoreceptor agonist was the most substantiated combination, but cardiovascular, respiratory, and thermoregulatory depression was presented. Ketamine alone, ketamine with acepromazine, and butorphanol with isoflurane were considered unsafe and ineffective. No study provided high quality of evidence. Other drugs (alfaxalone, droperidol, fentanyl, halothane, methadone, morphine, nitrous oxide, pentobarbital, and propofol) have been implemented but their anesthetic effects have not yet been evaluated or published.

Keywords anesthesia, anesthetic protocols, systematic review, Xenarthras.

5.1 Introduction

Xenarthras are a superorder differentiated into two clades, Pilosa (anteaters and sloths) and Cingulata (armadillos). The taxon is endemic to the American continent and mostly neotropical (SUPERINA; ABBA, 2020). There are currently 38 extant species, and about two-thirds (26) were globally categorized as threatened of extinction, near threatened, or had insufficient data (FEIJÓ *et al.*, 2022). In Brazil, there is confirmation of the occurrence of 18 species, where eight of them were considered within the same previous categories (ICMBIO, 2015).

Collection of biological information is requested to fill in the data for lacking species and to guide conservation actions for threatened species (FEIJÓ et al., 2022; ICMBIO, 2015; SUPERINA; ABBA, 2020). When specimens are captured to collect such information, anesthesia is advised as it can provide safety for both animals and investigators (CHINNADURAI *et al.*, 2016; HAWKINS; GUZMAN; PAUL-MURPHY, 2020). Furthermore, Xenarthrans populations are highly susceptible to anthropogenic impacts such as wildfire and roadkill (ARENALES *et al.*, 2020; RIBEIRO; SILVEIRA MIRANDA; RODRIGUES DE ARAÚJO, 2017; SILVA *et al.*, 2020; SUPERINA; ABBA, 2020). Alive but injured animals require clinical care, where anesthesia has an important role (DAHROUG *et al.*, 2009; DE SOUZA *et al.*, 2022; SILVA *et al.*, 2018). However, the taxon has noteworthy physiological and anatomical adaptations that should be considered when anesthetizing them (MORENO, 2018; WEST; CARTER; SHAW, 2014). As imperfect homeotherms, they have low body temperature that is influenced by environment temperature, and low metabolic rates with unusual oxygen consumption (ATTIAS *et al.*, 2018; CLIFFE *et al.*, 2018; DUARTE *et al.*, 2007; DUARTE; DA COSTA; HUGGINS, 1982; GILMORE; DA-COSTA; DUARTE, 2000; GIROUX *et al.*, 2022; MACCARINI *et al.*, 2015). They can also be anatomically difficult to intubate (BRAINARD *et al.*, 2008; FARO *et al.*, 2015).

Scattered anesthetic protocols in the literature may hinder decision-making, especially in emergency care. To the author's knowledge, there is no published document compiling anesthetic protocols for Xenarthrans besides books that can reasonably be harder to acquire. Therefore, this systematic review aimed to investigate (i) which anesthetic protocols have been used to anesthetize Xenarthrans, (ii) which

ones have been evaluated and considered safe and effective or not, and (iii) how Xenarthrans' physiological parameters responded to such anesthetic protocols.

5.2 Material and methods

A scoping search was previously conducted to identify commonly used terms, define eligibility criteria, refine the question, and identify where the relevant information could be expressed in the text (FOO *et al.*, 2021). A systematic search was then conducted using online databases, following the precluded by guidelines (FOO *et al.*, 2021; PAGE *et al.*, 2021a,b; RETHLEFSEN *et al.*, 2021; SARGEANT; O'CONNOR, 2020). Duplicates were removed. Reports were then first screened using the title, abstract, and methodology. Methodology was included because, during the scoping search, it was observed that some reports implemented anesthetic protocols for Xenarthrans but mentioned it neither in the title nor in the abstract. Reports that did not meet the first eligibility criteria described below were immediately excluded. Reports were then fully screened, and only reports that met all the eligibility criteria were included. Backward and forward search was conducted from the included reports, with the same processes applied until no new report could be included. Included reports were distinguished between reports that objectively evaluated anesthetic protocols and reports that implemented but did not evaluate the anesthetic protocol or were case-reports. Data was then collected, and the quality of evidence was assessed for reports that objectively evaluated anesthetic protocols.

5.2.1 Database and timespan

Systematic search was conducted from March to October of 2023. Online databases used were PubMed (March and April), Web of Science (May and June), Scopus (July and August), and Google Scholar (September and October). The timespan used for the systematic search was arbitrarily chosen from 1982 to 2022.

5.2.2 Search string and terms

("xenarthra" OR "sloth" OR "bradypus" OR "choloepus" OR "anteater" OR "cyclopes" OR "myrmecophaga" OR "tamandua" OR "armadillo" OR "cabassous" OR "calyptophractus" OR "chaetophractus" OR "chlamyphorus" OR "dasypus" OR

"euphractus" OR "priodontes" OR "tolypeutes" OR "zaedyus") AND (immobilization OR anesthesia OR restraint OR sedation OR immobilized OR anesthetized OR restrained OR sedated)

For Google Scholar, due to how the searching tool works, a search was conducted separately for each taxon from the search string and results were then combined.

5.2.3 Eligibility criteria

Were excluded reports that: i) did not contain Xenarthra species and anesthetic protocols in their title, abstract, and/or methodology; ii) evaluated or implemented anesthetic protocols for euthanasia purposes; iii) evaluated more taxons in addition to Xenarthrans; iv) and/or non-peer-reviewed reports (gray literature).

5.2.4 Collected data

Data was collected from reports as identical as possible to what authors described, and unclear or lack of information was not filled in with any interpretation from reviewers. Collected data was transcribed into a table. Data from reports that evaluated the anesthetic protocols were: species; the number of tested events; procedure category; anesthetic drugs and doses; antagonist drugs and doses; induction, maintenance, and recovery times; considerations about induction, maintenance, and recovery; degree of sedation/anesthesia, muscular relaxation, and analgesia; cardiac, respiratory, blood pressure, oxygen saturation, and body temperature effects; and if the protocol was judged to be safe and effective or not by the authors. Data collected from reports that implemented but did not evaluate anesthetic protocol or case-reports, were: species; anesthetic drugs and doses; and antagonist drugs and doses.

When authors described the assessment of an aforementioned aspect but did not address it in their results, it was transcribed into the table as “not reported”. When authors did not indicate the assessment of an aspect, nor address the aspect in their results, it was transcribed as “not assessed”. When authors addressed the aspect and considered it as within normal expectations, it was transcribed as “no alteration”. Additionally, to standardize information, the induction interval consisted of the time between anesthetic administration until achieving full immobilization. Maintenance

interval consisted of the time between full immobilization until first arousal or administration of the antagonist. Recovery interval consisted of the time between first arousal or antagonist administration until full recovery. For contextual purposes, procedures were subjectively categorized considering possible noxious stimuli, from highest to lowest, as: surgical, when incision and suture of tissue were performed; biological sampling, when invasive samples such as blood, tissue, or sperm were extracted; clinical, when physical examination, complementary exams and/or biometry was performed; and radio-collaring, when such device was fitted on the animal. The "safe and effective" aspect was extracted from the report's conclusion, and the quality of evidence was then assessed to evaluate the certainty of this conclusion.

5.2.5 Quality of evidence assessment

Study designs were assessed (RANGANATHAN; AGGARWAL, 2018) and the quality of evidence was graded using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system (SCHÜNEMANN *et al.*, 2013). The grading application was adapted to be as accurate as possible considering what was observed from the scoping search. Reports were scored as high (4), moderate (3), low (2), or very low (≤ 1) quality of evidence. Since all the included reports would necessarily be experimental studies, as an intervention (anesthesia) was applied, no grading up was assessed, and all reports started as high quality. Risk of bias was considered to downgrade the report in -1 (one or two limitations) or -2 (three or four limitations) if it: i) lacked randomization; ii) lacked concealment if it was randomized; iii) lacked blinding; iv) or had unclear criteria to determine the quality of anesthesia. If the report lacked a control or comparison groups, it was automatically downgraded in -2 regardless of other limitations. Inconsistency was considered only across reports with the same species and anesthetic protocol. Results were compared, and the less detailed study was downgraded if most results were mildly different (-1) or highly different/opposite (-2). Indirectness was considered to downgrade a report in -1 (one or two limitations) or -2 (three limitations) if anesthetic effects were not assessed through measurement of at least three physiological parameters (heart rate, respiratory rate, blood pressure, oxygen saturation, and body temperature), quality of anesthesia (sedation, muscle relaxation, or analgesia), and anesthetics intervals (induction,

maintenance, or recovery). Imprecision was considered to downgrade a report in -1 (one limitation) or -2 (both limitations) if it had five or fewer analyzed events ($n \leq 5$) or no statistical analysis was implemented. Publication bias was purposely accepted to represent only anesthetic protocols that have been implemented and peer-reviewed, serving as a reference for clinicians.

5.3 Results

The systematic search resulted in a total of 36,645 entries (PubMed = 155; Web of Science = 81; Scopus = 95; Google Scholar = 36,314). After removing duplicates, applying eligibility criteria, and backward and forward search, it was included a total of 16 reports that objectively evaluated anesthetic protocols and 99 reports that implemented but did not evaluate anesthetic protocol or were case-reports (Table 1). Local anesthetics are presented in the tables for a comprehensive purpose, but they were not accounted for protocols differentiation. Atropine was accounted for protocols differentiation but was not accounted as an anesthetic drug.

5.3.1 Evaluated anesthetic protocols

From the 16 reports that objectively evaluated anesthetic protocols, a single report (6.3%) was scored as moderate quality of evidence, 10 reports (62.5%) were scored as low, and five reports (31.3%) as very low. Considering risk of bias, all reports (16; 100%) were downgraded for lack of blindness, 11 (68.8%) for unclear criteria to determine the quality of anesthesia, eight (50%) for the lack of control, and five (31.3%) for the lack of randomization. Considering inconsistency, one report (6.3%) was downgraded for highly different results. Considering indirectness, two reports (12.5%) were downgraded for partial physiological parameters assessment, two (12.5%) for lacking quality of anesthesia, and two (12.5%) for lacking anesthetic intervals. Considering imprecision, four reports (25%) were downgraded for testing less or equal to five anesthetic events, and four (25%) for not testing results statistically. Across the reports, a total of 37 anesthetic studies (species:protocols) were conducted with 17 different drug combinations, from which mostly (14; 82.4%) were considered safe and effective by the authors, and three (17.6%) were considered unsafe and ineffective (Table 2).

Table 1 – Anesthetic protocols implemented for Xenarthra's anesthesia from 1982 to 2022.

(continue)

Subclade	Species	Evaluation	Protocols	References
Anteaters	*Cyclopes didactylus*	?	Ketamine (8) + Midazolam (0.5)	69
	Myrmecophaga tridactyla	✓	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.2) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01) or [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	51; 75
		✓	Ketamine (8.8) + Acepromazine (0.06) + Diazepam (0.3) + Buprenorfine (0.059) + {Isoflurane (1.1±0.1%)}	15
		?	{Isoflurane (2.5 to 5%)}	76
		?	Dexmedetomidine (0.01) + [Ketamine (3) + Midazolam (0.3)] + {Isoflurane (dose-response)} + <Bupivacaine (0.26ml/kg) + Morphine (0.1)>	4
		?	Dexmedetomidine (0.002) + Midazolam (0.3) + {Isoflurane}	114
		?	Ketamine (3) + Medetomidine (0.06) + {Isoflurane (2 to 5%)}	6; 21
		?	Ketamine (10; 11) + Midazolam (0.2; 0.4; 1)	13; 61; 62; 88; 94
		?	Ketamine (5; 7) + Midazolam (0.3; 5) + {Isoflurane (1ml/min)}	77; 78; 79; 115
		?	Ketamine (7) + Midazolam (0.3) + [Propofol (2.5)]	115
		?	Ketamine (10) + Midazolam (0.3) + [Propofol (1)] + {Isoflurane} + <Morphine (0.1) + Bupivacaine (0.2)>	8
		?	Ketamine (8) + Midazolam (0.3) + Methadone (0.2) + <Lidocaine (9)>	104
		?	Ketamine (10; 15) + Midazolam (0.5) + Morphine (0.1; 0.3) + {Isoflurane}	24; 68

Subclade	Species	Evaluation	Protocols	References
Anteaters	*Myrmecophaga tridactyla*	?	Ketamine (9.56; 9.7; 10; 12; 15) + Xylazine (0.5; 1; 1.6); Ketamine & Xylazine (6:1)	9; 22; 39; 89; 97
		?	Ketamine (6.5) + Xylazine (0.5) + {Isoflurane}	87
		?	Ketamine (8; 10) + Xylazine (0.5; 0.8; 1) + Midazolam (0.2; 0.5)	12; 39; 58
		?	Morphine (0.1) + [Propofol (1)] + {Isoflurane} + <Morphine (0.1) + Bupivacaine (0.2)>	8
		?	Tiletamine & Zolazepam (2; 4; 5)	4; 38; 66; 68; 72; 73; 92
		?	Tiletamine & Zolazepam (3; 4; 5) + {Isoflurane}	33; 57; 110
		?	Zolazepam (2,5)	24
	Tamandua tetradactyla	✓	Ketamine (10; 11.2±1.4; 15; 19.7±1.3) + Xylazine (1±0.1)	34
		?	{Isoflurane}	81; 85; 109
		?	Ketamine (4±0.25) + Dexmedetomidine (20±5) + Midazolam (0.1)	31
		?	Ketamine (10) + Midazolam (1)	94
		?	Ketamine (10) + Xylazine (1) + Atropine (0.04)	93
		?	Ketamine S (5) + Midazolam (0.15) + [Propofol (5)] + {Isoflurane (2%)} + <Lidocaine (3) + Ropivacaine (1)>	99
	Tamandua mexicana	?	{Isoflurane (3.5 to 4%)}	82

