Physiological effects of high and low medetomidine doses in combination with zolazepam-tiletamine for the immobilisation and anaesthesia of spotted hyenas *(Crocuta crocuta)*

By

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Submitted in fulfilment of the requirements for the degree of Master of Sciences (Veterinary Science) in the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria

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Declaration of originality

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Contents:

List of tables:

List of figures:

List of images:

Image 2: ECG pads placed on front paw-pads of immobilised spotted hyena.

Image 3: Immobilised hyena showing the setup of the spirometer with the ET tube connection and pulse oximeter.

Image 4: Dr Tordiffe collecting an arterial blood sample from the medial saphenous artery of one of the immobilised spotted hyena.

Image 5: Recovery position of a spotted hyena in the night room after extubation.

List of abbreviations:

α_2-adrenoceptor	alpha two adrenoceptor
%	percentage
±	plus-minus
=	equal to
°C	degree(s) celsius
AV	atrioventricular
BEecf	base excess in the extracellular fluid
beats min^{-1}	beats per minute
breaths min^{-1}	breaths per minute
cAMP	adenosine 3',5'-cyclic monophosphate
DAP	diastolic arterial pressure
ECG	electrocardiogram
$EtCO_2$	end-tidal carbon dioxide tension
ET-tube	endotracheal tube
Exp	expiratory tidal volume
FiO_2	fractional inspired oxygen concentration
g	gram(s)
GABA	gamma-aminobutyric acid
GIT	gastrointestinal tract

glu	glucose
HD	high dose
h	hour
Hb	haemoglobin concentration
HCl	hydrochloride
HCO_3	bicarbonate
HR	heart rate
Ht	haematocrit
iCa	ionised calcium
IM	intramuscular
Insp	inspiratory tidal volume
IPPV	intermittent positive pressure ventilation
IU	international units
IV	intravenous
K	potassium
kg	kilogram(s)
KXA	ketamine-xylazine-atropine
L	litre(s)
LD	low dose
MAP	mean arterial pressure
mg	milligram(s)
min	minute(s)
mL	millilitre(s)
mmHg	millimetre(s) mercury
mmol	millimole(s)
n	number of animals
Na	sodium

NMDA	*N*-methyl-*D*-aspartate
NMB	neuromuscular block
$PaCO_2$	arterial carbon dioxide tension
pH	negative log of hydrogen ion concentration
$P(A\text{-}a)O_2$	alveolar-arterial oxygen tension gradient
PAO_2	alveolar partial pressure of oxygen
PaO_2	arterial oxygen tension
P_B	barometric pressure
PCO_2	partial pressure of carbon dioxide
P_{H2O}	saturated vapor pressure for water at 37°C
PO_2	partial pressure of oxygen
RQ	respiratory quotient
RR	respiratory rate
SA	sinoatrial
SaO_2	haemoglobin oxygen saturation (in arterial blood calculated by i-STAT)
SAP	systolic
SC	subcutaneous
SD	standard deviation
SpO_2	peripheral oxygen haemoglobin saturation (in arterial blood, measured by pulse oximetry)
TCO_2	total carbon dioxide
yrs	years old

Summary:

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Co-promoter: Dr Roxanne Kate Buck

Department: Paraclinical Sciences

Degree: Master of Science (Veterinary Science)

Objective:

To compare the physiological effects of high and low dose medetomidine in combination with zolazepam-tiletamine for the immobilisation and anaesthesia of the spotted hyenas (*Crocuta crocuta*).

To identify a safe and effective medetomidine dose in combination with zolazepam-tiletamine in the immobilisation of the spotted hyenas (*Crocuta crocuta*).

Study design:

Prospective, blinded, randomised cross-over study

Animals:

Eight captive adult spotted hyenas, five males and three females, weighing a mean (± SD) of 65.2 (± 8.4) kg.

Material and methods:

All hyenas were immobilised on two separate occasions for at least 60 min with either a high (HD treatment – 0.04 mg kg^{-1}) or low dose (LD treatment – 0.02 mg kg^{-1}) of medetomidine in combination with zolazepam-tiletamine (1 mg kg^{-1}) administered via IM dart. Physiological variables and anaesthetic plane were recorded at 5-min intervals. Cardiorespiratory variables were monitored using a multiparameter monitor, spirometry, ECG, high definition oscillometer correlated with pulse oximeter and auscultated heart and respiratory rates. Rectal temperature was measured using a digital portable rectal thermometer. Two arterial blood samples were collected for blood gas analysis. The first arterial blood gas sample was collected 20 min after darting. Supplementary oxygen was then provided at 2 L min^{-1}. The second arterial blood gas sample was collected 50 min after darting. Once the 60-min anaesthetic period was completed, atipamezole was administered IM at five times the medetomidine dose and recovery was observed. Data are reported as mean ± SD; all variables were compared using two-way repeated measure ANOVA's and paired sample t-tests with post-HOC tests. Significance was determined at $p < 0.05$.

Results:

All hyenas were safely and successfully immobilised with time to recumbency of 4.2 (± 2.1) min and 7 (± 5.5) min for the HD and LD treatments, respectively. A stable cardiorespiratory function was maintained throughout anaesthesia. Blood oxygen saturation did decrease significantly after darting, indicating a mild to moderate hypoxaemia (SaO_2 range of 79-91%) possibly attributed to a ventilation-perfusion mismatch. End-tidal CO_2, base excess, partial pressure of

carbon dioxide and oxygen, bicarbonate and total carbon dioxide did significantly increase with oxygen supplementation, with pH decreasing slightly. Haemoglobin concentration and haematocrit significantly increased with the HD treatment, but remained within acceptable range for hyenas and other mammals. Glucose significantly increased slightly after drug administration. Ionised calcium increased slightly possibly with decreasing pH values. Heart rates were a mean of 42 and 40 beats min^{-1} for both HD and LD treatments, respectively. Both AV and SA blocks were observed, along with sustained hypertension during both treatments. MAP were a mean of 134.5 (± 25.7) mmHg and 123.5 (± 20.0) mmHg for HD and LD treatments, respectively. Cardiovascular effects did not differ between treatments. Vomition occurred during only one LD immobilisation (6%). Atipamezole, at five times the medetomidine dosage, successfully antagonised the effects of medetomidine in all hyenas. Time to standing was 26 min (± 15) and 26.4 min (± 23.1) for HD and LD treatments, respectively. Both treatments provided sufficient anaesthesia and calm recovery. Treatment HD provided more reliable and consistent results indicated by the small standard deviations for time to first signs of sedation, recumbency, handling, as well as time to sternal recumbency and standing during recovery.

Conclusions and clinical relevance:

Both low and high medetomidine doses, at 0.02 mg kg^{-1} and 0.04 mg kg^{-1} respectively, in combination with zolazepam-tiletamine at 1.0 mg kg^{-1} can provide adequate immobilisation and anaesthesia for routine field management and minor surgical procedures in the spotted hyena for at least 60-min duration. Minimal physiological differences were observed between the two treatments. Oxygen supplementation is advised with the use of this protocol in order to treat the hypoxaemia possibly caused by ventilation-perfusion mismatch. A decrease in heart rate was observed and was as low as 24 beats min^{-1}, mean 41 beats min^{-1} with an overall mean MAP of 129 mmHg. Therefore, cautious monitoring is suggested. All cardiovascular effects were expected, and within acceptable clinical range based on observations noted in other wildlife and domestic species and possibly related to medetomidine use. An adverse effect observed was

vomition which only occurred in 6% of the hyenas immobilised. All blood pH's were <7.35, most likely related to the strict carnivorous diet of the spotted hyena as well as the mild hypercapnia experienced. Atipamezole was found to effectively antagonise the effects of medetomidine at five times the dosage of medetomidine administered. The one advantage of using the low dose medetomidine treatment is the reduction in cost, which can be an important consideration in developing countries where these free-ranging animals inhabit.

Keywords: Hyena, *Crocuta crocuta*, medetomidine, hypoxaemia, immobilisation, zolazepam-tiletamine

Literature review

Many research studies have been conducted on spotted hyenas (*Crocuta crocuta*) (Hahn et al. 2007; Kruuk 1972; Seal et al 1970; Van Horn et al. 2004; Van Jaarsveld and Skinner 1992). Unfortunately, few have focussed solely on the anaesthesia of this species (Harthoorn 1976; Smuts 1973; Stander and Gasaway 1991; Van Jaarsveld et al. 1984; Van Jaarsveld 1988). Hyenas have often been selected for study due to their unique morphology, especially in terms of their reproductive organs and behavioural characteristics, but in order to work with these animals, they need to be safely and effectively restrained (Hahn et al. 2007). The forced restriction of movement of all or part of an animal can be either physical or chemical. Chemical restraint includes both immobilisation and anaesthesia. Chemical immobilisation is achieved using drugs that have a wide range of effects from light sedation to unconsciousness. Whereas general anaesthesia includes sedation, reversible loss of sensation and loss of consciousness. An animal that is anaesthetised is always immobilised, whereas, an immobilised animal may either be anaesthetised or not. Over the years many different anaesthetic and sedative protocols have been used to assist with essential research sample collection, population management, diagnostic imaging and for surgical procedures. It is common to use injectable anaesthetic drugs for immobilisations and quick procedures; and inhalation anaesthetic drugs as maintenance for longer surgical procedures.

Past and current practises in anaesthesia of the spotted hyena (*Crocuta Crocuta*).

Some of the injectable drug combinations that have been used for immobilisation and maintenance of anaesthesia are succinylcholine, ketamine-midazolam, zolazepam-tiletamine-medetomidine, ketamine-xylazine-atropine, zolazepam-tiletamine, phencyclidine-promazine and etorphine-xylazine combinations with halothane as maintenance (Ebedes 1973; Hahn et al. 2007; Harthoorn 1976; Kruuk 1972; Smuts 1973; Van Jaarsveld et al. 1984; Van Jaarsveld 1988).

Succinylcholine, a competitive depolarising neuromuscular blocking (NMB) drug, induces muscle paralysis and is not acceptable as a sole anaesthetic drug as it results in a conscious perception of pain and distress as well as paralysis of the respiratory muscles. Therefore, succinylcholine should not be used without concurrent anaesthetic drug administration or analgesia and ventilation support (Grimm et al. 2015). Previous studies have shown that succinylcholine resulted in recovery that was too rapid after immobilisation and had a high level of toxicity as overdoses were fatal to the spotted hyena (Kruuk 1972; Smuts 1973). Kruuk (1972) indicated that with the use of this drug, not all darted animals exhibited the effects and some still had the ability to move and bite and therefore had to be forcibly restrained.

Phencyclidine, a dissociative anaesthetic drug, has been described by Ebedes (1973) as the safest and most practical drug, at that time, that may be used for the immobilisation of carnivores, but side effects such as a rise in body temperature during warm days, hypersalivation, corneal desiccation, muscle spasticity and convulsions have been known to occur (Ebedes 1973). Phencyclidine in combination with promazine created a smooth induction period, but also caused hypersalivation, increased temperature and a prolonged recovery period (Seal et al. 1970).

Van Jaarsveld et al. (1984) investigated multiple immobilisation protocols on spotted hyenas including ketamine and xylazine immobilisation with intravenous administration of ketamine or Saffan or halothane inhalation anaesthesia for maintenance; and etorphine alone or in combination with xylazine for immobilisation with halothane inhalation anaesthesia for maintenance. The overall conclusion of this study was that the etorphine and xylazine combination was found to be the most effective for the immobilisation of wild spotted hyenas. Although ketamine does result in a smooth immobilisation, it also has a long induction period which increases the likelihood that the animal will not be easily located (Van Jaarsveld et al. 1984). Ketamine is also needed in large quantities therefore; the use of larger darts could lead

to tissue damage at the dart site. The quantities required for maintenance of anaesthesia were also found to be too costly (Van Jaarsveld et al. 1984). The one animal anaesthetised with saffan showed cyanosis and respiratory depression. The use of etorphine resulted in short induction times. When doses of etorphine were increased greater than 3 mg, the excitatory period was increased, and apnoea resulted. In one case, it was believed that etorphine was injected intravenously, as opposed to intramuscularly, which led to the animal developing cardiac failure (Van Jaarsveld et al. 1984).

Stander and Gasaway (1991) used a ketamine and xylazine mixture placed in bait in order to immobilise spotted hyenas. Tolazoline was used as an antagonist for xylazine. The drug protocol resulted in an effective and partially reversible immobilisation with much shorter recovery periods. The recovery period was reduced from a maximum of 3.5 h to 1.6 h in comparison to those reported in previous studies, such as in Smuts (1973) and Van Jaarsveld et al. (1984), using higher dosages of ketamine alone or in combination with xylazine.