Subclade	Species	Evaluation	Protocols	References
Anteaters	*Tamandua mexicana*	?	Ketamine (4) + Dexmedetomidine (0.04) + {Isoflurane (1%)} | Atipamezole (0.1, + 0.1 20min later)	82
Armadillos	*Cabassous tatouay*	?	Tiletamine & Zolazepam (0.2ml/kg)	100
	Cabassous unicinctus	✓	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	53
		✓	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	53
		?	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.2)	52
		?	Tiletamine & Zolazepam (0.2ml/kg) + Atropine (0.04ml/kg)	48
	Chaetophractus nationi	✓	Ketamine (15) + Xylazine (1) + Midazolam (0.4) | Yohimbine (0.22±0.05)	90
	Chaetophractus villosus	?	[Ketamine (30) + Acepromazine (0.3)]	10; 16; 17; 108
		?	Ketamine (30) + Acepromazine (0.3)	18; 84
		?	Ketamine (40) + [Pentobarbital (35)]	1; 19
		?	Ketamine + Medetomidine + {Isoflurane}	21
		?	Pentobarbital (35)	2
	Dasypus hybridus	?	Tiletamine & Zolazepam (0.2ml/kg)	100

Subclade	Species	Evaluation	Protocols	References
Armadillos	*Dasypus kappleri*	✓	Ketamine (7.7±0.4) + Medetomidine (0.077±0.0034) | Atipamezole (0.3851±0.0169)	35
		✓	Ketamine (40.5±1.7) + Xylazine (1)	35
		✓	Tiletamine & Zolazepam (8.5±0.2)	35
	Dasypus novemcinctus	✓	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	53
		✓	Ketamine (7.5±0.3) + Medetomidine (0.0756±0.0031) | Atipamezole (0.3781±0.0155)	35
		✓	Ketamine (15) + Medetomidine (0.07) + Butorphanol (0.1) | Atipamezole (0.31±0.03)	44
		✓	Ketamine (40±1.3; 40) + Xylazine (1±0.1; 1)	35; 44
		✓	Tiletamine & Zolazepam (8.5±0.3)	35
		X	Butorphanol (0.1) + {Isoflurane (1.5 to 2.5%)}	44
		X	Ketamine (65 to 89)	44
		?	{Isoflurane (2 to 3.5%)}	54; 55
		?	{Isoflurane (1.5 to 2.25%)} + [Propofol & Fentanyl (1:4, 4 to 6ml/h)]	96
		?	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.2)	52
		?	Fentanyl & Droperidol (0.11ml/kg)	43
		?	Ketamine (20) + {Isoflurane}	23

Subclade	Species	Evaluation	Protocols	References
Armadillos	*Dasypus novemcinctus*	?	Ketamine (25) + Acepromazine (0.3)	43
		?	Tiletamine & Zolazepam (0.2ml/kg; 4)	60; 100
		?	Tiletamine & Zolazepam (5) + Atropine (0.044)	91
		?	Tiletamine & Zolazepam + Medetomidine	25
	Dasypus septemcinctus	?	Ketamine (8) + Midazolam (1)	64
	Euphractus sexcinctus	✓	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	53
		✓	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	53
		✓	Ketamine (30) + Xylazine (0.5) + Midazolam (0.5) + Atropine (0.02)	7; 37
		?	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.2)	52
		?	Ketamine (7) + Butorphanol (0.4) + [Propofol (5)]	102
		?	Ketamine (7; 15) + Xylazine (1)	9; 14
		?	Ketamine (7) + Xylazine (1) + [Propofol (5)]	102; 103
		?	Tiletamine & Zolazepam (0.2ml/kg; 4)	60; 67; 100
	Priodontes maximus	✓	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	53

Subclade	Species	Evaluation	Protocols	References
Armadillos	*Priodontes maximus*	✓	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	53
		✓	Butorphanol (0.4) + Xylazine (1.2) + Midazolam (0.2) + {Isoflurane (2.5 to 1%)} | [Naltrexona (0.25) + Yohimbine (0.125)]	32
		?	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.2) | Naloxone (0.04) + Yohimbine (0.125) + Flumazenil (0.025)	27; 28; 52
		?	Ketamine (10) + Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.2) | Naloxone (0.04) + Yohimbine (0.125) + Flumazenil (0.025)	26; 59
		?	Ketamine (10) + Midazolam (0.2)	70
		?	Ketamine (10) + Midazolam (0.2) + Morphine (10) + {Sevoflurane (2.3 to 5%)}	105
		?	Tiletamine & Zolazepam (3.8±0.58)	101
	Tolypeutes matacus	✓	Ketamine (20 to 37) + Xylazine (0.62 to 1.25) | Yohimbine (0.121 to 0.141)	80
		✓	Tiletamine & Zolazepam (3.85 to 11.9)	80
		?	{Isoflurane (1 to 5%)}	46; 63
		?	Ketamine (25) + Xylazine (0.5) + Midazolam (0.5) + Atropine (0.02)	7
		?	Tiletamine & Zolazepam + Medetomidine + {Isoflurane}	25
	Tolypeutes tricinctus	?	Ketamine (8) + Midazolam (1)	65
	Zaedyus pichiy	?	{Sevoflurane (3 to 8%)} + Atropine (0.02) + <Lidocaine>	106

Subclade	Species	Evaluation	Protocols	References
Armadillos	*Zaedyus pichiy*	?	Ketamine (20) + Xylazine (1) + Midazolam (0.8) + <Lidocaine>	107
		?	Tiletamine & Zolazepam (15) + Xylazine (1) + <Lidocaine>	107
Sloths	*Bradypus torquatus*	?	Ketamine (1.3) + Acepromazine (0.1)	83
		?	Ketamine (4) + Medetomidine (0.03)	11
	Bradypus tridactylus	✓	[Chloralose (50)]	29; 30
	Bradypus variegatus	✓	Ketamine (2±0.01) + Dexmedetomidine (0.011±0.002) | Atipamezole (0.13)	49
		✓	Ketamine (2.5) + Medetomidine (0.02) | Atipamezole (0.1)	42; 65
		?	Xylazine (2)	5
		?	Ketamine (0.1ml/kg) + Dexmedetomidine (0.1ml/kg)	40
	Choloepus didactylus	✓	Ketamine (3) + Medetomidine (0.04) | Atipamezole (0.2) or [Atipamezole (0.2)]	111; 112
		✓	Ketamine (10) + Xylazine (1)	111; 112
		✓	Ketamine (3) + Xylazine (1) + Midazolam (0.2) | Yohimbine (0.125) + Flumazenil (0.125)	56
		✓	Tiletamine & Zolazepam (10)	111; 112
		X	Ketamine (10) + Acepromazine (0.1)	111; 112

Subclade	Species	Evaluation	Protocols	References
Sloths	*Choloepus didactylus*	?	Ketamine (4) + Dexmedetomidine (0.04) | Atipamezole (same volume as Dexmedetomidine)	98
		?	Ketamine (5) + Midazolam (0.5) + {Isoflurane}	95
		?	Ketamine (2.5) + Xylazine (0.3) + {Isoflurane} + <Lidocaine>	3
		?	Tiletamine & Zolazepam (3.8 to 4.3) + {Isoflurane}	36
	Choloepus hoffmanni	✓	Ketamine (2.26±0.53) + Dexmedetomidine (0.012±0.003) | Atipamezole (0.13)	49
		✓	Ketamine (2.67±0.25) + Dexmedetomidine (0.012±0.004) + Midazolam (0.1) | Atipamezole (0.22±0.05)	71
		✓	Ketamine (2.5) + Medetomidine (0.02) | Atipamezole (0.1)	42; 45; 65
		?	Alfaxalone (2 to 4) + Midazolam (0.5)	20
		?	Ketamine (2 to 6; 25)	47; 113
		?	Ketamine (0.2ml/kg) + Medetomidine (0.2ml/kg) | Atipamezole (0.2ml/kg)	74
		?	Ketamine (3) + Medetomidine (0.04) + {Isoflurane} | Atipamezole (0.2)	41
		?	Ketamine (5) + Midazolam (0.5) + Butorphanol (1)	50
	Choloepus spp	?	Ketamine (50)	86
		?	Ketamine (40) + {Halotane + Nitrous oxide}	86

Doses are encompassed by "()" and are presented in mg/kg unless otherwise noted. Missing doses means they were not reported by the studies. Doses separated by "±" stands for "mean±SD". Drugs separated by "&" are a single mixture. Antagonists are separated from anesthetics by "|". Administration route is intramuscular unless encompassed by "[]" for intravenous, "{}" for inhalant, and "<>" for local. Studied, safe and effective protocols are marked with "✓". Studied, unsafe and ineffective protocols are marked with "X". Unstudied but implemented protocols are marked with "?". References' numbers are correspondent to Apêndice X.

Table 2 – Quality of evidence assessed through adapted Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system of studies that evaluated anesthetic protocols for Xenarthra's anesthesia.

(continue)

Species and number of samples	Anesthetic protocol	Procedure	Safe and effective?	GRADE								Score	Quality of evidence	Reference
				Risk of bias	#	Inconsistency	#	Indirectness	#	Imprecision	#			
Myrmecophaga tridactyla *n* = 51	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.2) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01) or [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	Biological sampling; clinical; radio-collaring	Yes	Non-controlled; non-blinded; unclear criteria to determine muscle relaxation	-2	Unavailable	0	None	0	None	0	2	Low	51
Cabassous unicinctus *n* = 2	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	Biological sampling; clinical; radio-collaring	Yes	Non-randomized; non-blinded	-1	Unavailable	0	No quality of anesthesia assessed	-1	None	0	2	Low	53
Euphractus sexcinctus *n* = 22	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]		Yes											

Species and number of samples	Anesthetic protocol	Procedure	Safe and effective?	GRADE								Score	Quality of evidence	Reference
				Risk of bias	#	Inconsistency	#	Indirectness	#	Imprecision	#			
Priodontes maximus *n* = 6	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	Biological sampling; clinical; radio-collaring	Yes	Non-randomized; non-blinded	-1	Unavailable	0	No quality of anesthesia assessed	-1	None	0	2	Low	53
Cabassous unicinctus *n* = 10	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	Surgical; biological sampling; clinical; radio-collaring	Yes											
Dasypus novemcinctus *n* = 2	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)		Yes											

Species and number of samples	Anesthetic protocol	Procedure	Safe and effective?	GRADE									Score	Quality of evidence	Reference
				Risk of bias	#	Inconsistency	#	Indirectness	#	Imprecision	#				
Euphractus sexcinctus *n* = 4	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	Surgical; biological sampling; clinical; radio-collaring	Yes	Non-randomized; non-blinded	-1	Unavailable	0	No quality of anesthesia assessed	-1	None	0	2	Low	53
Priodontes maximus *n* = 27	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)		Yes											
Euphractus sexcinctus *n* = 10	Ketamine (30) + Xylazine (0.5) + Midazolam (0.5) + Atropine (0.02)	Surgical; biological sampling; clinical; radio-collaring	Yes	Non-controlled; non-blinded	-2	Unavailable	0	None	0	None	0	2	Low	37
Choloepus didactylus *n* = 3	Ketamine (3) + Xylazine (1) + Midazolam (0.2) | Yohimbine (0.125) + Flumazenil (0.125)	Clinical	Yes	Non-controlled; non-blinded	-2	Unavailable	0	None	0	No statistical analysis; ≤ 5 analyzed events	-2	0	Very low	56

Species and number of samples	Anesthetic protocol	Procedure	Safe and effective?	GRADE									Score	Quality of evidence	Reference
				Risk of bias	#	Inconsistency	#	Indirectness	#	Imprecision	#				
Priodontes maximus *n* = 3	Butorphanol (0.4) + Xylazine (1.2) + Midazolam (0.2) + {Isoflurane (1% to 2.5%)} | [Naltrexona (0.25) + Yohimbine (0.125)]	Biological sampling; clinical	Yes	Non-controlled; non-blinded; unclear criteria to determine sedation	-2	Unavailable	0	Partial physiological parameters*	-1	No statistical analysis; ≤ 5 analyzed events	-2	-1	Very low	32
Bradypus variegatus *n* = 12	Ketamine (2±0.01) + Dexmedetomidine (0.011±0.002) | Atipamezole (0.13)	Biological sampling	Yes	Non-controlled**; non-blinded; unclear criteria to determine sedation and muscle relaxation	-2	Unavailable	0	None	0	None	0	2	Low	49
Choloepus hoffmanni *n* = 14	Ketamine (2.26±0.53) + Dexmedetomidine (0.012±0.003) | Atipamezole (0.13)		Yes											
Chaetophractus nationi *n* = 6	Ketamine (15) + Xylazine (1) + Midazolam (0.4) | Yohimbine (0.22±0.05)	Biological sampling; clinical	Yes	Non-controlled; non-blinded; unclear criteria to determine sedation and muscle relaxation	-2	Unavailable	0	None	0	No statistical analysis	-1	1	Very low	90