Nearly 2000 anaesthetic procedures were carried out on hyenas in a research colony at the University of California, Berkeley (UCB) and no deaths had occurred (Hahn et al. 2007). One of the protocols, established more than 20 years ago at UCB for captive spotted hyenas, is a ketamine (4-6 mg kg^{-1}) –xylazine (1 mg kg^{-1}) –atropine (0.045 mg kg^{-1}) combination (KXA) (Hahn et al. 2007). Although, this protocol was effective for immobilisation, it also had many side effects. The drug combination induced vomition, poikilothermia, decreased ventricular myocardial contractility and a marked decrease in heart rate, reaching as low as 48 beats min^{-1}, which was attributed to the use of xylazine in the protocol. As has been known to occur in cats, ketamine caused the hyenas eyes to remain open which makes monitoring of anaesthetic depth more difficult. Hypersalivation has been known to occur with the use of this protocol. In addition, with this combination, lower weight animals frequently required sedatives such as diazepam to treat side effects believed to be induced by ketamine use such as extensor rigidity

and tonic-clonic movements or twitching. However, the use of diazepam was associated with respiratory depression, evident as periods of apnoea following bolus administration. Generalised seizure activity had also been observed with the use of this protocol (Hahn et al. 2007). Hyperthermia also occurred in some cases, possibly caused by a response to ketamine, due to an indirect increase of sympathetic tone, or a loss of thermoregulation due to the vasoconstrictive effects of xylazine, but this has not been proven (Hahn et al. 2007). Despite the many possible side effects, ketamine has also proven to offset the bradycardia that has been associated with xylazine (Hahn et al. 2007). Isoflurane at 1-2% was used as maintenance with the KXA immobilisation (Hahn et al. 2007). Yohimbine was always administered after the animals had been stabilised under isoflurane in order to prevent adverse effects brought about by the use of xylazine (Hahn et al. 2007). Atropine is a parasympatholytic anticholinergic agent which blocks the effect of acetylcholine at the muscarinic receptor (Clarke et al. 2014; Papich 2011). Atropine causes a decrease in respiratory and gastro-intestinal tract (GIT) secretions as well as a decrease in GIT motility, mydriasis, and an increase in heart rate due to an antivagal effect (Clarke et al. 2014; Papich 2011). This antivagal effect is the biggest reason for the use of atropine when xylazine is being used, as xylazine has been known to induce significant bradycardia when used (Clarke et al. 2014; Hahn et al. 2007;). However, atropine at high doses can cause an increase in myocardial oxygen demand as well as tachycardia (Papich 2011).

Commercially prepared drug combinations containing equal mixtures of zolazepam and tiletamine are available, such as Zoletil and Telazol, and commonly used in many carnivore species. In fact, the most commonly used drug combination for field immobilisation in hyenas at present is zolazepam-tiletamine with an estimated dosage of 5 mg kg^{-1} in captive spotted hyenas and up to 6.5 mg kg^{-1} in free-ranging hyenas (As reviewed by Hahn et al. 2007 in their communications with Kay Holekamp; Van Horn et al. 2004). Hahn et al. (2007) observed rough, prolonged recoveries when this protocol was used in the captive colonies and therefore, they do not recommend this drug combination for use in hyena at these doses (Hahn et al. 2014).

Zolazepam-tiletamine:

Tiletamine HCl is a dissociative anaesthetic drug which produces cataleptoid anaesthesia with excellent analgesia, whereas zolazepam HCl is a benzodiazepine agonist drug which produces sedation and muscle relaxation (Clarke et al. 2014; Grimm et al. 2015). Dissociative drugs, such as tiletamine, act on *N*-methyl-*D*-aspartate (NMDA), muscarinic, opioid and monoaminergic receptors and interact with voltage calcium channels. At the NMDA receptors, dissociative drugs act as non-competitive antagonists (Grimm et al. 2015). They bind to the phencyclidine-binding site thereby preventing the excitatory neurotransmitter, glutamate, from binding. This results in depression of the reticular, limbic and thalamocortical activating systems (Grimm et al. 2015). Benzodiazepines, such as zolazepam, enhance the affinity of the $GABA_A$ receptors for γ-Aminobutyric acid (GABA) which results in an increased chloride conduction and hyperpolarisation of postsynaptic cell membranes (Grimm et al. 2015). When tiletamine is administered alone it can cause clonic or tonic muscular movements and convulsive seizures. When combined with zolazepam, the possibility of these side effects is greatly reduced, and the combination becomes a potent dissociative immobilisation drug combination (Grimm and Lamont 2007; Van Jaarsveld 1988). During induction the zolazepam-tiletamine combination does not cause excitation (Papich 2011; Van Jaarsveld 1988). Dissociative anaesthetic drugs are also commonly combined with α_2-adrenoceptor agonists to enhance immobilisation resulting in acceptable muscle relaxation and analgesia (Grimm et al. 2015). Both intravenous and intramuscular administration methods are used for these combinations. Depending on the dosage administered, the drug combination of tiletamine and zolazepam could result in rapid and reliable immobilisation or general anaesthesia. The onset of action for this combination is 10 min with duration of anaesthesia dependant on drug dosage administered, which is usually longer with IM administration due to large drug volumes for distribution and a high lipid solubility. There are also variable elimination times between species as tiletamine is metabolised faster in cats than in dogs whereas zolazepam is eliminated faster in dogs (Grimm et al. 2015).

Zolazepam-tiletamine is able to be made up into high concentration solutions which decreases the overall drug volume and therefore a smaller dart may be used (Fahlman 2005). The zolazepam-tiletamine combination has been found to be successful in the immobilisation of many species of African carnivores, wolves, bears, and other wildlife species (Fahlman 2005; Larsen and Kreeger 2007; Papich 2011; Van Jaarsveld 1988; Vilà 1994; White et al. 1996). Previously, the use of zolazepam-tiletamine combinations in wild felids, specifically tigers, was thought to be inadvisable as adverse reactions such as seizures, ataxia, delayed recovery, hyper-reflexia, hindlimb paresis and deaths were known to occur (Grimm et al. 2015; Gunkel and Lafortune 2007). Further review of this topic indicated that the mortality rate in tigers administered zolazepam-tiletamine combinations were similar to those that occurred when other anaesthetic drugs were used in a variety of species. The review indicated that adverse side effects do occur but do not occur often enough to warrant contraindication in tigers (Kreeger and Armstrong 2010). When supplementation is necessary, it has been suggested to rather supplement with ketamine as opposed to further additional zolazepam-tiletamine administration as it results in shorter recovery times (Grimm et al. 2015; Larsen and Kreeger 2007). Drug elimination may also play a role in the shortening of recovery times, as in cats the duration of action of zolazepam is longer than tiletamine which results in the sedation effects lasting longer than those of tiletamine. Whereas in dogs, it is quite the opposite as the duration of action of tiletamine is longer than zolazepam (Grimm et al. 2015).

The use of zolazepam-tiletamine causes the eyes of those anaesthetised to remain open, thereby predisposing the animals to corneal desiccation, and therefore it has been suggested to always use an ointment or lubricant on the eyes during any period of sedation or anaesthesia (Papich 2011; Van Jaarsveld 1988).

A study in hyenas determined that both the laryngeal and pharyngeal reflexes, controlling the jaw tone and swallowing reflexes, were present when zolazepam-tiletamine was used for immobilisation but these effects did not prevent intubation (Grimm et al. 2015; Van Jaarsveld 1988). Zolazepam-tiletamine has been known to maintain protective reflexes such as swallowing or coughing, and other reflexes such as corneal and pedal. These effects are most likely due to the limited degree of muscle relaxation produced as cranial nerve and spinal reflexes remain preserved or active, it is therefore important to not confuse the protective reflexes with indications of inadequate anaesthesia (Clarke et al. 2014; Van Jaarsveld 1988). Any attempts to monitor the plane of anaesthesia using the palpebral, pedal and pinnal reflexes were observed to be not good enough due to the dissociative nature of this drug in hyenas, as these reflexes too remained functional in the study. A state of surgical anaesthesia was said to be obtained in the spotted hyena at ± 8 mg kg^{-1} (Van Jaarsveld 1988). A combination of zolazepam-tiletamine has been recommended for field use in hyenas as it has a high therapeutic index, low mortality rate, produces a smooth immobilisation lasting 75-120 min, is safe for use in a wide range of ages and physiological conditions such as those occurring in late stages of pregnancy. Although the recovery from zolazepam-tiletamine immobilisation is smooth, some "head flopping" does tend to occur. The rapid induction time with zolazepam-tiletamine immobilisation (2 min ± 40 sec) also reduces the risk of losing wild animals before they become recumbent or attacked by other predators (Van Jaarsveld 1988). The high concentration of the drug formulation also means that the hyenas may be darted with a 1 mL dart that minimises the risk of physical injury as well as minimising the risk of losing sight of the animal after darting (Van Jaarsveld 1988).

Medetomidine:

Medetomidine HCl is a highly selective, reversible and potent α_2-adrenoceptor agonist. It is synthetic, non-narcotic and dose-dependent in terms of depth and duration of sedation and analgesia (Cullen 1996; Papich 2011; Pypendop and Verstegen 1998). Medetomidine has been found to be more potent and have a higher α_2 agonist receptor affinity, more than 1000 greater,

than xylazine (Papich 2011; Pypendop and Verstegen 1998; Cullen 1996). Therefore, in comparison to xylazine, in dogs medetomidine has been shown to have better sedative qualities (Cullen 1996; Papich 2011). Medetomidine molecules are represented as two stereoisomers, namely the L-enantiomer and the D-enantiomer which affects the cardiovascular and nervous systems (Cullen 1996; Papich 2011). Medetomidine's effects can be antagonised or reversed by yohimbine, atipamezole or tolazoline.

α_2-adrenoceptors are found in the central and peripheral nervous system and located both pre- and post-synaptically, throughout the body's tissues and organs, where they facilitate endogenous catecholamines. Presynaptically they control the release of the sympathetic neurotransmitter, noradrenaline, from the adrenergic nerve endings (Cullen 1996; Grimm et al. 2015). α_2-adrenoceptor agonists do this by binding to presynaptic α_2-adrenoceptors thereby resulting in decreased sympathetic outflow, sedation and analgesia (Cullen 1996; Cullen 1999; Papich 2011). The postsynaptic α_2-adrenoceptors are found in blood vessels where they control vasoconstriction which can lead to an increased blood pressure. α_2-adrenoceptors can also be found extra-synaptically in vascular tissue and platelets (Grimm et al. 2015). Adrenoceptors have been classified into three distinct classes such as alpha$_1$, alpha$_2$ and beta; with alpha$_2$ receptors being subtyped into A, B, and C. A subtype D is also present but is believed to be a species variation of subtype A and therefore is not considered separately as it has similar functions and distributions (Grimm et al. 2015). Alpha$_{2A}$ receptors located in the brainstem and cortex facilitate supraspinal analgesia, sedation and centrally mediated hypotension and bradycardia. Alpha$_{2B}$ receptors located in the spinal cord and vascular tissue facilitate the early increased vascular resistance, spinal analgesia and peripherally mediated bradycardia. Alpha$_{2C}$ receptors located in the spinal cord modulate possible thermoregulation and spinal analgesia as well as anxiolysis (Grimm et al. 2015; Kamibayashi et al. 2000). α_2-adrenoceptors are G-coupled proteins and when stimulated inhibit adenyl cyclase activity which leads to a decrease in adenosine 3',5'-cyclic monophosphate (cAMP) concentrations inside cells. This is the most important result of

α_2-adrenoceptor stimulation as cAMP is essential for regulation of cellular function. Inhibition of platelet aggregation is also caused by α_2-adrenoceptor stimulation, but it does not require G-protein coupling to occur (Murrell and Hellebrekers 2005; Grimm et al. 2015). Medetomidine is also one of the few clinically available α_2-adrenoceptor agonists that contains an imidazole ring in its structure, which provides the opportunity for the drug to interact with imidazoline receptors (Murrell and Hellebrekers 2005). Other α_2-adrenoceptor agonists, such as dexmedetomidine, have demonstrated a cardioprotective effect, an enhancement of vagal tone and an antiarrhythmic effect via imidazoline receptors in the central nervous system (Murrell and Hellebrekers 2005; Kamibayashi et al. 2000).

Medetomidine may be given intravenously (IV), intramuscularly (IM) or subcutaneously (SC). Subcutaneous administration is more unreliable than IM and IV (Cullen 1996; Papich 2011). After 0.04 mg kg^{-1} IV administration peak sedation was produced between 10-20 min in dogs (Grimm et al. 2015). Medetomidine has been found to produce good sedative effects in small animals, cheetahs, primates, bears, lions, wolverines, wolves, snow leopards and many other wildlife species (Arun et al. 2016; Cattet et al. 1999; Fahlman 2005; Fahlman et al. 2005; Jalanka 1989; Jalanka and Roeken 1990; Johansson et al. 2013; Murrell and Hellebrekers 2005; Painer et al. 2012; Semjonova et al. 2017; Semjonova et al. 2018; Wenger et al. 2010).

A combination of ketamine (3-4 mg kg^{-1}) and medetomidine (0.035-0.045 mg kg^{-1}) has successfully been used to immobilise brown hyenas in Namibia (Hahn et al. 2014). This combination produced sedative effects within 3 min, recumbency in 7 min and 40-50 min of anaesthesia (Hahn et al. 2014). A few field immobilisations of the spotted hyena, using this combination, required supplemental ketamine IM administration to ensure complete recumbency. The ketamine-medetomidine combination produced a stable heart and respiratory rate with good muscle relaxation. Ptyalism and bradycardia were experienced in these animals with heart rates averaging 40-60 beats min^{-1}. Rectal temperatures were on average 37.8°C.

Hypoxia, <75% indicated via pulse oximetry, was initially observed during the immobilisation period but was found to improve to >90% overtime within 20-30 min of anaesthesia (Hahn et al. 2014).

Administration of medetomidine in dogs produces marked cardiovascular changes, such as hypertension, bradyarrythmias, increased systemic vascular resistance, and reduced cardiac output and oxygen delivery (Grimm et al. 2015; Murrell and Hellebrekers 2005; Pypendop and Verstegen 1998). Blood flow is preserved in vital organs such as the brain, heart, kidneys and liver. Oxygen requirements are reduced and blood flow to vital organs are maintained above hypoperfusion and oxygen debt levels (Grimm et al. 2015). Pypendop and Verstegen (1998) found that the above mentioned negative cardiovascular effects in canines were at peak levels at dosages as low as 0.05 mg kg^{-1} and that higher doses had minimal additional cardiovascular effects (Murrell and Hellebrekers 2005). In their study they found that bradycardia occurred and the changes in blood pressure and heart rate were enough to warrant further action, as the heart rate was as low as 24 beats min^{-1}. During phase one (initially after drug administration) in dogs, an increase in blood pressure results due to peripheral vasoconstriction. This peripheral vasoconstriction is caused by the activation of post-synaptic α_2-adrenoceptors in the peripheral vascular smooth muscle which is associated with an increased vagal tone as well as a decreased heart rate. Higher doses, above 0.02 mg kg^{-1}, have been shown to cause an increased duration of hypertension which has been associated with increased systemic vascular resistance. However, at lower dosages central effects dominate, and blood pressure decreases to values within normal acceptable range (Kuusela et al. 2000; Murrell and Hellebrekers 2005; Pypendop and Verstegen 1998). Normal blood pressure levels in healthy mammals, such as dogs and cats, are considered to be: SAP of 125-160 mmHg, MAP of 90 – 110 and DAP of 75 – 95 mmHg (Clarke et al. 2014). Medetomidine doses in dogs at 0.04, 0.08 and 0.16 mg kg^{-1} have proven to raise the blood pressure by 18-26% before decreasing due to peripheral vasopressor action (Cullen 1996; Vainio and Palmu 1989b). The exact cause for decrease in cardiac output are unknown but have

been suggested as possibly being due to a direct myocardial depressant effect, decrease in response to alpha$_2$ agonist-mediated increase in afterload or due to a drug-induced decrease of metabolic demands. The possibility of several of these mechanisms occurring together is also likely (Murrell and Hellebrekers 2005).

α_2-adrenoceptor agonists, when administered alone, do not produce a marked respiratory depression. Medetomidine, however, has been found to cause a slight decrease in respiratory rate and therefore minute ventilation that is thought to be centrally mediated (Vainio 1990). Arterial blood gas values in these cases do not indicate a significant change, including arterial partial pressure of carbon dioxide remaining the same or slightly decreased and pH remaining within normal limits (Clarke et al. 2014; Short 1992). Medetomidine when administered at 0.02, 0.04 and 0.08 mg kg^{-1} in dogs showed a minor insignificant decrease in arterial oxygen tension and increase in arterial carbon dioxide tension (Cullen 1996; Vainio 1989a). However, a study in dogs indicated that medetomidine at 0.05-0.1 mg kg^{-1} IV administration does produce a decrease in respiratory rate, respiratory drive and minute volume in conscious dogs (Lerche and Muir 2004). At the peak effect of medetomidine, decreased ventilation associated with increased arterial carbon dioxide tension may decrease the arterial oxygen tension. This period corresponds with the decreased heart rate and oxygen demand experienced therefore oxygenation of tissues is maintained (Pettifer and Dyson 1993). A study provided some evidence as to the possible cardioprotective effects of the drug particularly in those high risk patients that could develop or already have cardiac disease (Aantaa and Jalonen 2006).

There are adverse effects found in other organs systems when using medetomidine. In the gastro-intestinal system medetomidine causes inhibition of gastric secretions due to the activation of both central and peripheral α_2-adrenoceptors as well as vomition in both dogs and cats. Poikilothermia may occur due to CNS depression affecting normal thermoregulation concurrent with decreased muscular activity (Sinclair 2003). In the endocrine system, α_2-

adrenoceptor agonists have shown to cause hyperglycaemia due to decreasing insulin release, cats in particular have been found to be much more susceptible than dogs (Burton et al. 1997; Kanda and Hikasa 2008). In the skeletal system, spontaneous muscle contractions have been known to occur after administration of medetomidine in dogs and cats, even more commonly with excessive auditory stimulation (Cullen 1996; England and Clarke 1989; Papich 2011). Uterine motor activity has been known to decrease at 0.02 mg kg^{-1} and increase at 0.04 and 0.06 mg kg^{-1} in dogs (Cullen 1996).

Atipamezole

Atipamezole is an α_2-adrenoceptor antagonist specifically formulated to antagonise the effects of medetomidine (Cullen 1996; Murrell and Hellebrekers 2005). In brown hyena, atipamezole has be found to successfully reverse the effects of medetomidine, within 5 – 10 min after IM administration, at five times the administered medetomidine dosage (Hahn et al. 2014). Intravenous administration of the drug has been known to result in profound vasodilation if a high systemic vascular resistance is still present. If this vasodilation occurs without an increase in cardiac output and heart rate, this leads to marked hypotension (Grimm et al. 2015). IM administration of atipamezole has been known to be effective but some cases of prolonged sedation or partial reversal have been known to occur (Grimm et al. 2015). The prolonged sedation or partial reversal is suspected to be caused by decreased absorption of the drug due to vasoconstriction of muscle tissue as plasma concentration levels may not be high enough to cause antagonist effects. Additionally, atipamezole also has a shorter half-life than medetomidine (Grimm et al. 2015).

Medetomidine-zolazepam-tiletamine combinations

Currently, medetomidine-zolazepam-tiletamine combinations are successfully used in many free-ranging species such as wolves, primates, bears, snow leopards, cheetahs and lions (Arun

et al. 2016; Cattet et al. 1999; Fahlman 2005; Johansson et al. 2013; Painer et al. 2012; Stegmann and Jago 2016; Deem et al. 1998). Many studies have shown that the combination of a dissociative anaesthetic agent with a tranquiliser and/or sedative, leads to improvement of muscle relaxation as well as a reduction of the dissociative agent being used (Fahlman 2005; Hayama et al. 1989; Jalanka and Roeken 1990; Lewis 1993; Röken 1997; Painer et al. 2012). The most frequently used sedatives for this purpose are the α_2-adrenoceptor agonists, more specifically, xylazine and medetomidine (Fahlman 2005). When medetomidine is combined with zolazepam-tiletamine it has been found that the dosage of zolazepam-tiletamine may be reduced by at least 50% in a wide variety of species, thereby a small volume of the drug combination is only needed for a rapid and smooth induction (Cattet et al. 1999; Clarke et al. 2014; Fahlman 2005; Kreeger et al. 2002; Painer et al. 2012; Röken 1997). The rapid induction is due to the fact that medetomidine has a steep dose-response curve and therefore doses should rather be calculated based on body surface area as opposed to weight. This means that with medetomidine use, the recommended dose per kilogram decreases as the size of the animal increases (Cattet et al. 1999; Clarke et al. 2014; Fahlman 2005; Kreeger et al. 2002; Painer et al. 2012; Röken 1997). A decrease in the recommended dose meant that a smaller dart could be used which results in a reduced risk of darting impact injuries and an improvement of accuracy at long distances (Fahlman 2005). Larger dosages of zolazepam-tiletamine cause prolonged recoveries, therefore the reduction of the zolazepam-tiletamine dose is preferable as it cannot be antagonised whereas medetomidine can. The reversal effects of α_2-adrenoceptor antagonists, such as atipamezole, have been shown to shorten the recovery period from anaesthesia dramatically when α_2-adrenoceptor agonists have been used (Cattet et al. 1999; Fahlman 2005; Jalanka 1989; Jalanka and Roeken 1990).

During the Fahlman et al. (2005) study, free-ranging lions were successfully anaesthetised with 0.027-0.055 mg kg^{-1} medetomidine and 0.38—1.32 mg kg^{-1} zolazepam-tiletamine administered via IM darting. Induction was smooth, rapid, and good muscular relaxation was observed. Time

to recumbency was 6 min. Spontaneous movements were only witnessed in one lion after an anaesthetic time of 1 h, and therefore any handling in terms of changing the animals' position should occur early on during anaesthetic period. Oxyhaemoglobin saturation, heart and respiratory rates were stable throughout the anaesthetic period. Bradycardia and the inability of the pulse oximeter to provide readings were observed in a few lions and attributed to medetomidine use. Rectal temperatures increased within the first 30 min after darting, after which a decrease was observed. Duration of anaesthesia was predictable and rapidly reversible. Reversal was possible with atipamezole administered IM at 2.5 times the medetomidine dosage resulting in a smooth and calm recovery within 8-26 min. Lion's anaesthetised with zolazepam-tiletamine > 1 mg kg^{-1} experienced prolonged recoveries of 50-166 min duration (Fahlman et al. 2005).

As study by Stegmann and Jago (2006) showed that medetomidine (0.027 mg kg^{-1}) and zolazepam-tiletamine (2.9 mg kg^{-1}) has successfully been used in the immobilisation of cheetahs. Induction occurred within 16.8 min. Hypertension was found to be present in all animals with a mean systolic pressure of 248 mmHg and MAP of 188 mmHg. Hypertension and the high failure rate to obtain blood pressure readings via indirect methods were attributed to the peripheral vasoconstrictive effects of medetomidine. It was also noted that blood pressure measured indirectly using a cuff as opposed to directly using the artery were significantly lower. Both metabolic acidosis and hypoxia were observed during the anaesthetic period, without oxygen supplementation. When oxygen supplementation was provided, oxyhaemoglobin saturation, arterial partial pressure of oxygen and carbon dioxide were significantly increased (Stegmann and Jago 2006).

Deem et al. (1998) safely and successfully immobilised cheetahs with zolazepam-tiletamine (1.56 ± 0.07 mg kg^{-1}) and medetomidine (0.031 ± 0.0014 mg kg^{-1}) via IM darting. The combination resulted in a smooth and rapid induction and recumbency within 4.3 min. Anaesthesia was

induced for at least 87 min. The drug combination resulted in easy intubation and excellent muscle relaxation. Hypoxia and arrhythmias only developed in one cheetah but resolved once oxygen supplementation was provided. ECG indicated sinus arrhythmias in 3 cheetahs. Hypertension developed in all cheetahs and was attributed to medetomidine use with a MAP of 217 mmHg within 30 min after darting, and decreasing to 161.6 mmHg, with MAP significantly lower in those supplemented with oxygen. Pulse oximeter readings were difficult to obtain and was attributed to medetomidine's vasoconstrictive effects. Mild acidaemia was demonstrated in all cheetahs, although pH was significantly lower with those supplemented with oxygen. Post reversal sedation occurred within 30-45 min after IM administration of atipamezole in four cheetahs and lasted up to 4 hours. Post reversal sedation was attributed to low atipamezole dosages administered (0.155 mg kg^{-1} half IV and half SC) (Deem et al. 1998).

Importance of characterising the physiological effects of medetomidine

Hyenas appear to be more closely related to cats as opposed to dogs in terms of their dentition as well as physiological responses to xylazine and ketamine combinations, although they do appear to resemble dogs more in terms of their physical appearance (Hahn et al. 2007). It has therefore been suggested to veterinarians to use the domestic cat and large dog as references with regards to drug choices and dosages in this species. Although medetomidine is widely used in small animal anaesthesia, the potent cardiovascular effects of this drug are a concern (Murrell and Hellebrekers 2005). Hypoxaemia is a common side effect that has also been linked to the use of medetomidine in multiple wild carnivores such as bears and lions (Cattet et al. 1999; Wenger 2010).

Free-ranging wild animal conditions are unsuitable for controlled clinical studies, as it is not always possible to control every aspect of their environment, physiology and behaviour in the field. Therefore, for wild animals, it is very common for recommended anaesthetic drug doses to be extrapolated from other species as only a few controlled clinical trial studies are conducted

(Painer et al. 2012). A dosage of 1-2 mg kg^{-1} zolazepam-tiletamine and 0.01 mg kg^{-1} medetomidine has been suggested for use in the immobilisation of the spotted hyena but no indications were made as to the effects that the combination has on the species (Hahn et al. 2007).

Although the International Union for Conservation of Nature (IUCN) classifies hyenas as "least concern", it has been noted that currently the population is only high in protected reserves and has been gradually declining in all other areas due mostly to loss of habitats, maltreatment by humans (hunting, trapping and poisoning) especially when considered a threat to other wildlife species in the area and stock theft issues (IUCN 2017).

Due to ongoing research, conservation and rescue efforts, finding a safe anaesthetic protocol for hyena has become important, as few anaesthetic studies have characterised the physiological effects of the drug combinations used in the spotted hyena.

Overview of literature review

Based on the most commonly used drug combinations in wild animals and what has currently been used in the brown hyena, it appears that the medetomidine-zolazepam-tiletamine combination may be a desirable combination for use in the spotted hyena. The evidence suggests that with regards to medetomidine, particularly in dogs, there seems to be very minimal advantages to decreasing the drug dosage below 0.01 mg kg^{-1} as the duration of analgesia would be shortened whilst the cardiovascular changes remain the same. There also seems to be no advantage to increasing the dosage above 0.04 mg kg^{-1}, as doing so in dogs and cats only prolongs the adverse cardiovascular effects (Kuusela et al. 2000; Murrell and Hellebrekers 2005; Pypendop and Verstegen 1998). Atipamezole at 4-6 times the medetomidine

dose also appears to successfully reverse all effects of medetomidine in brown hyena (Hahn et al. 2014).

To this author's knowledge, no studies have been done in order to identify and characterise the physiological effects that these drugs cause during any chemical immobilisations of the spotted hyena. Many of the studies reviewed with regards to the spotted hyena have based their protocols on suggested doses only. This proposed drug combination does warrant further investigation before any recommendations can be made for field use, in order to identify and characterise the physiological effects of using either a high or low dose of medetomidine in combination with zolazepam-tiletamine as the combination of both medetomidine and zolazepam-tiletamine has proven to be effective in other wildlife species. Further investigation with regard to the effects of a medetomidine-zolazepam-tiletamine drug combination may lead to a much safer, rapidly reversible, physiologically stable, effective, easily adaptable, reliable and cheaper anaesthetic protocol for hyenas in captivity and in the wild.

Introduction

Historically, studies on the spotted hyena have been more focussed on their unique reproductive organs and behavioural characteristics and not the immobilisation of this species. The spotted hyena needs to be safely and effectively immobilised in order to assist with or conduct any number of studies, population management efforts, research sample collections as well as capture and translocation of this species. The ability to effectively immobilise the spotted hyena is essential for their protection as well as the protection of those working with them (Hahn et al. 2007).