Species and number of samples	Anesthetic protocol	Procedure	Safe and effective?	GRADE									Score	Quality of evidence	Reference
				Risk of bias	#	Inconsistency	#	Indirectness	#	Imprecision	#				
Choloepus hoffmanni *n* = 9	Ketamine (2.67±0.25) + Dexmedetomidine (0.012±0.004) + Midazolam (0.1) | Atipamezole (0.22±0.05)	Biological sampling; clinical	Yes	Non-controlled; non-blinded; unclear criteria to determine sedation and muscle relaxation	-2	Unavailable	0	None	0	None	0	2	Low	71
Tolypeutes matacus *n* = 12	Ketamine (20 to 37) + Xylazine (0.62 to 1.25) | Yohimbine (0.121 to 0.141)	Biological sampling; clinical	Yes	Non-blinded; unclear criteria to determine muscle relaxation	-1	Unavailable	0	None	0	None	0	3	Moderate	80
n = 18	Tiletamine & Zolazepam (3.85 to 11.9)		Yes											

Species and number of samples	Anesthetic protocol	Procedure	Safe and effective?	GRADE									Score	Quality of evidence	Reference
				Risk of bias	#	Inconsistency	#	Indirectness	#	Imprecision	#				
Dasypus novemcinctus *n* = 2	Butorphanol (0.1) + {Isoflurane (1.5% to 2.5%)}	Surgical	No	Non-randomized; non-blinded; unclear criteria to determine muscle relaxation	-2	Comparable with Fournier-Chambrillon *et al.* (2000) for ketamine + xylazine (KX) protocol: Mild different induction time and highly different recovery times (mean±SD); non reported heart rate, respiratory rate and SpO_2 for comparison	(-2 for KX)	None	0	No statistical analysis; ≤ 5 analyzed events for butorphanol + isoflurane (BI) and ketamine (K) protocols	-1 (-2 for BI and K)	1 (-1 for KX, K and BI)	Very low	44
n = 4	Ketamine (65 to 89)		No												
n = 19 *n* = 14	Ketamine (15) + Medetomidine (0.07) + Butorphanol (0.1) | Atipamezole (0.31±0.03)		Yes												
	Ketamine (40) + Xylazine (1)		Yes												
Myrmecophaga tridactyla *n* = 5	Ketamine (8.8) + Acepromazine (0.06) + Diazepam (0.3) + Buprenorfine (0.059) + {Isoflurane (1.1±0.1%)}	Surgical; clinical	Yes	Non-controlled; non-blinded; unclear criteria to determine muscle relaxation	-2	Unavailable	0	None	0	≤ 5 analyzed events	-1	1	Very low	15
Bradypus variegatus *n* = 15	Ketamine (2.5) + Medetomidine (0.02) | Atipamezole (0.1)	Biological sampling	Yes	*** Non-blinded	-1	Unavailable	0	No anesthetic intervals assessed	-1	None	0	2	Low	42

Species and number of samples	Anesthetic protocol	Procedure	Safe and effective?	GRADE								Score	Quality of evidence	Reference
				Risk of bias	#	Inconsistency	#	Indirectness	#	Imprecision	#			
Choloepus hoffmanni *n* = 26	Ketamine (2.5) + Medetomidine (0.02) | Atipamezole (0.1)	Biological sampling	Yes	*** Non-blinded	-1	Unavailable	0	No anesthetic intervals assessed	-1	None	0	2	Low	42
Dasypus kappleri *n* = 12	Ketamine (7.7±0.4) + Medetomidine (0.077±0.0034) | Atipamezole (0.3851±0.0169)	Biological sampling; clinical	Yes	Non-randomized; non-blinded; unclear criteria to determine sedation and muscle relaxation	-2	Comparable with Hernandez *et al.* (2010) for ketamine + xylazine protocol	0	None	0	None	0	2	Low	35
Dasypus kappleri *n* = 9	Ketamine (40.5±1.7) + Xylazine (1±0.0)		Yes											
Dasypus kappleri *n* = 10	Tiletamine & Zolazepam (8.5±0.2)		Yes											
Dasypus novemcinctus *n* = 17	Ketamine (7.5±0.3) + Medetomidine (0.0756±0.0031) | Atipamezole (0.3781±0.0155)		Yes											
Dasypus novemcinctus *n* = 18	Ketamine (40±1.3) + Xylazine (1±0.1)		Yes											
Dasypus novemcinctus *n* = 12	Tiletamine & Zolazepam (8.5±0.3)		Yes											

Species and number of samples	Anesthetic protocol	Procedure	Safe and effective?	GRADE								Score	Quality of evidence	Reference
				Risk of bias	#	Inconsistency	#	Indirectness	#	Imprecision	#			
Choloepus didactylus *n* = 30	Ketamine (10) + Acepromazine (0.1)	Biological sampling; clinical	No	Non-randomized; non-blinded; unclear criteria to determine sedation	-2	Unavailable	0	None	0	None	0	2	Low	111
n = 46	Ketamine (3) + Medetomidine (0.04) | Atipamezole (0.2) or [Atipamezole (0.2)]		Yes											
n = 89	Ketamine (10) + Xylazine (1)		Yes											
n = 37	Tiletamine & Zolazepam (10)		Yes											
Tamandua tetradactyla *n* = 7	Ketamine (11.2±1.4) + Xylazine (1±0.1)	Biological sampling; clinical	Yes	Non-randomized; non-blinded; unclear criteria to determine sedation and muscle relaxation	-2	Unavailable	0	None	0	None	0	2	Low	34
n = 10	Ketamine (19.7±1.3) + Xylazine (1±0.1)		Yes											

Species and number of samples	Anesthetic protocol	Procedure	Safe and effective?	GRADE										Score	Quality of evidence	Reference
				Risk of bias	#	Inconsistency	#	Indirectness	#	Imprecision	#					
Bradypus tridactylus *n* = 6	[Chloralose (50)]	Clinical	Yes	**** Non-blinded	-1	Unavailable	0	No quality of anesthesia assessed; partial physiological parameters; no anesthesia intervals assessed	-2	None	0	1	Very low	29

Doses are encompassed by "()" and are presented in mg/kg unless otherwise noted. Missing doses means they were not reported by the studies. Doses separated by "±" stands for "mean±SD". Drugs separated by "&" are a single mixture. Antagonists are separated from anesthetics by "|". Administration route is intramuscular unless encompassed by "[]" for intravenous, "{}" for inhalant, and "<>" for local. The "#" columns represent the downgraded values. References' numbers are correspondent to Apêndice X.

* Study address only heart and respiratory rates as physiological parameters;

** Groups are not compared;

*** Lack of randomization, and thus allocation concealment, wasn't considered a study limitation since all individuals received the same intervention;

**** Self-controlled study; lack of randomization, and thus allocation concealment, wasn't considered a study limitation since all individuals received the same intervention.

Considering quality of evidence and the cumulative number of trials (*n*) for each different drug combination (17), it was found moderate (*n* = 12), low (*n* = 133), and very low (*n* = 14) quality of evidence that ketamine-xylazine is safe and effective. Moderate (*n* = 18) and low (*n* = 59) quality of evidence that tiletamine-zolazepam is safe and effective. Low quality of evidence that ketamine-medetomidine (*n* = 116), butorphanol-detomidine-midazolam (*n* = 51), butorphanol-detomidine-midazolam-lidocaine-ketamine (*n* = 42), butorphanol-detomidine-midazolam-lidocaine (*n* = 30), ketamine-dexmedetomidine (*n* = 26), ketamine-xylazine-midazolam-atropine (*n* = 10), and ketamine-dexmedetomidine-midazolam (*n* = 9) are safe and effective. Very low quality of evidence that ketamine-xylazine-midazolam (*n* = 21), ketamine-medetomidine-butorphanol (*n* = 19), chloralose (*n* = 6), ketamine-acepromazine-diazepam-buprenorfine-isoflurane (*n* = 5), and butorphanol-xylazine-midazolam-isoflurane (*n* = 3) are safe and effective. Low quality of evidence that ketamine-acepromazine (*n* = 30) is unsafe and ineffective. Very low quality of evidence that ketamine (*n* = 4) alone and butorphanol-isoflurane (*n* = 2) are unsafe and ineffective (Table 2).

Considering how drugs were presented separately across the 37 anesthetic studies, ketamine was the most prevalent (26; 70.3%), followed by midazolam (13; 35.1%), butorphanol (11; 29.7%), xylazine (11; 29.7%), detomidine (8; 21.6%), medetomidine (6; 16.2%), tiletamine (4; 10.8%), zolazepam (4; 10.8%), dexmedetomidine (3; 8.1%), isoflurane (3; 8.1%), acepromazine (2; 5.4%), buprenorphine (1; 2.7%), chloralose (1; 2.7%), and diazepam (1; 2.7%).

Considering drug combinations across the 37 anesthetic studies, ketamine associated with an alpha2-adrenoreceptor agonist (a2-A) (xylazine, medetomidine or dexmedetomidine) was the most prevalent (19; 51.4%), without (14; 37.8%) or with the addition of midazolam (3; 8.1%), midazolam and atropine (1; 2.7%), or butorphanol (1; 2.7%). Ketamine was also tested alone (1; 2.7%), with acepromazine only (1; 2.7%), and with acepromazine, diazepam, buprenorphine, and isoflurane (1; 2.7%). The second most common combination was butorphanol with an a2-A (xylazine or detomidine) and midazolam (9; 24.3%), without (4; 10.8%) or with isoflurane (1; 2.7%) or ketamine (4; 10.8%) implemented during anesthesia maintenance. Butorphanol was also tested with

isoflurane only (1; 2.7%). The association of tiletamine and zolazepam was studied four times (10.8%). Chloralose was studied once (2.7%).

The anesthetic intervals of each protocol are presented in the Table 3. Quality of anesthesia and physiological effects are presented in the Table 4. Not all reports included the number of trials (*n*) that presented each alteration for physiological parameters, therefore results here-in represents the general array of alterations found across protocols studied. Ketamine paired only with an a2-A (xylazine, medetomidine, or dexmedetomidine) (14 studies) produced no alteration (2; 14.3%) or a decrease over time in heart rate (8; 57.1%) and bradycardia (4; 28.6%). One study (7.1%) did not report heart rate effects. For respiratory rate, it produced no alteration (6; 42.9%), a decrease and then increase over time (2; 14.3%), tachypnea (2; 14.3%), bradypnea (2; 14.3%), apnea (1; 7.1%), irregular breathing (1; 7.1%), or had lower mean rates when compared to other protocols (1; 7.1%) (ketamine-acepromazine and tiletamine-zolazepam). One study (7.1%) did not report respiratory rate effects. Blood pressure decreased over time (4; 28.6%) with initial hypertension (2; 14.3%), or it was not assessed (10; 71.4%). Oxygen saturation increased over time in two studies (14.3%) and in eight studies (57.1%) hypoxemia was presented. Oxygen saturation was not assessed in four studies (28.6%), and not reported in two studies (14.3%). Body temperature mostly decreased over time (6; 42.9%), with initial hypothermia (2; 14.3%) and the necessity of thermal intervention (1; 7.1%). It presented no alteration in two studies (14.3%) and was not reported in three studies (21.4%). With the addition of midazolam (3 studies), heart rate decreased over time (2; 66.7%) and bradycardia (1; 33.3%) was presented. Respiratory rate had no alteration (1; 33.3%), decreased over time (1; 33.3%) or apnea was presented (1; 33.3%). Blood pressure had no alteration (1; 33.3%) or hypertension was presented (1; 33.3%). Blood pressure not assessed in one of the studies (33.3%). Oxygen saturation had no alteration (2; 66.7%) or hypoxemia (1; 33.3%) was presented. Body temperature presented no alteration (3; 100%). The addition of midazolam and atropine (1 study) produced no alteration, an increase over time for respiratory rate, and a decrease over time for males' body temperature, while blood pressure and oxygen saturation were not assessed. For the addition of butorphanol (1 study) instead of midazolam, physiological parameters were either not reported or not assessed.

Table 3 – Anesthetic intervals of studied protocols for Xenarthra's anesthesia.