In this study, the physiological effects of high and low dose medetomidine in combination with zolazepam-tiletamine were studied in order to determine the possibility of the combination for field use. Zolazepam-tiletamine was integrated into the combination as it is a commonly used drug combination for field immobilisation (Hahn et al. 2007). Medetomidine was included for its muscle relaxant, analgesic, sedative and dose sparing effects as it allowed us to decrease the dosage of zolazepam-tiletamine used in this combination. The combination also allowed us to immobilise with a minimal drug volume and thereby a smaller dart was used, ensuring less trauma to the dart impact site.

Currently, no studies have been conducted in order to fully investigate and characterise the physiological effects that this combination of drugs have during any chemical immobilisations of the spotted hyena (*Crocuta crocuta*). The possible cardiovascular effects, that have been associated with the medetomidine use in many other species, were of particular interest in this study. Specifically, cardiovascular effects such as hypertension, bradycardia, arrhythmias, increased systemic vascular resistance, and reduced cardiac output and oxygen delivery (Murrell and Hellebrekers 2005; Pypendop and Verstegen 1998). Oxygen supplementation was also

added as the use of medetomidine has been known to induce hypoxaemia in a wide variety of wildlife species (Cattet et al. 1999; Fahlman 2005; Kreeger et al. 1986; Painer et al. 2012).

Aims and objectives

The aim of the study was to identify a safe and effective dose of medetomidine, when used in combination with zolazepam-tiletamine in the immobilisation and anaesthesia of the spotted hyena. The objective was to compare the physiological effects of medetomidine when used in low and high doses, in combination with zolazepam-tiletamine, during the immobilisation and anaesthesia of the spotted hyena.

Hypothesis

H_0: The immobilisation and physiological effects of medetomidine will not differ between the lower dose and the higher dose treatments, in combination with zolazepam-tiletamine.

H_1: The immobilisation and physiological effects of medetomidine will differ between the lower dose and the higher dose treatments, in combination with zolazepam-tiletamine.

Benefits arising from the experiment

This investigation assisted us in expanding our knowledge, identifying and understanding the physiological effects of medetomidine in combination with zolazepam-tiletamine in the spotted hyena. The knowledge gained from this study will assist us in improving the safety, effectiveness and reliability of immobilisation of the spotted hyena in both captivity and in the wild.

Material and methods

Experimental design

A prospective blinded, randomised cross-over study was performed.

Animals

Eight of thirteen adult captive-held spotted hyenas were included in this study. All hyenas were owned by Mr Kevin Richardson and housed at the Kevin Richardson Wildlife Sanctuary within the Dinokeng Nature Reserve in Gauteng province, South Africa (25°28'06.6''S 28°27'14.1'' E). The animals were housed in one-hectare camps containing six to seven hyenas in each camp. A number of trees were found within each camp to provide shade and shelter from the elements. These hyenas were immobilised for annual health checks, vaccination, contraception and translocation to new enclosures with an anaesthetic period of approximately 60 min.

Procedures

This study was approved by the University of Pretoria's Animal and Research Ethics Committees (Protocol number: V091-17). The data collection took place over two periods of two-day duration each, in August and October 2017. The animals were randomly allocated to either the lower (LD) or higher (HD) dose treatment, by drawing out of a hat prior to initial immobilisation. Four animals received the lower dose of medetomidine and four the higher dose of medetomidine during the first data collection period. The animals received the alternative dose during the second data collection period.

Immobilisation

Free access to water was provided, but food was withheld for over 36 hours prior to immobilisation as the hyenas are normally only fed once a week. Each hyena was identified and immobilised in their respective camps. The hyenas were remotely injected (darted) by an experienced veterinarian using a carbon dioxide powered projector, Dan-inject JM Special (Dan-inject 0037; Mod JM; Denmark) which was fitted with a 13mm barrel, using 1.5 ml Motsumi darts (Motsumi Darts; South Africa) with a 1-inch barbed needle that was placed into a suitable area of musculature in the hindquarters.

One of two immobilisation protocols were used in a cross-over design. Both treatments contained zolazepam-tiletamine (1.0 mg kg^{-1}) (Zoletil 50 mg mL^{-1}; Virbac; South Africa) with either LD (0.02 mg kg^{-1}) (Medetomidine 10 mg mL^{-1}; Kyron Prescriptions; South Africa) or HD (0.04 mg kg^{-1}) medetomidine.

The hyenas were dosed according to estimated body weight as visualised by the immobilisation team. During the second session, the drug doses were calculated based on the body weights measured in the first session. The time of darting and recumbency were recorded along with dart impact site. Quality of immobilisation was scored and recorded along with any adverse effects that were observed (Table 1).

Table 1: Scoring system for the capture and immobilisation quality of spotted hyena after immobilisation with a medetomidine-zolazepam-tiletamine combination.

Immobilisation quality during capture	
Score	Description
1	Calm, no excitement*, remains recumbent once down
2	Mild excitement* before recumbency, stays recumbent once down
3	Marked excitement*, multiple attempts to stand after period of recumbency
4	Intense excitement*, no sign of recumbency

*Excitement such as thrashing, running or fighting

Once the animal was recumbent, the darting team entered the camp and collected the animal once it was clear that it was safe to approach. Each hyena's tail was tugged, and their jaw tone was cautiously assessed, and once deemed moderately relaxed an ocular lubricant (Celluvisc; Allergen Pharmaceuticals, Westport, Ireland) was placed into the eyes of each animal and the animal was then blind-folded. The hyena's mass was determined by placing the hyena on a canvas stretcher that was then suspended from a self-zeroing and calibrating digital hanging crane scale (Modern step; USA) (Image 1) and thereafter placed on a supportive mattress just

outside the night rooms in right lateral recumbency. An indwelling catheter, 21-gauge (Jelco; Smiths Medical; Lancashire, UK) was placed aseptically, percutaneously into the cephalic or jugular vein. This was used as the primary access point for any further intravenous anaesthetic or emergency drugs deemed necessary as well as balanced isotonic crystalloid fluids (Ringer's lactate Fresenius 0.9%, Fresenius Kabi, South Africa) at 5 mL kg^{-1} h^{-1}.

Image 1: Weighing of spotted hyena after darting using a canvas stretcher suspended from a hanging crane scale with monitoring station in the background.

With an assistant holding the mouth open, the trachea was intubated in sternal recumbency with 11-12mm endotracheal (ET) tubes. The hyenas remained intubated for the duration of the anaesthetic period and breathed spontaneously. After 20 min, oxygen was provided at a flow rate of 2 L min^{-1} via a portable anaesthetic machine with a circle breathing system.

If depth of anaesthesia was insufficient to allow orotracheal intubation, ketamine was administered in boluses of 0.25 mg kg^{-1} IV to effect. Any additional medication deemed necessary were recorded on the data capture sheets provided for each animal.

Monitoring

Anaesthetic plane and physiological variables such as heart rate, respiratory rate, capillary refill time, temperature, non-invasive blood pressure (including SAP, DAP and MAP), spirometry, inspiratory and expiratory volumes, peripheral oxygen haemoglobin saturation, fraction inspired oxygen concentration and end tidal carbon dioxide tension were recorded at five-minute intervals. The cardiorespiratory variables were monitored continuously throughout the anaesthetic period using a multiparameter monitor (Datex Cardiocap 5; Datex-Ohmeda; Finland). Cardiorespiratory variable monitoring began within 10 minutes after time of darting, due to the delay caused by transportation of all hyenas from their respective camps to the monitoring station.

An electrocardiogram was used to measure heart rate, with three ECG leads attached to the hyena's paws in a limb lead configuration which were then connected to the multiparameter monitor (Image 2). A pulse oximeter transmission probe (Nonin 9847V; Nonin Medical INC; USA) was placed on the hyena's tongue in order to measure the pulse rate and peripheral oxygen haemoglobin saturation levels. Auscultated heart rate was correlated with the ECG and pulse oximeter rates. Blood pressure was measured indirectly using a high definition oscillometer with the cuff placed on the tail or hindlimb (VET HDO monitor; S + B medVet; Germany).

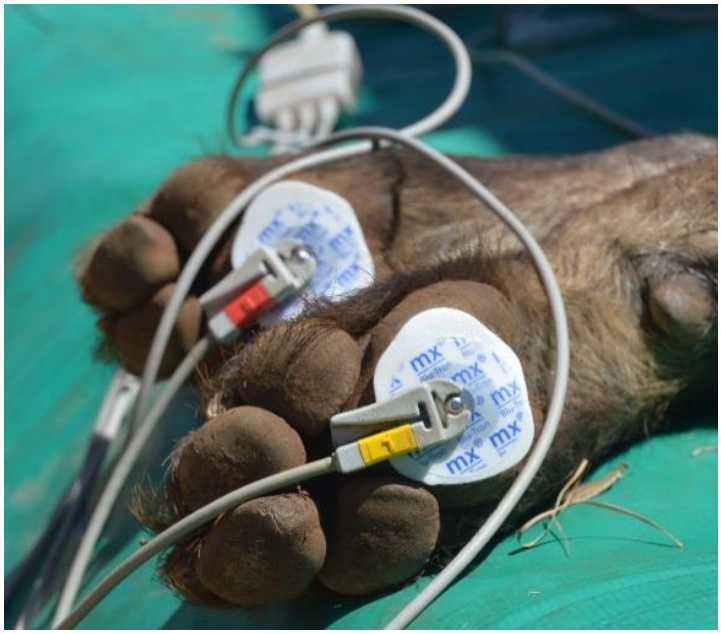


Image 2: ECG pads placed on front paw-pads of immobilised spotted hyena.

Image 3 Immobilised hyena showing the setup of the spirometer with the ET tube connection.

Respiratory rate was calculated from the capnograph and then correlated with the visual assessment of the thoracic wall movement. Inspiratory and expiratory tidal volume, end-tidal carbon dioxide and fractional inspired oxygen was evaluated using a pitot pneumotachograph and side stream gas sampler, 200 mL min^{-1}, which were attached to the ET tube (Image 3).

A digital portable rectal thermometer (Hanna CHECKTEMP 1; Hanna instruments; USA) was used to measure rectal temperature, accuracy of thermometer was within 0.1˚C.

Depth of anaesthesia was record at five-minute intervals and assessed based on palpebral reflexes, muscle tone and response to external stimuli. If depth of anaesthesia was insufficient, ketamine was administered in boluses of 0.25 mg kg^{-1} IV to effect.

Two arterial blood samples (1 mL per sample) were drawn for blood gas analysis at 20 and 50 minutes after darting time respectively, over a period of two respiratory cycles. The blood samples were collected aseptically from the medial saphenous artery (Image 4), which was palpated on the right stifle joint, with a 23-gauge needle and with pre-heparinized syringes (lithium heparin) and analysed immediately with an i-STAT portable blood gas analyser (i-STAT1 Portable Clinical Analyzer and i-STAT cartridges CG8+, Abbott Laboratories, USA). The blood gas analyser measured or calculated the blood pH, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), sodium (Na), ionised calcium (iCa), potassium (K), haematocrit (Ht), glucose (glu), haemoglobin (Hb), oxygen haemoglobin saturation (SaO_2), bicarbonate (HCO_3), total carbon dioxide (TCO_2) and extracellular base excess (BEecf). The blood samples were not corrected for body temperature. Lactate was analysed using a portable Lactate Pro blood lactate analyser (Lactate Pro; Arkray; Japan) but was only done for the eight hyenas (eight arterial and one venous) in the first session.

The alveolar-arterial oxygen tension difference, $P(A\text{-}a)O_2$, at standard temperature (37°C) was estimated from the equation: $PAO_2 = F_IO_2 (P_B - P_{H2O}) - (PaCO_2/RQ)$. PAO_2 = Partial pressure of alveolar oxygen, F_IO_2 = Fraction of inspired O_2, P_B = Barometric pressure (664 mmHg), P_{H2O} = Saturated vapor pressure for water at 37°C (47 mmHg), and RQ = Respiratory quotient (0.8). Respiratory quotient has not been calculated in hyena before and therefore values were extrapolated from values calculated in dogs and cats.

Any interventions deemed necessary or side effects observed during the anaesthetic period were recorded.

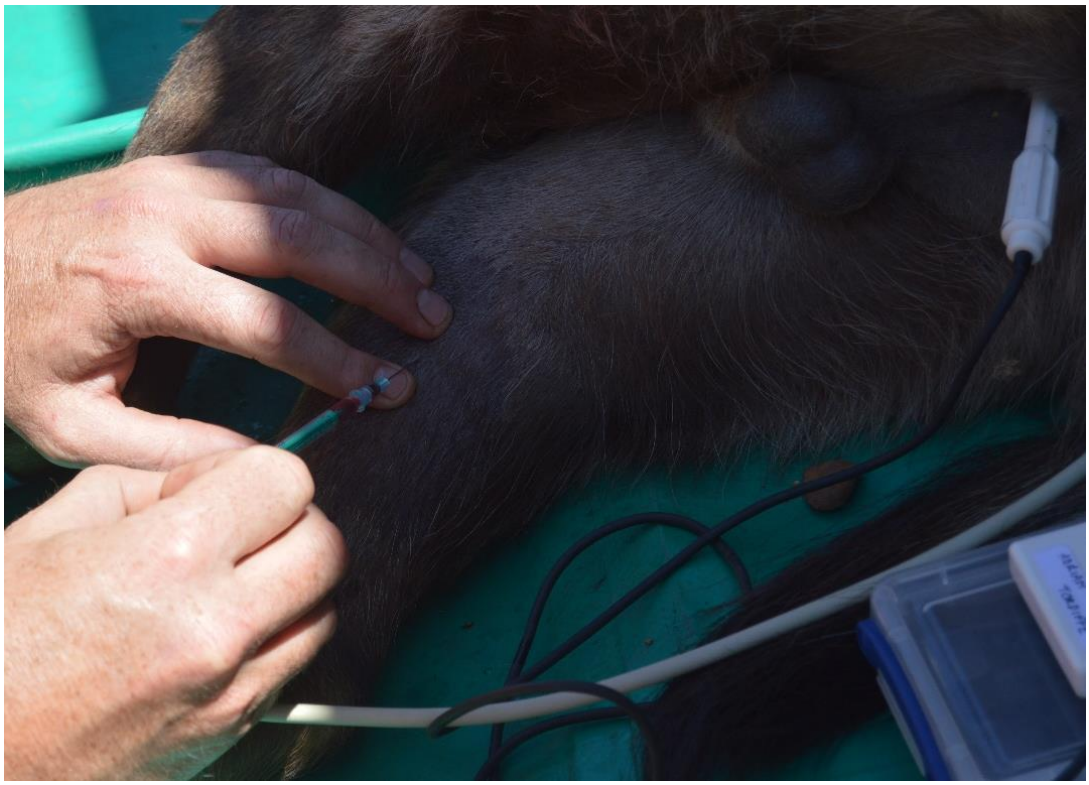


Image 4: Dr Tordiffe collecting an arterial blood sample from the medial saphenous artery of one of the immobilised spotted hyenas.

Recovery

60 min after darting, the hyenas were disconnected from all monitoring equipment before taken back to the night room for recovery. The endotracheal tube was removed. The dart site was cleaned and treated with tetracycline spray. The medetomidine was then reversed using atipamezole (5 mg mg^{-1} of medetomidine administered; 5 mg mL^{-1}; Zoetis; South Africa) IM.

The quality of recovery was observed and scored (Table 2). Recovery was broken up into two phases, early and late recovery for easy accuracy of assessment. “Early” was immediately after the reversal drug had been delivered and “Late” was once the animal was standing and then released into the enclosure.

Table 2: Scoring system for early and late recovery phases in spotted hyena immobilised with a medetomidine-zolazepam-tiletamine combination after administration of the α_2-adrenoceptor antagonist – atipamezole.

Early recovery		Late recovery	
Score	Description	Score	Description
1	A calm transition to an alerted state, one or two attempts to stand	1	No ataxia, normal movements
2	Quiet, easily startled, one or two attempts to stand	2	Mild ataxia, gait mostly normal or quickly returns to normal
3	Uncoordinated movements, easily startled, paddling,	3	Moderate ataxia, unsteady gait
4	multiple attempts to stand Thrashing, delirium, multiple attempts to stand	4	movements Pronounced ataxia for a long period of time after release (>10 minutes)

Image 5: Recovery position of a spotted hyena in the night room after extubation.

Data collection

An onsite data collection sheet was used for each individual animal (Addendum 1). This data sheet included the date, identification of the animal in question, sex as well as body mass. All clinical variables and data collected by each of the monitoring equipment used were recorded at 5-min intervals for the entire duration of anaesthesia.

All doses of any immobilisation, reversal or emergency drugs were recorded. The times of darting, first signs of sedation, recumbency, safe to handle, end of anaesthetic, extubation, reversal agent delivery, sternal recumbency, and standing were also recorded on the data collection sheets (Addendum 1). Any deviations that were deemed necessary were recorded as well.

All measured and calculated variables from the i-STAT machine were recorded, printed and placed on the data collection sheets immediately.

Rescue interventions

For the duration of this study the hyena's health, welfare and safety were our highest priority. Throughout each stage of the study from immobilisation to the recovery the following potential concerns and rescue interventions were to be followed if necessary.

Potential concerns:

1. Hypotension: MAP of less than 60 mmHg
2. Apnoea: No breathing for longer than 45 sec
3. Cardiovascular collapse: Cardiac arrest or decreased heart rate (bradycardia) in combination with hypotension
4. Hypoxaemia: Decreased oxygen haemoglobin saturation (SaO_2 or SpO_2 < 90%) or arterial partial pressure of oxygen (PaO_2 <80 mmHg)
5. Hypo-/hyperthermia: < 37.0˚C or > 38.5˚C

Rescue interventions:

1. Administration of a bolus of a balanced isotonic crystalloid fluid at 10-20 mL kg^{-1} over 10 min until MAP increases
2. Tracheal intubation followed by the attachment and use of an adult ambu bag for intermittent positive pressure ventilation (IPPV)

3. Anaesthesia will be stopped immediately; reversal drugs will be administered; standard cardiopulmonary resuscitation will be initiated
4. Administration of oxygen at 2 L min^{-1} via anaesthetic machine attached to the ET tube
5. Cooling with fans and cold water or heating with blankets were available but were not necessary

Statistics

The data obtained from this study were analysed using commercially available IBM SPSS Statistical software (IBM SPSS Statistics Version 25.0; International Business Machines Corp; Released 2017; NY, USA). Normality of the data were assessed by calculation of descriptive statistics, plotting histograms, and by performing Shapiro-Wilk tests for normality. Quantitative data were described using mean ± standard deviation and ranges. Paired sample t – tests and two-way repeated measure ANOVA's were used, followed by pairwise Wilcoxon signed ranked tests with Bonferroni correction of p-values for multiple post-hoc tests in order to compare the clinical and physiological variables taken over the 60-min immobilisation period within and between the lower and higher dose treatments. Significance was determined at $p < 0.05$.

Results

All eight spotted hyenas successfully completed the trial and were deemed healthy based on visual inspection before immobilisation and clinical examination during immobilisation. The sex, body mass and age of the study animals were obtained from historical records and measurements obtained during the study period (Table 3).

Table 3: Age, sex, body mass for each session and drug dosages administered based on weights measured for each spotted hyena immobilised with a medetomidine-zolazepam-tiletamine combination (HD and LD treatment).

Animal	Age	Sex	Body mass (kg) Session I -II	Medetomidine (mg kg^{-1}) HD Treatment	LD Treatment	Zolazepam-Tiletamine (mg kg^{-1}) Session HD - LD Treatment
Hyena 1	7 yrs	Female	77.6-76.8	0.040	0.020	0.97 – 1.00
Hyena 2	2 yrs	Male	52.7-53.2	0.038	0.021	0.95 – 1.00
Hyena 3	2 yrs	Male	54.7-54.9	0.040	0.020	1.00 – 1.01
Hyena 4	4 yrs	Male	67.7-70.4	0.038	0.019	0.96 – 0.97
Hyena 5	2 yrs	Male	60.3-61.9	0.039	0.022	0.97 – 1.08
Hyena 6	4 yrs	Female	74.2-74.9	0.040	0.022	1.00 – 1.08
Hyena 7	8 yrs	Female	67.9-67.0	0.040	0.022	1.02 – 1.11
Hyena 8	6 yrs	Male	63.3-65.1	0.044	0.020	1.11 – 1.00

All the spotted hyenas were successfully immobilised using the medetomidine and zolazepam-tiletamine combinations allowing at least 66 min of handling time (66-92 min), regardless of whether they received the high or low dose of medetomidine. One LD treatment anaesthetic period was extended to 90 min due to occupation of the recovery room without spontaneous recovery or incidences. After darting, behaviour exhibited and noted as first signs of sedation were licking of the lips, yawning, the inability to support their body weight as well as uncoordinated movement of the limbs. All hyenas were consistently observed achieving acceptable anaesthetic depth based on lack of palpebral reflexes, excellent muscle relaxation

and lack of response to external stimuli and manipulation. No spontaneous recoveries were observed.

Two different hyenas (one from each dosage treatment) required an intravenous ketamine bolus to facilitate tracheal intubation as mild swallowing reflexes were observed. The two hyenas, who received the additional ketamine boluses, were included in this study. Jaw tone throughout all immobilisations never fully disappeared but were low. The immobilisation and recovery scores also did not significantly differ between the dose treatments, although the immobilisation scores induced by the HD treatment were more consistent (Table 4). The immobilisation scores induced by the LD treatment were either 1 (n = 3), 2 (n = 3) and 3 (n = 1), no immobilisation score could be recorded for the eighth hyena, and for the HD treatment were 1 (n = 7) and 3 (n = 1). Individual drug doses were calculated for each immobilisation (Table 3). Only one hyena, during the LD treatment, vomited soon after darting and mild hypersalivation was seen in all hyenas. Four individual hyenas experienced arrhythmias during the anaesthetic period. Two experienced second degree atrioventricular blocks (AV) (n=2), one from each dose treatment, and another two experienced sinoatrial blocks (SA) (n=2), one from each dose treatment. All resolved during the anaesthetic period.

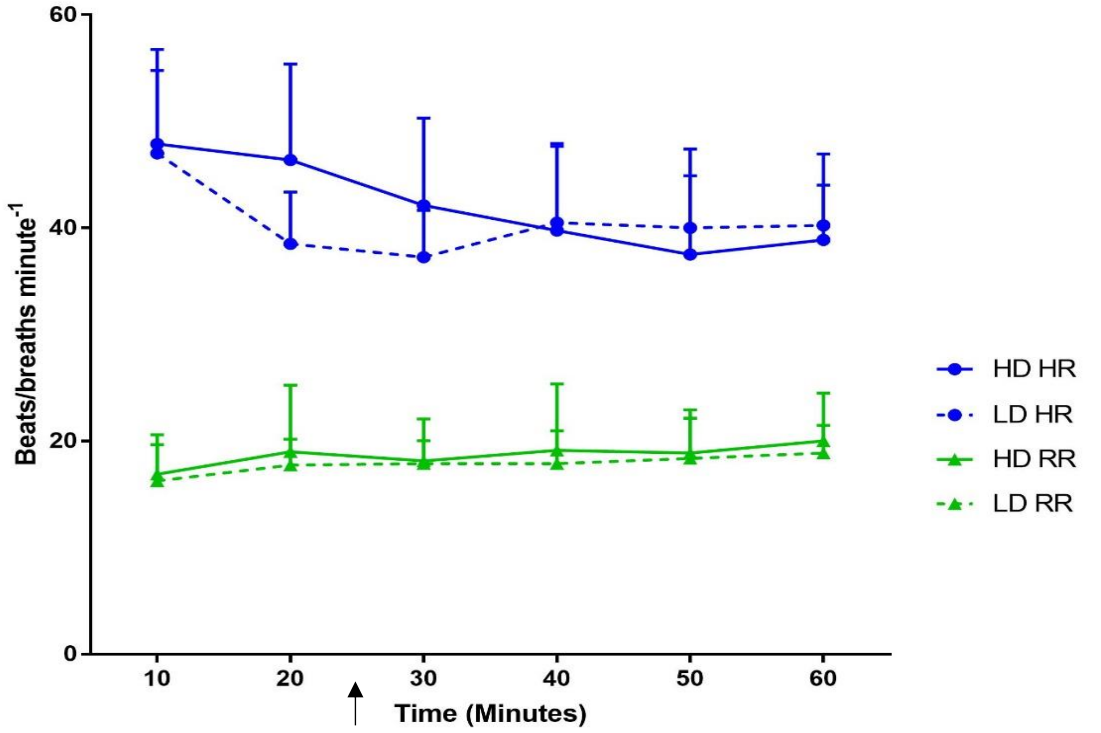
No significant differences were found when comparing the times (time feature) to first signs of sedation, recumbency, handling, sternal recumbency during recovery and standing times but the LD treatment caused a greater variability than the HD treatment for each studied time feature. After darting, all the hyenas were immobilised rapidly with minimal excitement and remained recumbent except for one individual that was highly excited before darting and attempted to stand multiple times during the LD treatment dose protocol but eventually remained recumbent after 18 min (twice as long as the second longest time to recumbency of both treatments).

*Table 4: Immobilisation features of high or low dose of medetomidine in combination with zolazepam-tiletamine to immobilise spotted hyenas (HD and LD treatment). Results are reported as mean ± standard deviation (Range) (*p<0.05, **p<0.01).*

Variables	HD Treatment	LD Treatment
Time to first signs of sedation	1.5 min ± 0.9 min (0.5 – 3.1 min)	2 min ± 1.2 min (0.7 – 4.2 min)
Time to recumbency (Immobilised)	4.2 min ± 2.1 min (0.6 – 7.4 min)	7 min ± 5.5 min (1.9 – 18.2 min)
Time to handling	8 min ± 1.5 min (5.8 – 10.4 min)	12.5 min ± 6.5 min (6.7 – 26.6 min)
Immobilisation score	1.3 ± 0.8 (1-3)	1.7 ± 0.8 (1-3)
Sternal recumbency (Recovery – after atipamezole administration)	21.8 min ± 14.9 min (6 – 44 min)	21.6 min ± 23.9 min (6 – 1 h 18 min)
Standing time	26 min ± 15 min (13 – 52 min)	26.4 min ± 23.1 min (8 – 1 h 19 min)
Early recovery score	1.5 ± 0.5 (1-2)	1.3 ± 0.5 (1-2)
Late recovery score	2.3 ± 0.5 (2-3)	2.1 ± 0.4 (2-3)

During anaesthesia muscle relaxation was excellent, mucous membranes were all pale pink and capillary refill time was less than 1 sec in all cases. Rectal temperatures ranged from 37.4 to 39.3 °C.

No differences were found when comparing the respiratory rates, inspiratory volumes, expiratory volumes, temperatures and blood pressures between dose treatments, but values were observed to have been stable over time (Table 5 and Figure 1). Inspiratory and expiratory tidal volume values decreased transiently 30 min after darting, this was not significant. No significant differences were found when comparing the heart rates between treatments, both treatment's heart rates decrease in the first 30 min with a greater drop observed during LD treatment but plateau from thereon (Figure 1a). The hyenas had an average heart rate of 42 beats min^{-1} for the HD treatment and 40 beats min^{-1} for the LD treatment, decreasing to as low as 24 and 28 beats min^{-1} during each treatment respectively. Overall the hyenas had an average MAP of 135 mmHg during the HD treatment and 124 mmHg during the LD treatment.