(continue)

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Myrmecophaga tridactyla *n* = 44	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.2) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01);	8.27±4.78	85.5±16.8	12±5.39	Quick and smooth	Supplementary doses needed to prolong anesthesia beyond 50±6.38 mins; shorter in juveniles than adults	Smooth and rapid	51
n = 7	or [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]			2.55±1.33			Faster with antagonist IV	
Cabassous unicinctus *n* = 2	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	2.5±1	60±85	3±0.5	Quick, smooth and adequate	*No difference for physiological parameters between groups (species and protocols) Not reported	*No difference between species for both protocols; less variation and faster recovery for the protocol without ketamine Not reported	53
Euphractus sexcinctus *n* = 22	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	3±2.5	78±25	2±4	Quick, smooth and adequate	Not reported	Not reported	

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Priodontes maximus *n* = 6	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	5.5±2.5	104±59.5	4±7	Longer induction compared to *E. sexcinctus* and *C. unicinctus*; quick, smooth and adequate	Longer immobilization compared to *E. sexcinctus* and *C. unicinctus*	Not reported	53
Cabassous unicinctus *n* = 10	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	2±1.5	98±44	3±6.7	Quick, smooth and adequate	Not reported	Not reported	
Dasypus novemcinctus *n* = 2	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	3.8±0.8	133.2±9.12	8.08±2.93	Quick, smooth and adequate	Not reported	Not reported	

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Euphractus sexcinctus *n* = 4	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	3.5±2	97±22	3.5±8.5	Quick, smooth and adequate	Not reported	Not reported	53
Priodontes maximus *n* = 27	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	5.5±3	137±39.5	6±8.5	Longer induction compared to *E. sexcinctus* and *C. unicinctus*; quick, smooth and adequate	Longer immobilization compared to *E. sexcinctus* and *C. unicinctus*	Not reported	
Euphractus sexcinctus *n* = 10	Ketamine (30) + Xylazine (0.5) + Midazolam (0.5) + Atropine (0.02)	1.7±05 males; 3.2±1.4 females	50	Not assessed	Shorter time for males	Good	Not assessed	37

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Choloepus didactylus *n* = 3	Ketamine (3) + Xylazine (1) + Midazolam (0.2) | Yohimbine (0.125) + Flumazenil (0.125)	3.8±0.6	25	13.7±0.6	Good	Good	Good	56
Priodontes maximus *n* = 3	Butorphanol (0.4) + Xylazine (1.2) + Midazolam (0.2) + {Isoflurane (1% to 2.5%)} | [Naltrexone (0.25) + Yohimbine (0.125)]	<5	40	5	No alteration	Partial reversal of xylazine required due to collateral effects (*n* = 2)	No alteration	32
Bradypus variegatus *n* = 12	Ketamine (2±0.01) + Dexmedetomidine (0.011±0.002) | Atipamezole (0.13)	4±1.3	40	11.6±5.6	Smooth, uneventful and rapid; supplementary dose needed (*n* = 1)	Not reported	Short	49
Choloepus hoffmanni *n* = 14	Ketamine (2.26±0.53) + Dexmedetomidine (0.012±0.003) | Atipamezole (0.13)	7.4±3.4	40	4±3.4	Smooth, uneventful and rapid; supplementary dose needed (*n* = 4)	Not reported	Short	

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Chaetophractus nationi *n* = 6	Ketamine (15) + Xylazine (1) + Midazolam (0.4) | Yohimbine (0.22±0.05)	6±3	50	10±4	No alteration	Not reported	No alteration	90
Choloepus hoffmanni *n* = 9	Ketamine (2.67±0.25) + Dexmedetomidine (0.012±0.004) + Midazolam (0.1) | Atipamezole (0.22±0.05)	6±3	50	10±4	Quick, smooth	Not reported	Not reported	71
Tolypeutes matacus *n* = 12	Ketamine (20 to 37) + Xylazine (0.62 to 1.25) | Yohimbine (0.121 to 0.141)	3.4±1.5	32.5±11.5	36.6±11.8	*No time difference between protocols No alteration	*No time difference between protocols; no difference in the occurrence of tachypnea nor apnea; with difference in the occurrence of hypothermia and muscle relaxation	*No time difference between protocols for recovery times, but different for first signs to full recovery	80
n = 18	Tiletamine & Zolazepam (3.85 to 11.9)	3.4±1.2	25.2±7.7	35.1±6.7	Slight salivation; shaking; initial tachycardia			
Dasypus novemcinctus *n* = 2	Butorphanol (0.1) + {Isoflurane (1.5% to 2.5%)}	17.5±3.5	41	32±2.1	Struggling	Best subjective quality; none was smooth	Smooth (100%); Shorter than ketamine and ketamine + xylazine	44

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Dasypus novemcinctus *n* = 4	Ketamine (65 to 89)	16	25,7	>3 hours	Shortest; dissociative movements; supplementary doses needed for all trials	Not reported	Not reported	44
n = 19	Ketamine (15) + Medetomidine (0.07) + Butorphanol (0.1) | Atipamezole (0.31±0.03)	12.2±6.9	30,1	13.7±5.4	Best subjective quality; supplementary doses needed (*n* = 1)	Recovering before the end of surgery (*n* = 1); best subjective quality; smooth (94%)	Smooth (100%); shortest	
n = 14	Ketamine (40) + Xylazine (1)	10.3±4.3	18,3	>3 hours	Best subjective quality	Recovering before the end of surgery (*n* = 1); best subjective quality; smooth (1%)	Not reported	
Myrmecophaga tridactyla *n* = 5	Ketamine (8.8) + Acepromazine (0.06) + Diazepam (0.3) + Buprenorfine (0.059) + {Isoflurane (1.1±0.1%)}	10 to 15	110 to 160	Not assessed	Not reported	Not reported	Not assessed	15

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Bradypus variegatus *n* = 15	Ketamine (2.5) + Medetomidine (0.02) | Atipamezole (0.1)	Not assessed	45	Not assessed	Not reported	Not reported	Smooth	42
Choloepus hoffmanni *n* = 26	Ketamine (2.5) + Medetomidine (0.02) | Atipamezole (0.1)	Not assessed	45	Not assessed	Supplementary dose needed (*n* = 4)	Not reported	Smooth	
Dasypus kappleri *n* = 8	Ketamine (7.7±0.4) + Medetomidine (0.077±0.0034) | Atipamezole (0.3851±0.0169);	4.3±1.8	30.4±6.2	15±10	*No time difference between groups Smooth, uneventful	*For all groups, 50 to 64% of the individuals never showed hypoxemia Shorter immobilization for females compared to ketamine + xylazine and tiletamine + zolazepam	Shorter with antagonist than without; With antagonist, shorter than ketamine + xylazine and tiletamine + zolazepam	35
n = 4	or without antagonist			Not reported				
Dasypus kappleri *n* = 9	Ketamine (40.5±1.7) + Xylazine (1±0.0)	2.8±0.6	71.1±34.7	82.3±50.2	Supplementary dose needed (*n* = 1); smooth, uneventful	Salivation (*n* = 1)	Longer recovery compared to *D. novemcinctus*	
Dasypus kappleri *n* = 10	Tiletamine & Zolazepam (8.5±0.2)	4±1.5	52.3±24.3 males; 98.4±33.7 females	31.7±26.7	Smooth, uneventful	Longer immobilization time for females than males	Not reported	

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Dasypus novemcinctus *n* = 13	Ketamine (7.5±0.3) + Medetomidine (0.0756±0.0031) | Atipamezole (0.3781±0.0155);	3.5±1.7	43.8±27.8	10	Smooth, uneventful	Cyanosis (*n* = 1)	Shorter with antagonist than without; With antagonist, shorter than ketamine + xylazine; restless (*n* = 2)	35
n = 4	or without antagonist			Not reported				
Dasypus novemcinctus *n* = 18	Ketamine (40±1.3) + Xylazine (1±0.1)	3.7±2.2	66.5±40	23.3±26.3	Smooth, uneventful	No alteration	Restless (*n* = 2)	
Dasypus novemcinctus *n* = 12	Tiletamine & Zolazepam (8.5±0.3)	3±0.5	53.1±38.7	57.5±67.1	Excitatory phase (*n* = 1); supplementary dose needed (*n* = 1)	Salivation (*n* = 1)	Not reported	
Choloepus didactylus *n* = 30	Ketamine (10) + Acepromazine (0.1)	6.33±2.25	44.4±10.7	29±13	Smooth, uneventful; supplementary dose needed (*n* = 1)	Not reported	Not statistically compared to other protocols; excitation (*n* = 1); recovery >2h and death in the following day (*n* = 1)	111

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Choloepus didactylus *n* = 23	Ketamine (3) + Medetomidine (0.04) | Atipamezole (0.2);	4.13±1.35	43.3±12.1	9.3±8.5	Smooth, uneventful; supplementary dose needed (*n* = 1)	Not reported	Shorter than ketamine + xylazine and tiletamine + zolazepam; quiet	111
n = 3	or [Atipamezole (0.2)];		41.6±8.9	1.5 to 2			Uneventful; shorter with antagonist IV	
n = 11	or first arousal before antagonist injection		Not reported	Not reported			Not reported	
n = 9	or without antagonist		Not reported	Not reported			Not reported	
n = 89	Ketamine (10) + Xylazine (1)	3.99±1.57	46.5±16.5	40.2±25.4	Smooth, uneventful; supplementary dose needed (*n* = 1)	Not reported	Not reported	
n = 37	Tiletamine & Zolazepam (10)	2.8±1.15	48.7±13.8	76.7±31.3	Shorter induction; smooth, uneventful; supplementary dose needed (*n* = 1)	Not reported	Some individuals required >3h	

Species and number of samples	Anesthetic protocol	Induction time (min)	Maintenance time (min)	Recovery time (min)	Considerations			Reference
					Induction	Maintenance	Recovery	
Tamandua tetradactyla *n* = 7	Ketamine (11.2±1.4) + Xylazine (1±0.1)	3±1.5	35±9.5	18.2±13.6	*No time difference between groups; smooth	Not reported	Smooth; Earlier first signs of recovery and time to first standing	34
n = 10	Ketamine (19.7±1.3) + Xylazine (1±0.1)	2.9±1	48.3±15.8	35.7±28.1	Smooth	Not reported	Smooth	
Bradypus tridactylus *n* = 6	[Chloralose (50)]	15	4 hours	Not assessed	Not assessed	Not reported	Not assessed	29

Doses are encompassed by "()" and are presented in mg/kg unless otherwise noted. Missing doses means they were not reported by the studies. Values separated by "±" stands for "mean±SD". Drugs separated by "&" are a single mixture. Antagonists are separated from anesthetics by "|". Administration route is intramuscular unless encompassed by "[]" for intravenous, "{}" for inhalant, and "<>" for local. References' numbers are correspondent to Apêndice X.
*Consideration is applied to all groups within the given study.

Table 4 – Quality of anesthesia and physiological effects of studied protocols for Xenarthra's anesthesia.

(continue)

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Myrmecophaga tridactyla *n* = 51	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.2) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01) or [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	Not assessed	Satisfactory	Satisfactory	Oscillating during first 20 min	No alteration	Not assessed	Oscillating during first 20 min	No alteration	51
Cabassous unicinctus *n* = 2	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	Adequate	Adequate	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	53
Euphractus sexcinctus *n* = 22	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	Adequate	Adequate	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Priodontes maximus *n* = 6	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> | [Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)]	Adequate	Adequate	Not assessed	Normal and constant after 20 mins	Prolonged apnea (*n* = 1); normal and constant after 20 mins	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	53
Cabassous unicinctus *n* = 10	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	Adequate	Adequate	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	
Dasypus novemcinctus *n* = 2	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	Adequate	Adequate	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Euphractus sexcinctus *n* = 4	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	Adequate	Adequate	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	53
Priodontes maximus *n* = 27	Butorphanol (0.1) + Detomidine (0.1) + Midazolam (0.1) + <Lidocaine (2)> + Ketamine (10, applied 20 mins later) | Naloxone (0.02) + Yohimbine (0.125) + Flumazenil (0.01)	Adequate	Adequate	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	Not assessed	Normal and constant after 20 mins	Normal and constant after 20 mins	
Euphractus sexcinctus *n* = 10	Ketamine (30) + Xylazine (0.5) + Midazolam (0.5) + Atropine (0.02)	Good	Good	Not assessed	Higher Initial in males than females	Increased over time during noxious stimuli	Not assessed	Not assessed	Decreased over time for males	37

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Choloepus didactylus *n* = 3	Ketamine (3) + Xylazine (1) + Midazolam (0.2) | Yohimbine (0.125) + Flumazenil (0.125)	Deep anesthesia	Included within anesthesia degree	Included within anesthesia degree	Slight decrease over time	No alteration	Hypertension	No alteration	No alteration	56
Priodontes maximus *n* = 3	Butorphanol (0.4) + Xylazine (1.2) + Midazolam (0.2) + {Isoflurane (1% to 2.5%)} | [Naltrexone (0.25) + Yohimbine (0.125)]	Deep sedation	Slight movements (*n* = 1)	Not assessed	No alteration	Bradypnea (*n* = 2)	Possible hypotension	Not assessed	Not assessed	32
Bradypus variegatus *n* = 12	Ketamine (2±0.01) + Dexmedetomidine (0.011±0.002) | Atipamezole (0.13)	Not reported	Not reported	Not assessed	Mild bradycardia	No alteration	Decreased over time	Not reported	Not reported	49
Choloepus hoffmanni *n* = 14	Ketamine (2.26±0.53) + Dexmedetomidine (0.012±0.003) | Atipamezole (0.13)	Not reported	Not reported	Not assessed	Mild bradycardia	No alteration	Decreased over time	Not reported	Not reported	
Chaetophractus nationi *n* = 6	Ketamine (15) + Xylazine (1) + Midazolam (0.4) | Yohimbine (0.22±0.05)	Great	Great	Not assessed	Bradycardia; increased during electro-ejaculation	Apnea (*n* = 2)	Not assessed	Hypoxemia (*n* = 1)	No alteration	90