60
40
20
0
Beats/breaths minute-1
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20
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Time (Minutes)
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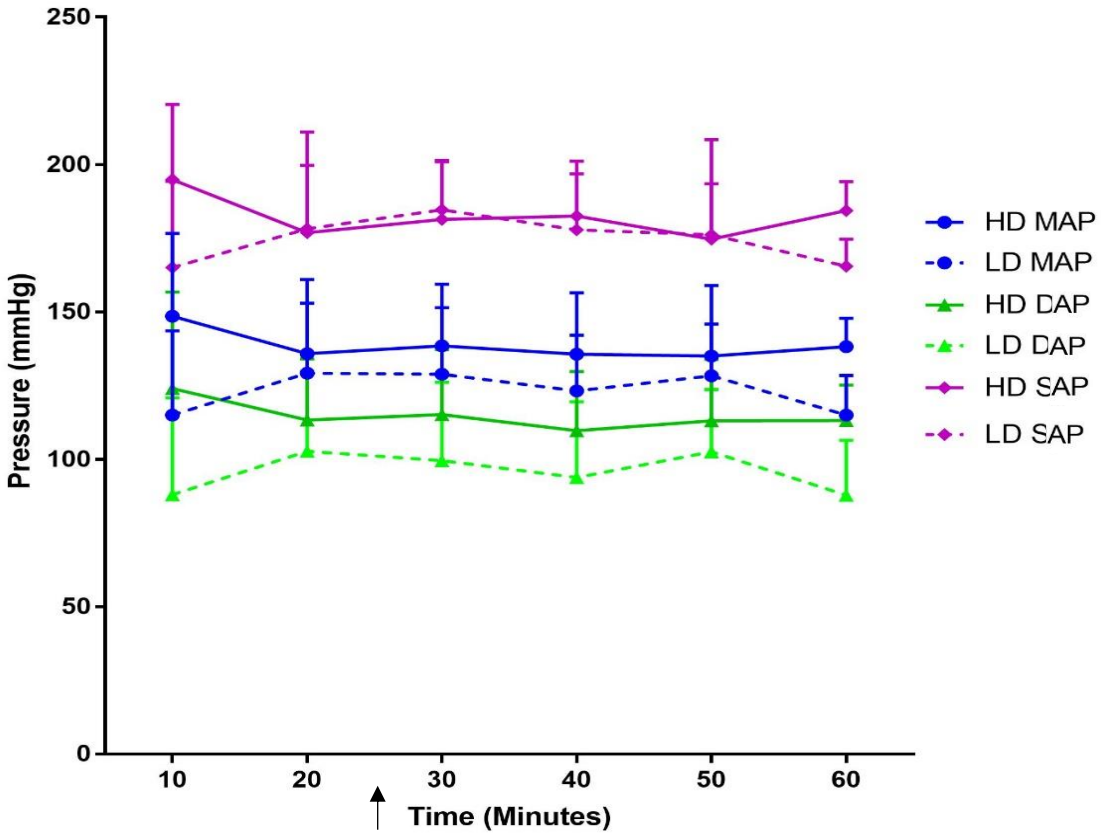
HD RR
LD RR


250
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Pressure (mmHg)
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Time (Minutes)
HD MAP
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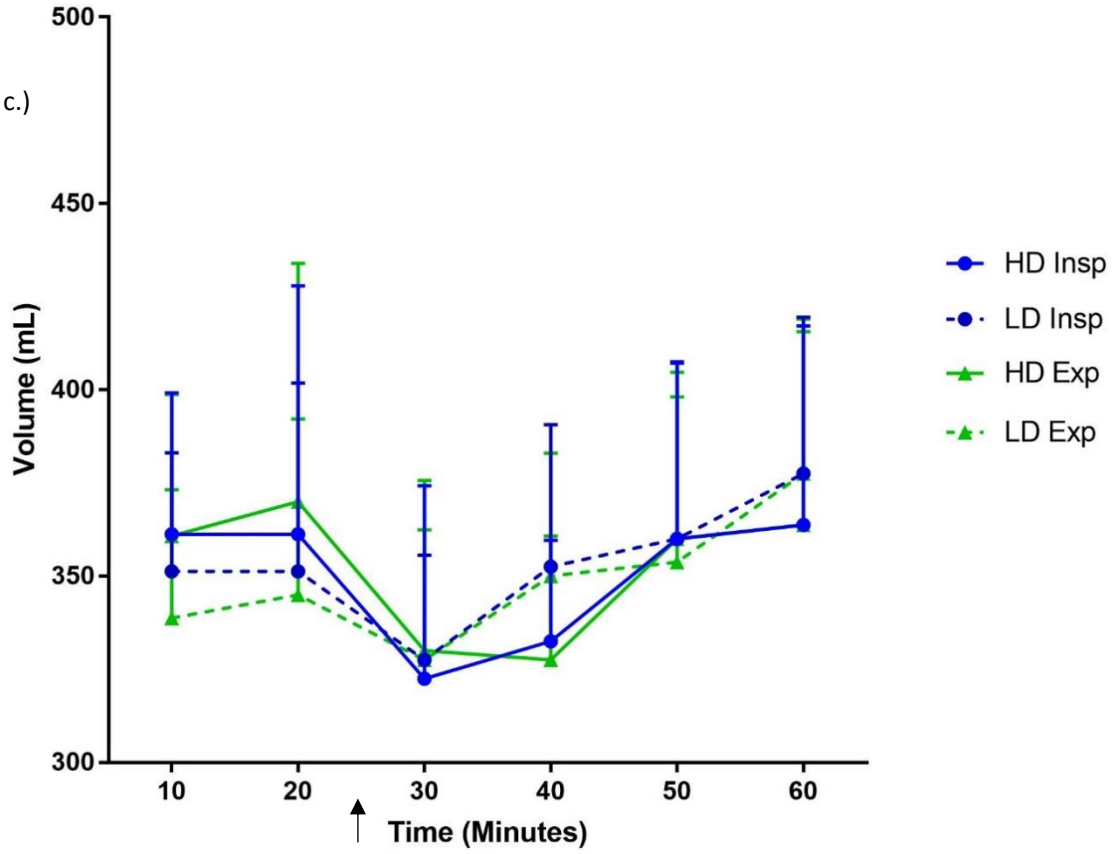

*Figure 1: Line graphs of heart – HR (beats min^{-1}) and respiratory rate – RR (breaths min^{-1}); (b) Mean arterial pressure - MAP, diastolic pressure – DAP and systolic pressure – SAP and (c) Inspiratory - Insp and expiratory - Exp volume (mL) plotted against time (minutes). Mean and standard deviation are reported at 10-minute intervals for the entire 60 minute anaesthetic period for both high (HD) and low (LD) dose of medetomidine in combination with zolazepam-tiletamine in eight spotted hyenas (*Crocuta crocuta*). Oxygen was administered at 25 minutes (arrow).*

*Table 5: Physiological variables in spotted hyenas given either a high or low dose of medetomidine in combination with zolazepam-tiletamine (HD and LD treatment). Results are reported as mean ± standard deviation (Range) (*p<0.05, **p<0.01).*

Variables	HD Treatment	LD Treatment
Heart rate (beats min^{-1})	42 ± 8.6 (24-62)	40 ± 6.6 (28-60)
Systolic BP (mmHg)	182.6 ± 21.6 (111-239)	173.2 ± 20.1 (111-222)
Diastolic BP (mmHg)	111.7 ± 22.5 (59-183)	96.9 ± 23.1 (52-139)
Mean arterial BP (mmHg)	134.5 ± 25.7 (37-201)	123.5 ± 20.0 (69-161)
Respiratory rate (breaths min^{-1})	19 ± 4.6 (12-32)	17.8 ± 3.3 (11-30)
Inspiratory volume (mL)	343.5 ± 50.6 (230-480)	354 ± 57.6 (110-550)
Expiratory volume (mL)	343.7 ± 49.9 (250-490)	349 ± 58.9 (80-580)
SpO_2 (%)**	94.9 ± 6.4 (78-100)	94.4 ± 5.0 (82-100)
End tidal CO_2 (mmHg)**	42.7 ± 5.9 (26 - 57)[a]	42.2 ± 4.3 (30 - 51)[a]
Rectal temperature (°C)	38.0 ± 3.7 (37.4-39.3)	38.5 ± 0.6 (37.4-39.0)

[a] = statistically significant difference over time; [b] = statistically significant difference between treatments

Significant differences were found with regards to some arterial blood gas variables between dose treatments, where haemoglobin (p = 0.024) and haematocrit (p = 0.025) were found to be higher in the HD treatment (Table 6). Arterial partial pressure of oxygen (PaO_2; p = 0.000), arterial partial pressure of carbon dioxide ($PaCO_2$; p = 0.000), partial pressure of alveolar oxygen (PAO_2; p = 0.000), oxygen saturation (SaO_2; p = 0.000), total carbon dioxide (TCO_2; p = 0.000), calculated $P(A\text{-}a)O_2$ (p = 0.000), fractional inspired oxygen concentration (FiO_2; p = 0.000), end-tidal carbon dioxide tension ($EtCO_2$; p = 0.034), peripheral oxygen haemoglobin saturation (SpO_2; p = 0.000), bicarbonate (HCO_3; p = 0.000) and base excess (BEecf; p = 0.000) (Table 6) changes significantly increased over time. Arterial pH significantly (p = 0.025) decreased over time, between the two blood arterial samples analysed. Ionised calcium (iCa; p = 0.016) and glucose (glu; p = 0.039) significantly increased between the 20- and 50-min blood sample collection points for both treatments.

Mild (PaO_2 of 60 – 79 mmHg) to moderate (PaO_2 of 40-59 mmHg) hypoxaemia was observed in all the hyenas (PaO_2 range of 43 - 66 mmHg, SaO_2 range of 73-91%) prior to oxygen supplementation. SaO_2, PaO_2, PAO_2 and $P(A\text{-}a)O_2$ increased in all hyena immobilised to above normal limits with a maximum (100%), eight-, seven- and seven-fold increase, respectively (Table 6). PaO_2:FiO_2 for this study, before oxygen supplementation, is approximately 271.43 based on average values observed in this study between both treatments. SpO_2 was found to slowly increase over time till oxygen supplementation was provided, where the values plateaued between 97 – 100% (Figure 2). TCO_2, BEecf and $PaCO_2$ were increased with oxygen supplementation (Table 6). Mild hypocapnia (28-35 mmHg) was observed in 5 immobilisations before oxygen supplementation. Mild hypercapnia (45 – 54 mmHg) was observed in 12 immobilisations, eight from the HD treatment and four from the LD treatment, after oxygen supplementation but $PaCO_2$ remained below 55 mmHg. The remaining $PaCO_2$ values throughout the anaesthetic period were within normal limits for dogs (Clarke et al. 2014). End tidal carbon

dioxide remained stable below 40 mmHg but increased with oxygen supplementation till it plateaued at below 45 mmHg. A pH range of 7.23 - 7.33 was recorded from the hyenas.

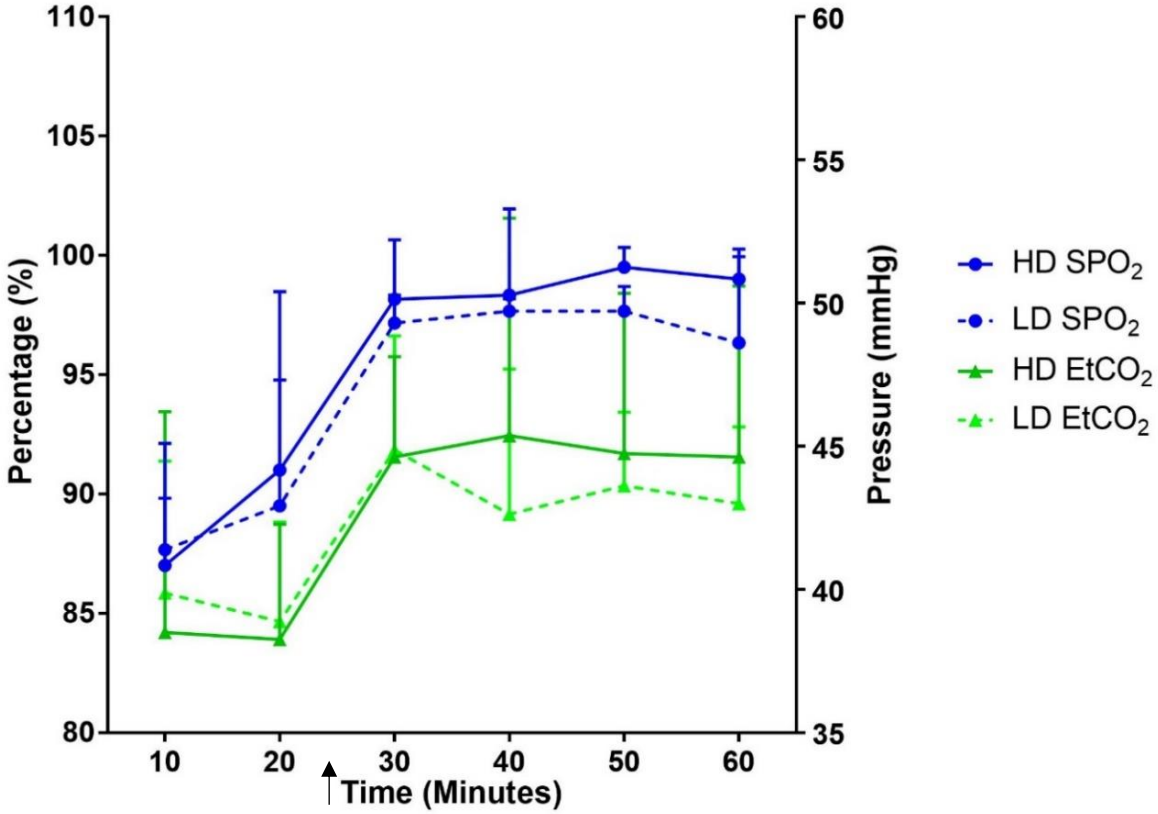

Figure 2: *Line graph of end tidal volume - $EtCO_2$ (mmHg) and SpO_2 (%) plotted against time (minutes). Mean and standard deviation are reported at 10-minute intervals for the entire 60 minute anaesthetic period for both high (HD) and low (LD) dose of medetomidine in combination with zolazepam-tiletamine in eight spotted hyenas (Crocuta crocuta). Oxygen was administered at 25 minutes (arrow).*

Only fourteen lactate measurements were made, five during the HD treatment and seven from the LD treatment, and values ranged from being too low to be read by the device and never exceeding 1.3 mmol L^{-1}. No significant differences were found, between treatments and samples, for potassium and sodium and both were within normal values (Hahn et al. 2007).