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Choloepus hoffmanni *n* = 9	Ketamine (2.67±0.25) + Dexmedetomidine (0.012±0.004) + Midazolam (0.1) | Atipamezole (0.22±0.05)	Great	Excellent	Not assessed	Decreased over time	Decreased over time	No alteration	No alteration	No alteration	71
Tolypeutes matacus *n* = 12	Ketamine (20 to 37) + Xylazine (0.62 to 1.25) | Yohimbine (0.121 to 0.141)	Not assessed	Great (75%) and good (25%)	Not assessed	No alteration	Intense initial tachypnea	Not assessed	Not assessed	Decreased over time (33,3%), thermal intervention required	80
n = 18	Tiletamine & Zolazepam (3.85 to 11.9)	Not assessed	Regular (55,6%) and good (44,4%)	Not assessed	No alteration	Irregular breathing, tendency to tachypnea (22%); apnea (4%)	Not assessed	Not assessed	Not reported	
Dasypus novemcinctus *n* = 2	Butorphanol (0.1) + {Isoflurane (1.5% to 2.5%)}	Appropriate (100%)	Included within anesthesia degree	Not assessed	Not reported	Not reported	Not assessed	Not assessed	Not reported	44
Dasypus novemcinctus *n* = 4	Ketamine (65 to 89)	Appropriate (0%)	Included within anesthesia degree	Not assessed	Not reported	Not reported	Not assessed	Not assessed	Not reported	

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Dasypus novemcinctus *n* = 19	Ketamine (15) + Medetomidine (0.07) + Butorphanol (0.1) | Atipamezole (0.31±0.03)	Adequate (*n* = 17)	Included within anesthesia degree	Not assessed	Not reported	Not reported	Not assessed	Not assessed	Not reported	44
n = 14	Ketamine (40) + Xylazine (1)	Appropriate (84%)	Included within anesthesia degree	Not assessed	Not reported	Not reported	Not assessed	Not assessed	Not reported	
Myrmecophaga tridactyla *n* = 5	Ketamine (8.8) + Acepromazine (0.06) + Diazepam (0.3) + Buprenorfine (0.059) + {Isoflurane (1.1±0.1%)}	Indication of being satisfactory	Adequate	Suggestively effective	Bradycardia	No alteration	Not assessed	Light hypoxemia; higher between 10 to 70 mins	No alteration	15
Bradypus variegatus *n* = 15	Ketamine (2.5) + Medetomidine (0.02) | Atipamezole (0.1)	Sufficient	Good	Not assessed	Higher pre-anesthesia than anesthetized; decreased over time; bradycardia	Higher pre-anesthesia than anesthetized; decreased and then increased over time	Higher than *C. hoffmani* while anesthetized; males higher than females; decreased over time; initial hypertension	Mild hypoxemia	Males higher than females	42

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Choloepus hoffmanni *n* = 26	Ketamine (2.5) + Medetomidine (0.02) | Atipamezole (0.1)	Sufficient	Good	Not assessed	Higher than *B. variegatus* in pre-anesthesia; higher pre-anesthesia than anesthetized; decreased over time; bradycardia	Higher than *B. variegatus* while anesthetized; higher pre-anesthesia than anesthetized; decreased and then increased over time	Males higher than females; decreased over time; initial hypertension	Mild hypoxemia	Males higher than females	42
Dasypus kappleri *n* = 12	Ketamine (7.7±0.4) + Medetomidine (0.077±0.0034) | Atipamezole (0.3851±0.0169)	Complete to deep anesthesia (42%)	Good to excellent (82%)	Not assessed	Decreased over time	No alteration	Not assessed	SpO_2<80% (*n* = 1)	No alteration	35
Dasypus kappleri *n* = 9	Ketamine (40.5±1.7) + Xylazine (1±0.0)	Complete to deep anesthesia (67%)	Good to excellent (78%)	Not assessed	Decreased over time	No alteration	Not assessed	SpO_2<80% (*n* = 1)	No alteration	
Dasypus kappleri *n* = 10	Tiletamine & Zolazepam (8.5±0.2)	Complete to deep anesthesia (100%)	Good to excellent (70%)	Not assessed	Decreased over time; Higher initial than ketamine + medetomidine	Lower initial and middle than ketamine + medetomidine	Not assessed	Not assessed	No alteration	

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Dasypus novemcinctus *n* = 17	Ketamine (7.5±0.3) + Medetomidine (0.0756±0.0031) | Atipamezole (0.3781±0.0155)	Complete or deep anesthesia (82%)	Better than tiletamine & zolazepam (88%)	Not assessed	Decreased over time	Lower initial and middle compared to *D. kappleri*	Not assessed	SpO_2<80% (*n* = 4)	Initial <32 (*n* = 1)	35
Dasypus novemcinctus *n* = 18	Ketamine (40±1.3) + Xylazine (1±0.1)	Complete to deep anesthesia (61%)	Better than tiletamine & zolazepam (89%)	Not assessed	Decreased over time	No alteration	Not assessed	SpO_2<80% (*n* = 2)	Decreased over time; initial <32 (*n* = 1)	
Dasypus novemcinctus *n* = 12	Tiletamine & Zolazepam (8.5±0.3)	Complete to deep anesthesia (75%)	Not satisfactory (50%)	Not assessed	No alteration	Lower initial than ketamine + xylazine and ketamine + medetomidine	Not assessed	Assessed only in two individuals, SpO_2>90%	Decreased over time; initial <32 (*n* = 1)	
Choloepus didactylus *n* = 30	Ketamine (10) + Acepromazine (0.1)	Complete anesthesia (36%)	Most poor or moderate (*n* = 27)	Not assessed	No alteration	Highest mean rates; tachypnea (*n* = 13)	Not assessed	Not assessed	Decreased over time	111
n = 46	Ketamine (3) + Medetomidine (0.04) | Atipamezole (0.2) or [Atipamezole (0.2)]	Complete or deep anesthesia (60%)	Highest degree; most good or excellent (*n* = 45)	Not assessed	Decreased over time; bradycardia (*n* = 2)	Lower mean rates; apnea (*n* = 1); bradypnea (*n* = 4)	Not assessed	Increased over time; hypoxemia (*n* = 3)	Decreased over time	

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Choloepus didactylus *n* = 89	Ketamine (10) + Xylazine (1)	Complete or deep anesthesia (60%)	Good or excellent (70%)	Not assessed	Decreased over time; bradycardia (*n* = 6)	Tachypnea (*n* = 4); bradypnea (*n* = 2); irregular breathing (*n* = 1)	Not assessed	Increased over time; hypoxemia (*n* = 1)	Decreased over time	111
n = 37	Tiletamine & Zolazepam (10)	Complete or deep anesthesia (60%)	Good or excellent (70%)	Not assessed	Decreased over time; bradycardia (*n* = 1)	Tachypnea (*n* = 3); bradypnea (*n* = 2)	Not assessed	Lowest mean rates; hypoxemia (*n* = 5)	Decreased over time	
Tamandua tetradactyla *n* = 7	Ketamine (11.2±1.4) + Xylazine (1±0.1)	Light anesthesia (*n* = 4) and complete anesthesia (*n* = 3)	Moderate (*n* = 1), good (*n* = 4) and excellent (*n* = 2)	Not assessed	No alteration	No alteration	Not assessed	Not assessed	Decreased over time	34

Species and number of samples	Anesthetic protocol	Sedation/ Anesthesia	Muscular relaxation	Analgesia	Heart rate	Respiratory rate	Blood pressure	Oxygen saturation	Body temperature	Reference
Tamandua tetradactyla *n* = 10	Ketamine (19.7±1.3) + Xylazine (1±0.1)	Light anesthesia (*n* = 3) and complete anesthesia (*n* = 7)	Good (*n* = 4) and excellent (*n* = 6)	Not assessed	Higher initial rates; decreased over time	No alteration	Not assessed	Not assessed	Higher; decreased over time	34
Bradypus tridactylus *n* = 6	[Chloralose (50)]	Not assessed	Not assessed	Not assessed	Increased over time	Not assessed	Decreased over time	Not assessed	Not assessed	29

Doses are encompassed by "()" and are presented in mg/kg unless otherwise noted. Missing doses means they were not reported by the studies. Values separated by "±" stands for "mean±SD". Drugs separated by "&" are a single mixture. Antagonists are separated from anesthetics by "|". Administration route is intramuscular unless encompassed by "[]" for intravenous, "{}" for inhalant, and "<>" for local. References' numbers are correspondent to Apêndice X.

The combination of butorphanol with an a2-A (xylazine or detomidine) and midazolam (8 studies), with or without the addition of ketamine, produced oscillating heart rate and oxygen saturation during the first 20 mins after injection (1; 12.5%) and normal and constant values after 20 mins (7; 87.5%). Respiratory rate presented no alteration (1; 12.5%) or prolonged and initial transitory apnea, with normal and constant values after 20 mins from the injection (7; 87.5%). Blood pressure was not assessed (8; 100%). Body temperature presented no alteration (1; 12.5%) or normal and constant values after 20 mins from the injection (7; 87.5%). When isoflurane was used instead of ketamine (1 study), it presented no alteration in heart rate, bradypnea, and possible hypotension, while oxygen saturation and body temperature were not assessed.

The combination of tiletamine with zolazepam (4 studies) produced no alteration (2; 50%) for heart rate, a decrease over time (2; 50%), and a higher initial rate when compared to other protocol (ketamine-medetomidine) (1; 25%), and bradycardia (1; 25%). Initial and middle respiratory rate was lower when compared to other protocols (2; 50%) (ketamine-xylazine and ketamine-medetomidine), and irregular breathing (1; 25%), bradypnea (1; 25%), apnea (1; 25%) and tachypnea (2; 50%) were presented. Blood pressure was not assessed (4; 100%). Oxygen saturation presented hypoxemia and lower mean values when compared to other protocols (1; 25%) (ketamine-acepromazine, ketamine-xylazine, and ketamine-medetomidine). It was not assessed or only assessed for too few individuals in the other studies (3; 75%). Body temperature had no alteration (1; 25%) or decreased over time (2; 50%), and initial hypothermia (1; 25%). Body temperature was not reported in one study (1; 25%).

Ketamine with acepromazine (1 study) presented no alteration for heart rates, highest mean respiratory rates when compared to other protocols (ketamine-xylazine, tiletamine-zolazepam, and ketamine-medetomidine) and tachypnea, and a decrease over time for body temperature, while blood pressure and oxygen saturation were not measured. For ketamine with acepromazine, diazepam, buprenorphine, and isoflurane (1 study) bradycardia was presented, light hypoxemia with higher mean values from 10 to 70 mins after injection, and no alteration for respiratory rate and body temperature, while blood pressure was not assessed. Chloralose (1 study) alone presented an increase over time for heart rate and a decrease over time for blood pressure, while respiratory rate, oxygen saturation, and body temperature were not assessed. Physiological

parameters for ketamine alone (1 study) and butorphanol with isoflurane (1 study) were either not reported or not assessed.

5.3.2 Unevaluated anesthetic protocols

Across the 99 reports that implemented but did not evaluate anesthetic protocol or were case-reports, a total of 128 anesthetic protocols were applied with 46 different drug combinations (Table 1).

Considering how drugs separately were present across the 128 anesthetic protocols, ketamine was the most prevalent (79; 61.7%), followed by midazolam (42; 32.8%), isoflurane (33; 25.8%), zolazepam (26; 20.3%), tiletamine (25; 19.5%), xylazine (24; 18.8%), medetomidine (12; 9.4%), butorphanol (11; 8.6%), acepromazine (9; 7%), detomidine (9; 7%), propofol (8; 6.3%), dexmedetomidine (6; 4.7%), morphine (6; 4.7%), pentobarbital (3; 2.3%), fentanyl (2; 1.6%), sevoflurane (2; 1.6%), alfaxalone (1; 0.8%), chloralose (1; 0.8%), droperidol (1; 0.8%), halothane (1; 0.8%), methadone (1; 0.8%) and nitrous oxide (1; 0.8%).

A cyclohexamine (ketamine or tiletamine) paired with a benzodiazepine (midazolam or zolazepam) was the most prevalent (58; 45.3%) combination, without (28; 21.9%) or with the addition of a general anesthetic (isoflurane, sevoflurane or propofol) (11; 8.6%), a2-A (detomidine, dexmedetomidine, medetomidine or xylazine) (9; 7%), opioid (butorphanol, fentanyl, methadone or morphine) (2; 1.6%), or a mix of them all (8; 6.3%). The combination of a cyclohexamine with an a2-A was the second most common (42; 32.8%), without (19; 14.8%), or with the addition of a general anesthetic (9; 7%). Cyclohexamine combined only with a general anesthetic was presented twice (1.6%) and once (0.8%) with the addition of an opioid. The combination of cyclohexamine with a phenothiazine (acepromazine) was the third most common (9; 7%). Cyclohexamine was also implemented with a barbiturate (pentobarbital) (2; 1.6%) or alone for ketamine (3; 2.3%). General anesthetics were vastly implemented (40; 31.3%) but used alone in only eight reports (6.3%), and with the addition of an opioid in two reports (1.6%). The combination of a benzodiazepine, an alpha2-agonist, and an opioid was presented seven times (5.5%). The combination of alfaxalone with midazolam (0.8%), fentanyl with droperidol (0.8%) or the use alone of

chloralose (0.8%), pentobarbital (0.8%), xylazine (0.8%) or zolazepam (0.8%) were all reported once.

5.4 Discussion

The combination of a cyclohexamine with either or both a2-A and benzodiazepine was widely implemented and considered safe and effective by the reports that evaluated the combinations (FOURNIER-CHAMBRILLON et al., 2000; FOURNIER-CHAMBRILLON; FOURNIER; VIÉ, 1997; GASPAROTTO et al., 2017; HANLEY et al., 2008; HERNANDEZ et al., 2010; KINNEY et al., 2013; LESCANO G. et al., 2014; MORENO, 2011; OROZCO, 2011; ROJAS; BERMÚDEZ; ENCISO, 2013; VOGEL; DE THOISY; VIÉ, 1998), from a very low to moderate quality of evidence. Ketamine is a cyclohexamine that induces dissociative anesthesia, acting mainly as a noncompetitive antagonism of the N-methyl D-aspartic acid (NMDA) receptor, with most noteworthy effects as an increase in heart rate and blood pressure, catalepsy, and somatic analgesia. Alpha2-adrenoreceptor agonists reduce activity on the central and peripheral nervous system through hyperpolarization of neurons and inhibiting norepinephrine release on presynaptic terminals, resulting in dose-dependent sedation, muscle relaxation, and analgesia, but with a decrease in heart rate and blood pressure and disruption of thermoregulation. Other undesirable effects includes respiratory depression, hypoxemia, and transitory initial hypertension, which are related to the drug's lack of specificity over receptors and/or administration route. Among the a2-A implemented in the reports, xylazine is the less selective, while dexmedetomidine is the most selective, producing better immobilization with fewer side effects. Benzodiazepine induces postsynaptic hyperpolarization through enhancement of the γ-Aminobutyric acid (GABA) receptors, leading to sedation and muscle relaxation with minimal cardiorespiratory depression, and anxiolytic and anticonvulsant benefits. Ketamine combined with an a2-A has been widely implemented for wild mammal anesthesia. They synergistically produce good immobilization with reduced required dosage, and the side effects of one drug are counterbalanced by the other to some extent (GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020; JALANKA; ROEKEN, 1990; KLEIN; KLIDE, 1989). Expected qualities of this combination were overall met for Xenarthra's anesthesia, such as adequate immobilization, short

induction, and fast recovery. On the other hand, cardiovascular, respiratory and body temperature depression were observed across studies (FOURNIER-CHAMBRILLON et al., 2000; FOURNIER-CHAMBRILLON; FOURNIER; VIÉ, 1997; HANLEY et al., 2008; HERNANDEZ et al., 2010; KINNEY et al., 2013; LESCANO G. et al., 2014; OROZCO, 2011; VOGEL; DE THOISY; VIÉ, 1998). This result may be especially relevant for Xenarthrans due to their physiological particularities – low metabolic rate, sensitive blood pressure, and imperfect homeothermy, with lower body temperature and unusual oxygen consumption (ATTIAS *et al.*, 2018; CLIFFE *et al.*, 2018; DUARTE *et al.*, 2007; DUARTE; DA COSTA; HUGGINS, 1982; GILMORE; DA-COSTA; DUARTE, 2000; GIROUX *et al.*, 2022; MACCARINI *et al.*, 2015). Ideally, the addition of a benzodiazepine should allow a reduction of the required doses of ketamine and a2-A, consequently reducing side effects as well (GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020; KLEIN; KLIDE, 1989). However, similar results were found when a benzodiazepine, specifically midazolam, was added to the ketamine and a2-A protocol (LESCANO G. *et al.*, 2014; MORENO, 2011; ROJAS; BERMÚDEZ; ENCISO, 2013). In the study where atropine was used with the previous combination, no cardiac depression was reported (GASPAROTTO *et al.*, 2017). Atropine is an anticholinergic that, among other effects, causes an increase in heart rate, therefore, possibly correcting bradycardia induced by anesthetics (GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020). For the combination of a cyclohexamine and benzodiazepine, specifically tiletamine with zolazepam was evaluated (FOURNIER-CHAMBRILLON et al., 2000; OROZCO, 2011; VOGEL; DE THOISY; VIÉ, 1998). One advantage of this combination is that it is commercialized as a pre-ready mixture, but is often associated with longer immobilization and, consequently, recovery times (GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020). In general, it produced less consistent anesthesia quality (sedation and muscle relaxation) for Xenarthras compared to ketamine and a2-A protocols, especially regarding the most modern a2-A such as medetomidine and dexmedetomidine. Proportionally, tiletamine with zolazepam had less cardiac and thermoregulation depression, but more respiratory side effects, while blood pressure and oxygen saturation were mostly not assessed (FOURNIER-CHAMBRILLON et al., 2000; OROZCO, 2011; VOGEL; DE THOISY; VIÉ, 1998). For this combination,

antagonizing the benzodiazepine was not studied, unlike ketamine with an a2-A where the antagonist was often studied (FOURNIER-CHAMBRILLON et al., 2000; HANLEY et al., 2008; HERNANDEZ et al., 2010; KINNEY et al., 2013; OROZCO, 2011; VOGEL; DE THOISY; VIÉ, 1998). The use of a benzodiazepine antagonist should aid reducing the associated prolonged recovery (GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020; KLEIN; KLIDE, 1989). The combination of ketamine with only midazolam was not evaluated, although it has been implemented.

Neither cyclohexamines, a2-A, nor benzodiazepines are recommended to be implemented alone for wildlife anesthesia due to their side effects and unreliable immobilization (CHINNADURAI *et al.*, 2016; GRIMM *et al.*, 2015; JALANKA; ROEKEN, 1990; KLEIN; KLIDE, 1989). For Xenarthra's anesthesia, ketamine alone was, as expected, considered unsafe and ineffective (HERNANDEZ *et al.*, 2010), with very low quality of evidence. While a2-A or benzodiazepines alone have not been evaluated, there was a report for each implementing them as such.

Ketamine with acepromazine was also considered unsafe and ineffective by the report that evaluated the combination (VOGEL; DE THOISY; VIÉ, 1998), with low quality of evidence, although it was a common combination for reports that did not evaluate the anesthetic protocol. Acepromazine is a phenothiazine that acts in multiple receptors, primarely blocking dopamine ones, resulting in sedation and muscle relaxation (GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020). However, both anesthetic aspects were considered insufficient for this combination. Similar to a2-A, vascular and thermoregulation depression may be important factors to consider especially for Xenarthra's anesthesia. In the study, the combination of ketamine and acepromazine was compared against tiletamine with zolazepam and ketamine with an a2-A. It was the only combination that didn't produce any cardiac alteration but had marked tachypnea, with the highest respiratory rates. All of them had a similar decrease in body temperature. No combination had blood pressure measured, and only ketamine with acepromazine did not have oxygen saturation measured (VOGEL; DE THOISY; VIÉ, 1998). Noteworthy, a general disadvantage of acepromazine when compared to a2-A, benzodiazepines, or opioids, is that it has no available antagonist (GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020).

Opioid drugs can be classified as full agonist, partial agonist, antagonist, and agonist-antagonist according to how they interact with the opioid receptors delta (DOP), kappa (KOP), and mu (MOP). Ultimately, the agonism of an opioid receptor will prevent nociceptive transmission (analgesia), but with other effects depending on the receptor. Effects can also vary across species and populations. The antagonist, however, does not produce a functional response, but rather blocks competitively the agonism of the receptor (GRIMM *et al.*, 2015). Butorphanol was the most common opioid implemented across reports. It acts as an antagonist to partial agonist of MOP and agonist of KOP, producing mild analgesia, with lesser sedation and respiratory depression (GRIMM *et al.*, 2015). The combination of butorphanol, detomidine, and midazolam was considered safe and effective (KLUYBER *et al.*, 2020, 2021), with low quality of evidence. For Xenarthrans, the combination interfered unclearly with cardiac, respiratory, oxygen saturation, and thermoregulation during the first 20 mins after the drug's injection, while blood pressure was not assessed. Adequate immobilization was achieved. The analgesic and sedative effect of opioids are enhanced when combined with an a2-A, however, so is the cardiovascular and thermoregulatory disruption (CHABOT-DORÉ *et al.*, 2015; GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020). One advantage of the combination of an opioid, a2-A, and benzodiazepine is that all three drugs have respective antagonists that can be administered to revert the anesthesia partially or fully at any necessary moment (KLUYBER *et al.*, 2020, 2021). There were no differences in physiological parameters when ketamine was administered 20 mins after the initial injection to prolong anesthesia during surgery, but recovery was lengthier (KLUYBER *et al.*, 2020). The combination of ketamine, medetomidine, and butorphanol was also considered safe and effective (HERNANDEZ *et al.*, 2010), with very low quality of evidence. Although adequate immobilization was also achieved, physiological parameters were either not assessed or not reported.

General anesthetics, such as propofol and inhalants, were present in about a third of the implemented but unevaluated anesthesia, although inhalant anesthetics were only studied in three reports (CARREGARO; GERARDI; HONSHO, 2009; FALZONE *et al.*, 2013; HERNANDEZ *et al.*, 2010). All three resulted in very low quality of evidence. Isoflurane was the inhalant anesthetic implemented across them. The major

advantages of inhalant anesthetics are the adjustable dose-related anesthesia depth and fast recovery after ceasing the anesthetic inflow. On the other hand, respiratory and cardiovascular depression are associated (GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020). Due to Xenarthra's anatomy, it's considered impractical to intubate them for inhalant anesthesia (BRAINARD *et al.*, 2008; FARO *et al.*, 2015), therefore facemasks have been used (CARREGARO; GERARDI; HONSHO, 2009; FALZONE *et al.*, 2013; HERNANDEZ *et al.*, 2010). A potential concern with this method is the lack of mechanical ventilation for a taxon that can undergo apnea for long periods (KLUYBER *et al.*, 2020, 2021; LESCANO G. *et al.*, 2014; WEST; CARTER; SHAW, 2014), jeopardizing the quality of anesthesia and the patient's safety. Inhalant anesthesia can also be hard to implement in field conditions due to the necessary equipment (CHINNADURAI *et al.*, 2016). For the three reports that evaluated inhalant anesthetics, one used captive animals (FALZONE *et al.*, 2013) and the other two used wild animals but performed the anesthesia inside facilities (CARREGARO; GERARDI; HONSHO, 2009; HERNANDEZ *et al.*, 2010). All three reports used facemasks. In one study, butorphanol alone did not provide adequate induction as premedication to allow maintenance with isoflurane. Therefore, the combination of butorphanol and isoflurane was considered unsafe and ineffective (HERNANDEZ *et al.*, 2010). Physiological parameters were either not reported or not assessed. The combination of butorphanol, xylazine, midazolam, and isoflurane was considered safe and effective (FALZONE *et al.*, 2013). The concomitant use of such drugs can reduce the necessary dose (minimum alveolar concentration – MAC) of isoflurane and cover its lack of analgesia for noxious procedures. However, they also potentiate the cardiovascular, respiratory, and thermoregulatory depression (GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020). In fact, two of the three anesthetized animals with this combination required partial reversion of xylazine due to bradypnea. No cardiac side effects were noted, but possible hypertension occurred, while other physiological parameters were not assessed. The combination of ketamine, acepromazine, diazepam, buprenorphine, and isoflurane was also considered safe and effective (CARREGARO; GERARDI; HONSHO, 2009). Buprenorphine is a partial MOP agonist opioid, that produces a less potent analgesia, but also less cardiovascular and respiratory depression (GRIMM *et al.*, 2015). In this case, the quality of anesthesia

was subjectively considered adequate, with no respiratory or thermoregulatory effects. However, bradycardia and light hypoxemia were described. A balanced anesthesia combines multiple drugs, aiming to reduce the required dosage of each, while covering unconsciousness, muscle relaxation, and analgesia with minimal side effects (CHINNADURAI *et al.*, 2016; GRIMM *et al.*, 2015; HAWKINS; GUZMAN; PAUL-MURPHY, 2020). Chloralose was also evaluated as a general anesthetic, which was considered safe and effective (DUARTE *et al.*, 1989), with very low quality of evidence. However, in that study, anesthesia was evaluated only regarding cardiovascular effects and its response when a hypotensive agent was administered. Chloralose is mostly used as long-lasting anesthesia for laboratory animals and is currently unwarranted for other uses (GRIMM *et al.*, 2015).

A limitation of this study was the unvalidated method to determine quality of evidence, as the application of the GRADE system had to be adapted. Further studies are encouraged to better understand the effect of anesthetic protocols on Xenarthrans. These should preferably be performed and reported clearly, utilizing standardized evaluation methods of all relevant information – physiological parameters (especially those likely to be affected by the administered drugs), time intervals (induction, maintenance, and recovery) and quality of anesthesia (sedation, muscle relaxation, and analgesia). To improve the quality of evidence, risk of bias should be avoided as much as possible (SCHÜNEMANN *et al.*, 2013). A comprehensible limitation to determine safety for Xenarthra's anesthesia is the lack of normal (unrestrained) physiological values across species. Utilizing unanesthetized control groups can pose challenge and dangerous to both animals and researches (CHINNADURAI *et al.*, 2016; GRIMM *et al.*, 2015). Hanley and collaborators (2008) collected heart and respiratory rates during physical restraint before administering anesthetics, however, collected information occurs during stressful stimulation that can bias the results and negatively interfere with the anesthesia. Finally, there was also a large number of reports that used Xenarthras under anesthesia but lacked corresponding published data on the anesthetic effects, meaning an underutilization or absence of adequate collected data.

5.5 Conclusion

Considering the results of this systematic review, protocols that included combinations of cyclohexamines, alpha2-adrenoreceptor agonist, benzodiazepines, opioids, and/or inhalant anesthetics were considered safe and effective for Xenarthra's anesthesia. The combination of ketamine with an alpha2-adrenoreceptor agonist was the most substantiated, yet it presented cardiovascular, respiratory, and thermoregulatory depression. Ketamine alone, ketamine with acepromazine, and butorphanol with isoflurane were considered unsafe and ineffective. No study provided high quality of evidence. Other drugs (alfaxalone, droperidol, fentanyl, halothane, methadone, morphine, nitrous oxide, pentobarbital, and propofol) have been implemented, but their anesthetic effects have not yet been evaluated or published.