Early and late recovery scores for all hyenas were calm with few attempts to stand (Table 2). All hyenas were observed for 24 hours after recovery from anaesthesia with no ill effects reported.

One individual hyena was observed to have the longest recovery times for both treatments (almost twice as long as the second longest recovery time during each treatment).

*Table 6: Arterial blood gas results and calculated variables in spotted hyenas given either a high or low dose of medetomidine in combination with zolazepam-tiletamine. (HD and LD treatment). With and without oxygen supplementation. Results are reported as mean ± standard deviation (Range) (*p<0.05, **p<0.01).*

Variables	**HD Treatment**	**LD Treatment**	**HD Treatment**	**LD Treatment**
Time (oxygen supplementation)	**20 min (without oxygen)**		**50 min (with oxygen)**	
SaO_2 (%)** [a]	84.7 ± 4.2 (79-91)	85.3 ± 5.8 (73-89)	100 ± 0 (All 100)	100 ± 0 (All 100)
PaO_2 (mmHg)** [a]	56.0 ± 5.7 (48-66)	57.7 ± 7.1 (43-64)	450.4 ± 40.8 (382-506)	455.4 ± 52.5 (366-501)
FiO_2** [a]	0.21 ± 0.01 (0.2-0.21)	0.21 ± 0 (0.20-0.21)	0.99 ± 0.02 (0.96-1)	0.98 ± 0.02 (0.96-1)
PAO_2** [a]	81.4 ± 3.5 (82.9-129.6)	83.7 ± 5.6 (77.7-92.7)	547.1 ± 12.0 (526.3-540.1)	544.2 ± 10.1 (533.4-610.8)
$PA\text{-}aO_2$** [a]	25.4 ± 3.9 (19.3-31.7)	26.0 ± 10.0 (17.7-45.2)	96.7 ± 37.6 (42.3-157.1)	92.8 ± 53.0 (9.9-172.0)
TCO_2 (mmol L^{-1})** [a]	18.5 ± 1.6 (17-21)	17.8 ± 1.7 (16-20)	23.5 ± 2.3 (22-28)	22.8 ± 1.7 (21-25)
$PaCO_2$ (mmHg)** [a]	37.1 ± 3.2 (32.4-42)	35.1 ± 3.8 (29.5-41.5)	50.1 ± 3.4 (46.2-54.7)	47.6 ± 4.4 (42.9-53)
pH* [a]	7.28 ± 0.03 (7.2-7.3)	7.29 ± 0.01 (7.2-7.3)	7.25 ± 0.02 (7.2-7.3)	7.26 ± 0.01 (7.2-7.3)
Base excess (mmol L^{-1})** [a]	-9.2 ± 1.9 (-6-(-11))	-9.8 ± 1.5 (-8-(-11))	-5.0 ± 2.5 (0-(-7))	-5.7 ± 1.6 (-4-(-8))

Bicarbonate (mmol L^{-1})** [a]	17.5 ± 1.7 (15.7-20.2)	16.8 ± 1.5 (15.1-18.7)	22.1 ± 2.1 (20.4-26.2)	21.4 ± 1.6 (19.5-23.1)
Sodium (mmol L^{-1})	141.3 ± 3.5 (134-144)	141.9 ± 3.5 (136-144)	141.4 ± 1.0 (140-143)	143.0 ± 2.5 (140-147)
Potassium (mmol L^{-1})	4.3 ± 0.2 (4.1-4.5)	4.5 ± 0.3 (4.2-4.7)	4.4 ± 0.2 (4.1-4.7)	4.5 ± 0.3 (4.2-4.6)
Ionised calcium (mmol L^{-1})*[a]	1.4 ± 0.1 (1.3-1.5)	1.5 ± 0.1 (1.3-1.6)	1.5 ± 0.04 (1.4-1.6)	1.5 ± 0.1 (1.43-1.59)
Glucose (mg dL^{-1})*[a]	157.3 ± 21.5 (126-191)	160.9 ± 21.9 (136-192)	171.6 ± 22.3 (134-219)	166.0 ± 22.9 (139-200)
Haematocrit (%)*[b]	38.4 ± 2.9 (33-42)	37.7 ± 2.2 (36-42)	38.3 ± 2.3 (36-43)	36.1 ± 1.5 (34-43)
Haemoglobin (g dL^{-1})*[b]	13.1 ± 1.0 (11.2-14.3)	12.8 ± 0.5 (11.6-14.3)	13.0 ± 0.8 (12.2-14.6)	12.3 ± 0.5 (11.6-14.6)

[a] = statistically significant difference over time for both treatments; [b] = statistically significant difference between treatments

Discussion

The results of this investigation indicate that the combined effects of medetomidine, whether at 0.02 mg kg^{-1} or 0.04 mg kg^{-1} dose, and zolazepam-tiletamine at 1.0 mg kg^{-1} provide sufficient and partially reversible immobilisation and anaesthesia. Both treatments proved to be safe and effective for use in the spotted hyenas with a rapid and calm immobilisation, lack of mortalities, small volume of drug necessary for darting thereby minimising the risk of injury, a predictable and controlled duration of anaesthesia and a calm recovery. The dose increase of 0.02 mg kg^{-1} to 0.04 mg kg^{-1} had no influence on cardiorespiratory variables thereby assuring a high degree of safety, particularly considering that weight is usually estimated, during in field practical applications for many minimally invasive procedures in these animals (clinical examinations, vaccinations, blood collections, placement of radio collars etc). The physiological effects identified and characterised did not differ between the two dose treatments. The reduction in cost of medetomidine and atipamezole is the only real advantage with using the lower dose as opposed to the higher dose, as this can be an important consideration in some developing countries where most of these free-ranging animals inhabit.

To the author's knowledge, this is the first study taking an in-depth look at the physiological effects of this particular drug combination in spotted hyenas. It is for this reason that a comparison cannot be made with any other similar studies in this species except for a few values indicated in Hahn (2007). It was noted that there was a large difference in standard deviations between both treatments with a large number of the LD treatment standard deviations being greater than the HD treatment. The variation in standard deviations was noted with regard to the time to first signs of sedation, recumbency, handling, sternal recumbency during recovery and standing times. It was also noted that the immobilisation scores were less variable during the HD treatment, based on standard deviation values. The variation in standard deviations and immobilisations scores indicate that the HD treatment is the most predictable of the two.

No hyperthermia, frequent episodes of vomition, tonic-clonic like movements or extensor rigidity were witnessed as has been known to occur with ketamine-xylazine-atropine combinations (Hahn *et al.,* 2007). No incidences of hypothermia or hyperthermia occurred as has been found with medetomidine use in cats and wild Felidae (Fahlman et al. 2005; Johansson et al. 2013). An adverse effect that occurred during this protocol was vomition in only one hyena (6%) during the LD treatment. In terms of medetomidine use in dogs and cats, the most common adverse finding in cats is vomition (Granholm et al. 2006). Vomition after IM administration has been known to occur in 8-20% of dogs and up to 90% in cats (Cullen 1996; Grimm and Lamont 2007). With regard to medetomidine use, vomition is caused by the activation of the chemoreceptor trigger zone of the area postrema, close to the locus coeruleus in the brain (Grimm and Lamont 2007; Sinclair 2003). Mild to moderate pseudoptyalism did occur and is most likely attributed to the use of the dissociative agent.

The heart rate and blood pressure of conscious spotted hyenas has not been documented and therefore the objective comparison of those observed during anaesthesia is not possible. Normal blood pressure values for dogs and cats range from: 100 -160 mmHg for SAP, 60-100 mmHg for DAP and 80-120 mmHg for MAP, but these do vary depending on body positioning, stress levels and the method of measurement (Grimm et al. 2015). Brown et al. (2007) indicates hypertension in domestic dogs and cats as SAP being >150 mmHg. The elevated blood pressure found in the hyenas of this study are of concern as systemic hypertension has been known to damage a plethora of tissues. A severe acute hypertension may cause oedema and haemorrhage anywhere in the body, but the primary organs of concern are the lungs and brain (Brown and Henik 1998; Grimm et al. 2015). Sustained systemic hypertension has been observed to result in an increase stiffness of the arterial walls in dogs, specifically those of the coronary arteries. The kidneys are more susceptible to injury due to hypertension in dogs and cats (Brown and Henik 1998; Grimm et al. 2015). Although the preglomerular arterioles normally actively

constrict during periods of hypertension, thereby protecting the renal glomeruli, in cases of animals with renal insufficiency the preglomerular arterioles are more dilated and therefore unable to effectively respond to the changes in blood pressures (Brown and Henik 1998). This lack of response to hypertension results in glomerular hypertension which causes glomerular damage which progresses to a decrease in renal function. Furthermore, the increased afterload as a result of systemic hypertension, may result in left ventricular hypertrophy, secondary valvular insufficiency, diastolic dysfunction, ocular retinopathy or choroidopathy, retinal detachment and encephalopathy (Brown and Henik 1998; Grimm et al. 2015). Concern in this regard, including all these possible effects, is usually only raised when MAP or SAP values exceed 140 or 180 mmHg, respectively, which was not the case based on the average blood pressure results obtained in this study. It is also important to note that non-invasive techniques, such as those used in this study, do tend to indicate more variability in comparison to direct arterial blood pressure, overestimate blood pressure in hypotensive animals and underestimate SAP (Grimm et al. 2015). The cuff placement being too loose tends to elicit extremely high values due to the excessive cuff pressure needed to occlude the underlying artery (Grimm et al. 2015). Another study using direct blood pressure measurements may be warranted to further investigate these effects fully. Small vessel size due to vasoconstriction, such as that elicited by medetomidine, may also interfere with accurate measurements using the non-invasive technique (Grimm et al. 2015). The blood pressure findings can be explained by similar findings in dogs (Murrell and Hellebrekers 2005). Initially the blood pressure increases due to peripheral vasoconstriction that was brought about by post synaptic stimulation of the vascular smooth muscle α_2-adrenoceptors. This is then followed by a baroreceptor reflex mediated heart rate decrease which leads to sinus bradycardia (Murrell and Hellebrekers 2005). Higher doses of medetomidine in dogs, > 0.02 mg kg^{-1} have been known to cause longer duration hypertension that has been associated with an increase in systemic vascular resistance (Kuusela et al. 2000; Murrell and Hellebrekers 2005). This indicates that hypertension is partially dose dependant, whereas, at lower doses the blood pressure initially increases and then decreases to values

observed prior to drug administration and below, due to the central effects predominating (Kuusela et al. 2000; Grimm et al. 2015). Dissociative drugs, such as tiletamine, have also been known to increase blood pressure due to an increase in sympathetic tone, possibly further contributing to the hypertension found in the study (Clarke et al. 2014). This increased sympathetic drive also has the potential of possibly increasing the risks of cardiac arrythmias as well as increased myocardial oxygen demand, whereas, in contrast, α_2-adrenoceptor agonists prevent catecholamine-induced arrhythmias and reduce myocardial oxygen demand (Clarke et al. 2014; Pettifer and Dyson 1993).

If a comparison were to be made using the Schmidt-Nielson equation for mammals, namely 241 X $M_b^{-0.25}$, then the average heart rate calculated for hyenas, based on average weight of those in this study, would be considered as 85 beats min^{-1} (Schmidt-Nielson 1997). In this study the heart rate is 50% of the average heart rate calculated for this species, which would be considered bradycardic for the spotted hyena. Bradycardia in dogs were a common finding with medetomidine usage at a range of doses of 0.04 – 0.16 mg kg^{-1} within 2-4 min of both IM and IV administration (Vainio 1989a; Vainio and Palmu 1989b). The bradycardia has shown to be partly due to a central decrease in sympathetic tone and partly due to a vagal physiological response mediated by the baroreceptor reflex to the early stages of hypertension and increased afterload (Maze and Tranquilli 1991). The bradycardia is then followed by a decrease in myocardial oxygen consumption which ensures the heart's oxygen requirements are still met (Murrell and Hellebrekers 2005).

Two cases of second-degree AV and SA blocks each were observed during the study, but all resolved without treatment. It has been suggested that the dramatic decrease in heart rate found in dogs may be a form of sinus bradycardia (Pypendop and Verstegen 1998; Ueyema et al. 2008). Both bradycardia and associated bradyarrhythmias, such as first- and second-degree AV blocks, have commonly been found in multiple medetomidine studies in dogs (Pypendop and

Verstegen 1998; Short 1992; Sinclair 2003; Ueyema et al. 2008). The bradycardia with associated bradyarrhythmias as well as cardiac output reduction is a physiological change brought about by the baroreceptor mediated reflex induced by the initial hypertension caused by medetomidine (Pypendop and Verstegen 1998; Sinclair 2003). These arrhythmias have been deemed non-life threatening (Sinclair 2003). Multiple studies indicate that the decreased cardiac output induced by these drugs do not cause hypoperfusion of any vital organs in healthy animals as the blood flow to these organs are preserved at the expense of less vital organs (Grimm et al. 2005; Lawrence et al. 1996).