6 ARTIGO 2

FIRST REPORT OF CHEMICAL IMMOBILIZATION AND PHYSIOLOGICAL PARAMETERS FOR FREE-RANGING MANED SLOTH (*BRADYPUS TORQUATUS*), USING A COMBINATION OF KETAMINE AND MEDETOMIDINE

(Aceito, em processo de publicação: *Journal of Zoo and Wildlife Medicine*)

FIRST REPORT OF CHEMICAL IMMOBILIZATION AND PHYSIOLOGICAL PARAMETERS FOR FREE-RANGING MANED SLOTH (*BRADYPUS TORQUATUS*), USING A COMBINATION OF KETAMINE AND MEDETOMIDINE

RESUMO

A preguiça-de-coleira (*Bradypus torquatus*) é uma espécie endêmica de dois estados brasileiros e ameaçada de extinção, com muitos aspectos biológicos desconhecidos que são necessários para guiar ações conservacionistas. Existem estudos anestésicos para outras espécies de preguiças, no entanto, não existem estudos para a preguiça-de-coleira. Informações anestésicas foram registradas de 12 preguiças-de-coleira de vida livre, imobilizadas a campo para coleta de dados. Indivíduos foram anestesiados com uma combinação de cetamina (4.0 mg/kg) e medetomidina (0.03 mg/kg), antagonizada com atipamezol (0.1 mg/kg). Tempo para indução e recuperação foram coletados e comparados entre sexo e faixa etária. Após a indução e até a administração do antagonista, foram registrados os parâmetros fisiológicos (frequência cardíaca, frequência respiratória, saturação de oxigênio e temperatura retal) a cada 10 minutos de anestesia, e estatisticamente avaliados no tempo. A indução foi rápida (3.21±0.76), mas a recuperação foi longa (113.3±18) quando comparada outros estudos. Não houve diferença nos tempos de indução e recuperação em relação ao sexo e faixa etária. Frequência cardíaca, saturação de oxigênio e temperatura retal mantiveram-se estáveis durante o procedimento. A frequência respiratória diminuiu ao longo do tempo de 18.25±7.03 para 13.17±3.66 movimentos por minuto. Nossos resultados indicam que a combinação descrita de cetamina e medetomidina é uma escolha segura e eficaz para a anestesia de preguiças-de-coleira.

Palavras-chave: preguiça-de-coleira; vida-livre; anestesia; cetamina; medetomidina.

Abstract The maned sloth (*Bradypus torquatus*) is an endemic and endangered species of two Brazilian states, with much unknown biological information needed to direct conservation actions. Other sloth species have been studied regarding anesthesia; however, there is a lack of anesthesia research for the maned sloth. Anesthetic data was collected from 12 free-ranged maned sloths that were immobilized for a field examination. Individuals were anesthetized using a combination of ketamine (4.0 mg/kg) and medetomidine (0.03 mg/kg) and antagonized with atipamezole (0.1 mg/kg). Time to induction and recovery were recorded and compared with sex and age classes. After the induction and until antagonist administration, physiological parameters (rectal temperature, heart rate, respiratory rate, and oxygen saturation) were recorded every 10 minutes during anesthesia and were statistically evaluated over time. Induction was fast (3.21±0.76), but recovery was longer (113.3±18) when compared to other studies. Induction and recovery times were not different across sex or age classes. Rectal temperature, heart rate, and oxygen saturation remained stable throughout the procedure. Respiratory rate significantly decreased over time, from 18.25±7.03 to 13.17±3.66 movements per minute. Our results indicate that the described combination of ketamine and medetomidine is a safe and effective choice for anesthesia of maned sloths.

6.1 Introduction

The genus Bradypus contains two subgenus of three-toed sloths: Bradypus, comprising three species (*B. variegatus*, *B. tridactylus*, and *B. pygmaeus*), and Scaeopus comprising two species (*B. torquatus* and *B. crinitus*) (MIRANDA *et al.*, 2022). The maned sloth (*B. torquatus* Illiger, 1811) is endemic to the Atlantic Forest - a threatened biome and hotspot of biodiversity (REZENDE *et al*., 2018). The population distribution of the species was considered limited to four Brazilian states (DE OLIVEIRA MOREIRA *et al*., 2014), but a recent taxonomic review showed that the distribution is smaller. The *B. torquatus* species occupies only the Sergipe and Bahia states, while *B. crinitus* (Gray, 1850) occupies the Espírito Santo and Rio de Janeiro states (MIRANDA *et al*., 2022). The latest assessment, made before the taxonomic review, considered the species vulnerable and with a lack of necessary biological information to direct conservation actions (CHIARELLO A *et al*., 2022).

To collect biological information, the capture of wild individuals is often necessary and anesthesia can provide a safe operating procedure for both animals and researchers (CHINNADURAI *et al.*, 2016). Previous studies revealed that unanesthetized *B. variegatus* can present blood pressure and heart rate changes due to the approximation of humans without necessarily exhibiting behavioral changes. This physiological response was more prominent if the animal was manually restrained for longer periods (DUARTE *et al*., 2007; DUARTE; DA COSTA; HUGGINS, 1982; GILMORE; DA-COSTA; DUARTE, 2000). Additionally, the species' blood pressure is highly sensitive to catecholamines (DUARTE *et al*., 1987). Another noteworthy characteristic of the genus Bradypus is that the environmental temperature influences its body temperature, considered low when compared to other mammals. The genus also have a low metabolic rate and the body temperature regulates the activity budget (CHIARELLO, 1998; CLIFFE *et al*., 2018; GILMORE; DA-COSTA; DUARTE, 2000; GINÉ *et al*., 2015). Therefore, these physiological aspects should be considered when restraining the species.

There are a few studies addressing the anesthesia of other sloth species (HANLEY et al., 2008; KINNEY et al., 2013; LESCANO G. et al., 2014; MORENO, 2011; VOGEL; DE THOISY; VIÉ, 1998), and the combination of ketamine with an alpha2-adrenergic receptor agonist (a2-A) has been indicated as the best option.

Commonly described side effects of this combination were bradycardia, bradypnea, and blood pressure variances. However, to the authors' knowledge, there are only two studies (BERNARDES *et al*., 2022; PINDER, 1993) that mention maned sloths being anesthetized and none addressed physiological effects. Therefore, the species lacks a literature-established anesthetic protocol. This study evaluates anesthetic data collected during a biological study (BERNARDES et al., 2022) to determine if the ketamine and medetomidine combination provides safe and effective field immobilization in maned sloths.

6.2 Material and methods

This study was carried out under the legal approval of the Sistema de Autorização e Informação em Biodiversidade (SISBIO) No. 67274-2, issued by Ministério do Meio Ambiente (MMA) through Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

The study took place at Reserva Ecológica da Sapiranga, located in the municipality of Mata de São João, Bahia, Brazil. Captures occurred from March to April 2020. Once a team member spotted an individual during an active search, a climber accessed the tree canopy to hand catch it. All claws were wrapped with tape to keep them closed during physical restraint. Thereafter, the animal was placed in a bag and lowered to the forest floor using a rope. Animals were weighed using a scale, measured from head to tail with a flexible tape, aged based on body mass and body length (infants <1 kg and <30 cm; juveniles 1-2.1 kg and 30-40 cm; sub-adults 1.7-4.7 kg and 41-59 cm; adults >4.4 kg and >59 cm), and sexed based on the pelage and genitalia (LARA-RUIZ; CHIARELLO, 2005).

A combination of ketamine (Vetnil, Louveira, São Paulo, 13294-100, Brazil; 100mg/ml; 4 mg/kg) and medetomidine (CP-Pharma, Burgdorf, Lower Saxony, 31303, Germany; 1 mg/ml; 0.03 mg/kg) (KM) was administered IM (semimembranosus or semitendinosus muscles) for chemical immobilization. Once the procedure was complete, atipamezole (Zoetis, Parsippany-Troy Hills, New Jersey, 07054, United States; 5 mg/ml; 0.1 mg/kg) was administered as antagonist, half the dose IV (cephalic vein) first and immediately after, the other half IM (vastus lateralis muscle). Induction time was considered from KM administration until the animal ceased responding to

external stimuli and was fully immobilized for manipulation. Time to initial arousal was considered from the antagonist administration until the animal presented the first voluntary movement. Recovery time was considered from the antagonist administration until the animal was able to move and grasp normally. After induction, rectal temperature (RT), heart rate (HR), respiratory rate (RR), and oxygen saturation (SPO2) were recorded every 10 minutes until antagonist administration. While anesthetized, biological samples were collected for another study. After recovery, animals were released at the base of the tree where they were captured.

To compare the induction and recovery times between sex and age classes (adult and subadult), the Mann-Whitney U tests were applied, one test for each analyzed time. Repeated-measures ANOVA were used to evaluate the variation of the physiological parameters measured throughout the procedure. For significant results, a post-hoc Bonferroni correction's test was applied to compare the pairwise variation. HR and RR's distribution residues did not attend the assumption of sphericity. Therefore, the Greenhouse-Geisser correction was used, and the degrees of freedom were recalculated accordingly. The residuals of the obtained SPO2 values did not follow a normal distribution, thus a Friedman's test was used for them. All analysis were conducted using the R statistical software.

6.3 Results

Twelve animals were included in this study (adult males = 4; adult females = 3; sub-adult females = 4; adult non-identified sex = 1). The mean body weights were 4.51±1.14 kg (adults 5.10±0.72 kg; sub-adults 3.47±1.01 kg). The mean final volume of the anesthetics was 0.316 ml (ketamine 0.18±0.045 ml; medetomidine 0.135±0.034 ml). In general, the protocol produced a light anesthesia depth, sufficient to carry over with the procedures. Animals maintained palpebral reflexes, partial muscle relaxation, and no response to external stimuli.

The mean values and SD for anesthetic times and physiological parameters are demonstrated in Table 5. For the Mann-Whitney U tests, two individuals were excluded from the analysis because one was not possible to be sexed and the other did not have a recovery time record (n = 10; adult males = 4; adult females = 2; sub-adult females = 4). The Mann-Whitney U tests demonstrated that there were no differences in the induction

and recovery times over sex (induction W = 12; $p = 0.98$ and recovery W = 8; $p = 0.44$) or age classes (induction W = 9.5; $p = 0.66$ and recovery W = 14.5; $p = 0.67$) (Figure 2). Sex and age classes combined were not considered because there was no animal in the sub-adult male category. The repeated-measures ANOVA demonstrated that there was a significant difference between the RR measured throughout the procedure ($F(2.02, 22.29) = 6.71$; $p < 0.01$). The post-hoc Student's t-test with Bonferroni correction demonstrated that the RR mean at 50 min was lower than the RR mean at 20 ($p_{20;50} < 0.01$) and 30 min ($p_{30;50} < 0.01$), and the RR mean at 40 min was lower than the RR mean at 20 min ($p_{20;40} < 0.05$). There was no significant change in time for HR, RT, and SPO2 (Figure 3), although SPO2 was only measured on 9 of 12 animals due to technical issues.

Table 5 – Anesthetic time (n = 10) and physiological parameters (n = 12) (mean±SD) for the anesthesia of maned sloths (*Bradypus torquatus*), with the combination of ketamine and medetomidine and antagonized with atipamezole.

Anesthetic intervals	Parameters	Mean	±SD	Mann-Whitney U tests						rmANOVA test		Friedman test
				Sex classes (males = 4; females = 6)	Median (±IQR)		Age classes (adult = 6; subadult = 4)	Median (±IQR)			post-hoc Student's t-test with Bonferroni correction	
					Female	Male		Subadult	Adult			
Induction	Time (min)	3.21	0.76	W = 12; p = 0.98	3.1 (±0.49)	2.9 (±1.1)	W = 9.5; p = 0.66	3.12 (±0.26)	2.8 (±0.75)			
Maintenance	Heart rate (bpm)	58.04	7.74							F (1.47, 13.28) = 3.11; p = 0.0889		
	Respiratory rate (bpm)	16.42	4.53							F (2.02, 22.29) = 6.71; p = 0.0051		
	At 10 mins	18.25	7.03								a	
	At 20 mins	19	6.18								ab	
	At 30 mins	16.67	4.21								abc	
	At 40 mins	15	4.93								acd	
	At 50 mins	13.17	3.66								ad	
	Rectal temperature (ºC)	35	0.67							F (4, 40) = 0.56; p = 0.6955		
	Oxygen saturation (%)	97.67	1.69									X^2 (4) = 0.61538; p = 0.9613
	Time (min)	58	6.51									
Recovery	Time to initial arousal (min)	2.38	1.41									
	Time to full recovery (min)	113.3	18	W = 8; p = 0.44	108 (±12.5)	121 (±29)	W = 14.5; p = 0.67	108 (±22.5)	110 (±20.2)			

Applied statistical analysis are shown for each parameter and considered significantly different with a $p < 0.05$. Respiratory rates measured throughout the procedure are represented with letters (a, b, c, d) where values with the same letter are not significantly different. Induction time was considered from anesthetic administration until the animal ceased responding to external stimuli and was fully immobilized for manipulation. Maintenance time is considered the time length between the end of the induction phase and the injection of the antagonist. Recovery times are considered to begin after the injection of the antagonist.

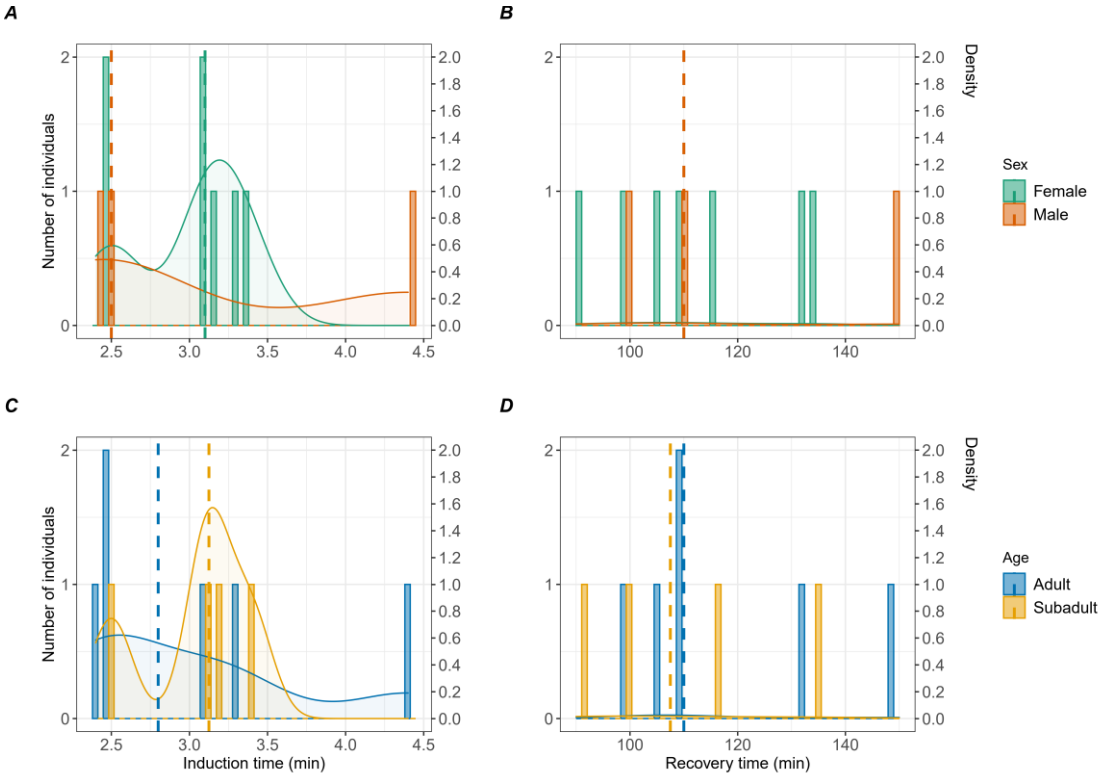

Figure 2 – Histograms of the Mann-Whitney U statistic comparing the induction and recovery times between sex and age classes for maned sloths (*Bradypus torquatus*; n = 10) immobilized with the combination of ketamine and medetomidine and antagonized with atipamezole. The dashed, vertical lines represent the median of each time, and the curves are the density related to each median.

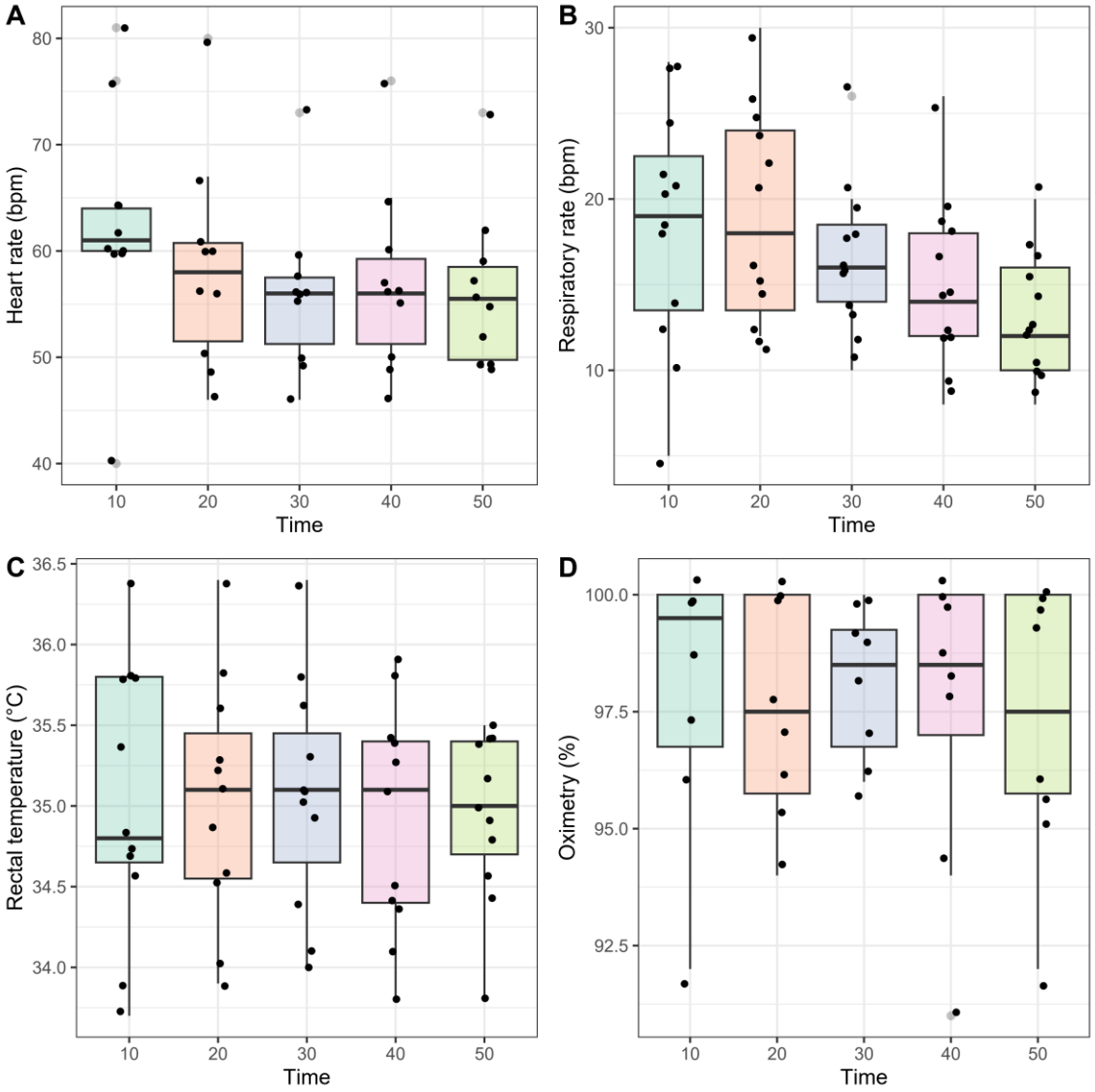

Figure 3 – Box and whisker plots of physiological parameters (heart rate, respiratory rate, rectal temperature, and oxygen saturation) measured every 10 mins throughout the anesthetic procedure of maned sloths (*Bradypus torquatus*; n = 12) with the combination of ketamine and medetomidine and antagonized with atipamezole.

6.4 Discussion

Induction was smooth for all animals and faster on average (3.21±0.8 min) than previously reported in a KM study in sloths (4.13±1.35 min). While recovery was smooth and time to initial arousal was faster (2.38±1.41 compared to 4.5±1.8 min), time to full recovery was longer (113.3±18 compared to 10±7.9 min) (VOGEL; DE THOISY; VIÉ, 1998). Since age and sex did not interfere with recovery time in our study, it is assumed that this discrepancy was due to differences in evaluation methodology, environment, and target species biology.

Physiological parameters, except for the RR, remained stable throughout the procedure. Due to the lack of known normal physiologic parameters of unanesthetized and unrestrained maned sloths, values of other species (HANLEY *et al*., 2008; KINNEY *et al*., 2013; LESCANO G. *et al*., 2014; MORENO, 2011; VOGEL; DE THOISY; VIÉ, 1998) were used to compare to the data in this study.

The RT mean (35±0.67 ºC) was within expected for sloth species (32.7 to 35.5 ºC) (GILMORE; DA-COSTA; DUARTE, 2000), although higher than other studies (varying from 31.8±0.8 to 34.72±1.43) (HANLEY *et al*., 2008; LESCANO G. *et al*., 2014; MORENO, 2011). In a study with *C. didactylus*, RT decreased over time (0.5 to 1.3 ºC) regardless of the anesthetic combination used, but initial RT varied between 32 to 38º C (VOGEL; DE THOISY; VIÉ, 1998). Sloths have unique metabolic changes to control body temperature according to the environment temperature (CLIFFE *et al.*, 2018; GILMORE; DA-COSTA; DUARTE, 2000; GINÉ *et al.*, 2015) and a2-A are known to interfere with the thermogenesis in homeotherms, which both could explain the difference across findings.

The HR mean (58±7.74 bpm) was similar to previously found across sloth species under anesthesia (varying from 46±13 to 79±21 bpm) (HANLEY *et al*., 2008; KINNEY *et al*., 2013; LESCANO G. *et al*., 2014; MORENO, 2011; VOGEL; DE THOISY; VIÉ, 1998), which could be considered bradycardic when compared with unanesthetized values (from 60 to 130 bpm) (GILMORE; DA-COSTA; DUARTE, 2000; HANLEY *et al.*, 2008). The same has been found in previous studies with a combination of ketamine (10 mg/kg) and acepromazine (0.1 mg/kg) (VOGEL; DE THOISY; VIÉ, 1998); ketamine (2±0,01 and 2,26±0,53 mg/kg) and dexmedetomidine (0,011±0,002 and 0,012±0,003 mg/kg) (KINNEY *et al*., 2013); and ketamine

(2,67±0,25 mg/kg), dexmedetomidine (0,012±0,004 mg/kg), and midazolam (0.1 mg/kg) (MORENO, 2011). Nevertheless, the ketamine with dexmedetomidine studies used a shorter evaluation period (25 and 30 min, respectively). Previous studies using a KM protocol and longer evaluation periods found a decrease in the HR mean over time, but doses were different (ketamine 2.5 mg/kg and medetomidine 0.02 mg/kg (HANLEY *et al*., 2008); ketamine 3 mg/kg and medetomidine 0.04 mg/kg (VOGEL; DE THOISY; VIÉ, 1998). Therefore, it implies that the doses proportion in the present study was more synergetic, where ketamine balanced the heart depression caused by the a2-A association.

The RR mean (16.42±4.53 bpm) decreased over time and was higher than previous studies with anesthetized sloths (varying from 6.4±2.3 to 14±8 bpm) (HANLEY *et al*., 2008; KINNEY *et al*., 2013; LESCANO G. *et al*., 2014; MORENO, 2011; VOGEL; DE THOISY; VIÉ, 1998). The first assessment (at 10 min) presented high variability (18.25±7.03 bpm) that did not differ from any other time. Similar variability has been previously reported (VOGEL; DE THOISY; VIÉ, 1998). Thereafter, the mean value stabilizes (at 20 min, 19±6.18 bpm) and significantly reduces onwards (at 50 min, 13.17±3.66). The opposite has been reported for KM combination, with a pre-anesthesia peak (35.7±19 bpm) followed by a decrease (at 10 min, 7.7±4.1) and then increasing over time again (at 45 min, 9.3±4.6) (HANLEY *et al*., 2008). Another study found no change over time for KM and other protocols (ketamine and acepromazine; ketamine and xylazine; tiletamine and zolazepam), but KM presented lower RR mean among them (from 14±8 to 11.5±5 bpm). However, KM was the only combination to not induce clinical respiratory complications (VOGEL; DE THOISY; VIÉ, 1998). The initial RR mean in the present study could be considered tachypneic, mostly due to stress from physical restraint time, reaching normal or close to normal values towards the end (GILMORE; DA-COSTA; DUARTE, 2000; HANLEY *et al.*, 2008). Alternatively, assuming that the values were normal from the beginning and then decreased to bradypneic, it could be due to the effect of ketamine wearing off and consequently allowing the negative effects of the a2-A.

Considering the lack of clinical signs and the maintenance of subjective safe levels of SPO2 (97.67±1.69 %), it implies that the RR decrease did not cause any health issues for the individuals in the present study. However, a more thorough investigation

is suggested as SPO2 alone does not necessarily indicate good tissue perfusion. It can also be inquired if the RR decrease could be due to metabolic adaptations to maintain the adequate body temperatures, since sloths have unique metabolic consumption of O2 for that purpose (CLIFFE *et al.*, 2018). Therefore, blood pressure, external temperature measurement and hemogasometry could help further explain the results herein. Additionally, the effect of capture and physical immobilization until anesthetics administration should be considered in future, as the increase in catecholamines from stress can interfere in the anesthetic process, especially when using an a2-A.

6.5 Conclusion

The combination of ketamine (4 mg/kg) and medetomidine (0,03 mg/kg) provided an efficient chemical immobilization of maned sloths, allowing relative safe clinical examination and biological sample collection. Extrapolating from other sloth species' physiological parameters, slight bradycardia and tachypnea may have been present, with RR decreasing over time, but without any clinical implication or need of intervention. Induction time was fast, but total recovery was long even with the use of atipamezole as antagonist, likely due to the species' metabolism. Both mentioned times were independent from sex and age classes.

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Apêndice A – Lista de referências em ordem numérica dos 115 relatos que implementaram anestesia em Xenarthras entre 1982 e 2022.

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