Domestic and wild canids and felids have also been known to experience bradycardia, arrhythmias and, or hypertension, which have been deemed clinically insignificant, commonly observed and attributed to medetomidine use (Fahlman et al. 2005; Kreeger and Seal 1986; Kreeger et al. 1987; Larsen et al. 2002; Murrell and Hellebrekers 2005; Semjonova et al. 2017; Semjonova et al. 2018; Sladky et al. 2000; Stegmann and Jago 2006; Wenger 2010). Sustained hypertension has in fact been known to occur when α_2 -adrenoceptor agonists are used in combination with dissociative drug (Larsen and Kreeger 2007).

Medetomidine has shown to induce a decreased central nervous system sympathetic outflow and increase parasympathetic vagal tone. The changes of the sympathetic and parasympathetic tones are secondary to increases in systemic vascular resistance and cardiac work due to marked vasoconstriction and decrease cardiac output (Hahn et al. 2007; Mich et al. 2008; Murrell and Hellebrekers 2005; Ueyema et al. 2008). This vasoconstriction side effect was further confirmed during the trial period as multiple attempts were necessary at times to obtain readings of heart rate, blood pressure and SpO_2 values from some of the equipment used to monitor around peripheral regions of the body such as the tail, tongue and limbs (Fahlman et al. 2005). It is important to note that the indirect measurement of blood pressure is usually considered

unreliable due to the bradycardia and vasoconstriction induced by α_2-adrenoceptor agonists (Clarke et al. 2014).

The lack of significant differences between the cardiovascular variables during the LD or HD treatment effects are consistent with a study in dogs, which also found that once a particular threshold was reached (0.05 mg kg^{-1} in canines) the maximum cardiovascular effects were obtained and increasing the dose further did not significantly influence the degree of cardiovascular changes and will not elicit a more extreme response, proving that the dose dependency on cardiovascular variables is limited (Pypendop and Verstegen 1998).

Mild to moderate hypoxaemia was recorded in all hyenas based on blood gas analysis before oxygen supplementation, regardless of the dose of medetomidine administered. Hypoxaemia specifically refers to the insufficient oxygenation of blood that is necessary to meet metabolic requirements. Hypoxaemia may result from ventilation/perfusion mismatching, decreased fraction of inspired oxygen, airway obstruction, shunting, hypoventilation, diffusion impairment, abnormal haemoglobin such as with methaemoglobinaemia or an increase in oxygen consumption (Lee and Drobatz, 2004; Mich et al. 2008; Moller et al. 1991; Mosley and Gunkel 2007). $EtCO_2$ values above 50 mmHg act as predictive indicators for hypoventilation, in this study average $EtCO_2$ values never exceeded 50 mmHg and $PaCO_2$ values were within normal range for mammals (Lee and Drobatz, 2004). No indications of hypoventilation, methaemoglobinaemia or airway obstructions were observed. The fraction of inspired oxygen was always maintained at an average of 21% throughout all anaesthetic procedures prior to oxygen supplementation, after oxygen supplementation it was then increased to an average of 98%. Both ventilation/perfusion mismatches and physiological shunting have been known to cause increased venous admixtures as a result of α_2-agonist administration (Mich et al. 2008). The alveolar-arterial oxygen tension differences that were calculated do indicate that the hypoxaemia observed is due to an impaired oxygen exchange, as gradients greater than 20

mmHg indicate a venous admixture. PaO_2:FiO_2 can act as a rough indicator of a shunt if the value is <200 and in this study the value is greater than 200. The A shunt was ruled out as supplementation of oxygen caused an increase in PAO_2 thereby suggesting that the cause of the hypoxaemia is likely to be due to a ventilation-perfusion mismatch. Ventilation-perfusion mismatches are the most common cause of hypoxaemia in anaesthetised and immobilised patients as they result in an insufficient gas exchange thereby impairing oxygen and carbon dioxide diffusion (Lee and Drobatz 2004; Mosley and Gunkel 2007). A mismatch is common in immobilised patients as with recumbent animals the normal compensatory mechanisms are changed which may cause gas exchange to become less effective than usual. Some of the identified factors that cause this are anatomical features, drug effects and body positioning. It is usually suggested that patients be positioned to minimise pressure placed on the thorax such as the sternal or lateral recumbency, these animals were placed in lateral recumbency. Based on the results obtained from this study, it is therefore recommended that oxygen supplementation be provided in order to counteract the hypoxaemia observed during anaesthesia over prolonged periods of time with medetomidine-zolazepam-tiletamine combinations. Inspiratory and expiratory tidal volume values decreased 30 min after darting but this was attributed to the supplementation of oxygen occurring at the time which affected the initial readings.

All hyenas initially experienced a pH <7.35 with a decreased base excess and bicarbonate levels, with a $PaCO_2$ within normal range. But with oxygen supplementation base excess, bicarbonate and $PaCO_2$ increased and pH decreased slightly. Wild felids and domestic cats are strictly carnivorous and research in cats indicate that amino acids containing sulphur that are metabolised, contribute to metabolic acidosis as these sulphurs containing amino acids produce additional acid when catabolised (Cook et al. 1996; Fahlman et al. 2005). The catabolism of acid containing sulphur has been found to produce 2 mol of hydrogen ions for each mole of amino acid that has been catabolised to sulphate. Cats in particular, have been found to selectively

choose or change their diets in order to maintain their acid-base homeostasis (Cook et al. 1996). Compounds containing histidine, essential amino acids such as carnosine and anserine found exclusively in the muscle and nervous tissue of animals, have been known to be excellent pH buffers. The pH <7.35 could be correlated to the diet and this could possibly be a normal occurrence in the spotted hyenas as they too are strict carnivores (Cook et al. 1996; Fahlman et al. 2005).

Haematocrit, haemoglobin, total carbon dioxide, sodium and potassium levels measured in all the hyenas were within normal range with glucose levels being slightly above normal based on the serum chemistry results in Hahn et al. (2007). Hyperglycaemia has been identified as a typical neuroendocrine response in α_2-adrenoceptor agonists, due to a decrease in insulin release and cell response but has been classified as not clinically significant (Abdel el Motel and Sharp 1985; Grimm and Lamont 2007; Osman and Nicholson 1991). Although, a decrease in circulating insulin does influence potassium concentrations. It was observed by Gunkel and Lafortune (2007) that initially in cheetahs, tigers and cougars, no clinical signs are present but overtime if hyperkalaemia is left untreated and coupled with pronounced acidosis, cardiac arrest may occur. Therefore, it is suggested that in these species blood gas monitoring should occur every 15-30 min under prolonged anaesthetic conditions (Gunkel and Lafortune 2007). Medetomidine has been observed to suppress both sympathoneural and adrenomedullary activities decreasing the levels of catecholamines, along with decreased insulin release and lipolysis, and an increase in plasma levels of glucose (Ambrisko and Hikasa, 2002a; Ambrisko and Hikasa, 2002b). It was also noted that medetomidine had an increased efficacy in reducing plasma epinephrine levels in a dose dependant manner (Ambrisko and Hikasa, 2002a; Ambrisko and Hikasa, 2002b). This is a possible concern as a decrease in corticosteroids could result in an Addisonian-like crisis where potassium would increase with associate bradycardia.

It was noted that a mild increase of ionised calcium occurred over time after drug administration (Table 6). Studies have indicated that ionised calcium does change in relation to changes in pH (Wang et al. 2002). Changes in pH have been found to alter the fraction of acidic amino acid residues that are charged on albumin thereby altering the amount of calcium ions bound and free. Decreases in pH cause ionised calcium to rise. The value of ionised calcium can be mathematically corrected for pH value i.e. Corrected iCa (at pH 7.4) = Measured iCa x [1-0.53 x (7.40 - measured pH)] but the calculation is only valid for measurements within 7.2 – 7.6 pH values (Baird 2011). The corrected ionised calcium values based on pH value is 1.37 ± 0.083 mmol L^{-1} (1.18 – 1.52).

Lactate has proven to be a marker of tissue oxygenation in dogs and cats. Lactate values in those hyenas tested in this study never exceeded 1.3 mmol L^{-1} which is well within the normal range of 0.3- and 0.5-2.5 mmol L^{-1}, in dogs and cats respectively. The lactate values obtained indicated that despite the hypoxaemia experienced by these animals, adequate tissue oxygenation still occurred (Siragusi et al. 2017).

Atipamezole effectively antagonised the effects of medetomidine at five times the dose of medetomidine administered and recovery of all animals were uneventful. It was observed that the matriarch of the pack took the longest to reach sternal recumbency out of all the hyenas during both dose treatments. This could be due to the stripping of sympathetic tone caused by medetomidine use as well as a delay with recovery of noradrenaline. It was also noted that the one individual hyena with the longest time to recumbency, during the lower dose immobilisation, was also the lowest on the hierarchy in the pack and therefore always picked one by his pack members. No spontaneous recoveries occurred in the one immobilisation case that experienced a delayed reversal agent administration, due to occupied recovery rooms.

It was also noted that the aseptic placement of indwelling catheters (21-gauge), percutaneously into the cephalic vein were more difficult than placement in the jugular vein, most likely due to the complex anastomoses present in the cephalic veins of hyenas (Hahn et al. 2007).

Overall there were little observed differences in physiological effects between the LD and HD treatments containing different doses of medetomidine. Some limitations of this study; due to the use of wild animals as the trial subjects, was that baseline measurements could not be obtained before the use of the above-mentioned protocol for immobilisation for comparative purposes; the difficulty recording the first 5 - 10 min of data after immobilisation due to time taken to retrieve the animal as well as the use of indirect as opposed to direct technique used to obtain blood pressure measurements. This thereby limits our ability to fully interpret the physiological effects of the protocol developed in comparison to baseline physiological variables for this species.

Conclusion

The medetomidine-zolazepam-tiletamine combination provided a reliable and controlled immobilisation and anaesthesia in the captive spotted hyena for potential use in the field, regardless of whether a high or low dose of medetomidine was used. Considering the risk of hypoxaemia, possibly due to ventilation/perfusion mismatch, and bradycardia it is suggested that cautious monitoring and oxygen supplementation are essential when using this protocol. All cardiovascular effects were expected, and within acceptable clinical range based on observations noted in other wildlife and domestic species, and possibly related to medetomidine use. The physiological effects do not differ when the drug dose of medetomidine is increased from 0.02 mg kg^{-1} to 0.04 mg kg^{-1}. A possible adverse effect is vomition which only occurred in 6% of the hyenas immobilised. All blood pH's were <7.35, most likely related to the strict carnivorous diet of the spotted hyena and the mild hypercapnia experienced. Atipamezole

was found to effectively antagonise the effects of medetomidine at five times the dose of medetomidine administered. The one advantage of using the low dose medetomidine treatment is the reduction in cost for both medetomidine and atipamezole, which can be an important consideration in developing countries where these free-ranging animals inhabit.

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Addendum

Contents:

Tasneem Anthony Tel: 076 152 9834

DATA COLLECTION SHEET FOR HYENA STUDY – Session _____ **Date: _____**

Patient Identification:_____ **Sex: _____** **Age: _____** **Weight: _____**

Immobilization Agent: Zolazepam/Tiletamine **Dose: _____** **Amount: _____** **Route: _____** **Impact Site: _____** **Time: _____**

Immobilization Agent: Medetomidine **Dose: _____** **Amount: _____** **Immobilisation quality score: ____** **Time to first sign of sedation: _____**

Time to recumbency: _____ **Safe to approach time: _____** **Fluid Therapy: _____** **Dose: _____** **Drip rate: _____**

Additional Medication: _____ **Dose: _____** **Amount: _____** **Route: _____** **Time: _____**

Additional Medication: _____ **Dose: _____** **Amount: _____** **Route: _____** **Time: _____**

Actual time																	
Time	**0**	5	10	15	**20**	25	30	35	**40**	45	50	55	**60**	65	70	75	**80**
Anaesthetic plane																	
CRT																	
Heart rate																	
Resp rate																	
BP – Sys/Dia																	
BP - Mean																	
SpO_2																	
Et CO_2																	
Temp																	
O_2 Flow																	
FiO2																	
Insp Vol																	
Expir Vol																	
Blood gas sample																	

Tasneem Anthony Tel: 076 152 9834

Reversal Agent: Atipamezole **Dose:** _____ **Amount:** _____ **Route:** _____ **Time:** _____

ET Tube size: _____ **Sternal recumbency time:** _____ **Standing Time:** _____ **Early Recovery Score:** _____

Extubation Time: _____ **Late Recovery score:** _____

Comments:

_____

_____

_____

_____

_____

Scoring Tables:

Table 1: Scoring system for the capture and immobilisation quality

Immobilisation quality during capture	
Score	Description
1	Calm, no excitement, remains recumbent once down
2	Mild excitement before recumbency, stays recumbent once down
3	Marked excitement, multiple attempts to stand after period of recumbency
4	Intense excitement, no sign of recumbency

*Excitement such as thrashing, running or fighting

Table 2: Scoring system for early and late recovery phases

Early recovery		Late recovery	
Score	Description	Score	Description
1	A calm transition to an alerted state, one or two attempts to stand	1	No ataxia, normal movements
2	Quiet, easily startled, one or two attempts to stand	2	Mild ataxia, gait mostly normal or quickly returns to normal
3	Uncoordinated movements, easily startled, paddling, multiple attempts to stand	3	Moderate ataxia, unsteady gait movements
4	Thrashing, delirium, multiple attempts to stand	4	Pronounced ataxia for a long period of time after release (>10 minutes)

Animal Ethics Approval Certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Cardiovascular effects of high and low medetomidine doses in combination with zolazepam/tiletammine for the immobilization of spotted hyenas**
PROJECT NUMBER	**V091-17**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr Tasneem Anthony**

STUDENT NUMBER (where applicable)	**u_10352547**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPESIES	**Spotted hyena (*Crocuta crocuta*)**
NUMBER OF ANIMALS	**15**
Approval period to use animals for research/testing purposes	**Sept 2017- Sept 2018**
SUPERVISOR	**Dr Adrian SW Tordiffe**

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 31 August 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15