Ketamine-butorphanol-medetomidine versus butorphanol-midazolam-medetomidine immobilisation of serval (*Leptailurus serval*)

By

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Abstract

Objective To compare ketamine-butorphanol-medetomidine (KBM) to butorphanol-midazolam-medetomidine (BMM) for chemical capture (immobilisation) of serval (*Leptailurus serval*).

Study design Blinded, randomised immobilisation trial.

Animals 23 free-ranging captures (KBM: 5 females, 6 males; mean weight 10.7 kg; BMM: 10 females, 2 males; mean weight 9.6 kg).

Methods Free-ranging serval were cage trapped and then immobilised using the randomly assigned drug combination delivered via a blow dart into the gluteal muscles. Prior to darting, a stress score was assigned (0: Calm; to 3: markedly stressed). The drug combinations were dosed based on estimated body weights: KBM - 8.0, 0.4 and 0.08 mg kg^{-1}, respectively; BMM - 0.4, 0.3 and 0.08 mg kg^{-1}, respectively. Time to first handling, duration of anaesthesia and recovery times were recorded. Physiological variables were recorded at five-minute intervals and arterial blood was sampled 20 minutes after instrumentation for arterial blood gas analysis. Atipamezole (5 mg kg^{-1} medetomidine) and naltrexone (2 mg kg^{-1} butorphanol) were administered intramuscularly for recovery. Data, presented using mean ± standard deviation values, were analysed using student t-test, general linear mixed model and Spearman's rank correlation.

Results The dose based on actual body weights were 8.7 ± 1.5, 0.4 ± 0.08 and 0.09 ± 0.02 mg kg^{-1} for KBM; and 0.5 ± 0.07, 0.4 ± 0.01 and 0.09 ± 0.05 mg kg^{-1} for BMM. Time to first handling was 611 ± 165 seconds for KBM and 800 ± 228 seconds for BMM (p = 0.033). Both combinations produced a physiological stable immobilisation that lasted for at least 35

minutes. Recovery was rapid and calm overall, but ataxia was noted in KBM. Stress score was positively and strongly correlated to blood glucose (r^2 = 0.788; p = 0.001) and temperature (r^2 = 0.634; p = 0.015).

Conclusion and clinical relevance Both combinations produce similar effective immobilisation that were physiologically stable in serval. Overall, BMM is recommended because it is fully antagonisable. It is essential to provide a calm, quiet environment before drug administration to avoid capture-induced hyperglycaemia and hyperthermia.

Keywords: Serval, ketamine-butorphanol-medetomidine, butorphanol-midazolam-medetomidine, stress induced hyperglycaemia, stress induced hyperthermia.

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List of abbreviations

α	alpha
%	percentage
°C	degrees Celsius
BE	base excess
BMM	butorphanol-midazolam-medetomidine
Ca^{2+}	ionised calcium
cHb	carbamylated haemoglobin
$cHCO^{3-}$	bicarbonate
Cl	chloride
cm	centimetre(s)
Creat	creatinine
Dia BP	diastolic arterial blood pressure
E	East
$P_E'CO_2$	end tidal carbon dioxide
GPS	global positioning system
Hct	haematocrit
IM	Intramuscular
IV	Intravenous
KBM	ketamine-butorphanol-medetomidine
Kg	kilogram(s)
L $minute^{-1}$	litre(s) per minute
m	meter(s)
max	maximum
Mean BP	mean arterial blood pressure
mg	milligram(s)
mg kg^{-1}	milligram(s) per kilogram
mg mL^{-1}	milligram(s) per millilitre
min	minimum
ml	millilitre(s)
ml kg^{-1}	millilitre(s) per kilogram
ml kg^{-1} hr	millilitre(s) per kilogram per hour
mm	millimetre(s)
mmHg	millimetres Mercury
n	number of animals
Na	sodium
NIBP	non-invasive arterial blood pressure
NR	no reading
$PaCO_2$	arterial partial pressure carbon dioxide,
PaO_2	arterial partial pressure oxygen
pH	negative log of hydrogen ion concentration
S	South
SpO_2	peripheral oxygen-haemoglobin saturation
Sys BP	systolic arterial blood pressure
T	time intervals between data collection points in minutes
Temp	temperature

Introduction

To date, very little research has been done on serval (*Leptailurus serval*) in their entirety and even less comparing different drug protocols used to chemically capture (immobilise) them. A possible reason for this is the relative difficulty associated with studying free-ranging felids, which include, limited areas in which these cats are found, difficulties in hand capture and darting and their nocturnal behaviour (Herbst & Mills 2010). This paucity presented a great opportunity to do further investigation into the use of different sedative drugs and their effects on the cardiopulmonary systems, duration and quality of immobilisation, and quality of recovery.

There has been no formal research on the appropriate drug combinations to immobilise and induce general anaesthesia in free-ranging serval. Serval are medium sized felids, they are too small to compare to other large felids and too big to compare with domestic cats (*Felis catus*), allometric extrapolation of immobilisation drug doses from other size cats is ineffective and unpredictable (Carregaro et al. 2016). Furthermore, most of the published drug doses used in serval are from habituated populations which often require conservative doses to achieve immobilisation compared to free-ranging animals (Langan et al. 2000). Therefore, investigating efficacy and safety of different drug combinations for immobilisation provides us with future opportunities to study these illusive free-ranging cats safely and collect valuable data that can aid conservation efforts in the future.

Literature Review

Serval

Serval are approximately 96-120 cm in length from shoulder to tail and can weigh in the range of 8-13 kg (Stuart & Stuart 2015). They are typically found in savannah areas closely associated with abundances of water, reed beds and prey, especially rodents (Stuart & Stuart 2015). Their specific habitats and limited geographic locations make them particularly susceptible to habitat destruction, urbanisation and agricultural development (Stuart & Stuart 2015; Thiel 2015). Serval are currently on the least threatened list on the International Union for Conservation of Nature (IUCN) red list (Thiel 2015). A decline in serval population can result in a rapidly growing rodent population which could translate into an increase in rodent associated disease prevalence and crop destruction (Geertsema 1984).

Carnivore populations are under increasing threat because of habitat destruction and fragmentation (Creel 2001; Edwards et al. 2018). Furthermore, human activities including poaching and illegal selling of their skins, exotic pet trade, rodenticides and hunting serval by farmers have further negative impacts on serval populations (Geertsema 1984; Bowland 1990; Ramesh & Downs 2013; Thiel 2015). With serval restricted to a niche environment, they are particularly vulnerable to habitat destruction (Edwards et al. 2018), and like so many other species, we could imminently start witnessing declines in their numbers. With the limited knowledge we have on serval we are not well equipped in helping conserve this species. There is a recognised need for an increase in conservation efforts for this species (Thiel 2015; Edwards et al. 2018). Research of safe immobilisation protocols and capture techniques provide us with opportunities to safely and intimately study these illusive felids. Immobilisation of serval provide opportunities for collaring and tracking giving further insight

into their home ranges, distributions, social dynamics and hunting patterns which will prove useful in future conservation efforts.

Immobilising Drugs of Interest

Due to the fact that serval are free-ranging animals, they are fractious, and handling poses a great threat to both them and the handler alike. As a result, intravenous (IV) induction is dangerous, logistically challenging to near impossible in free-ranging serval. The preferred method of administrating drug(s) used to immobilise free-ranging animals, like serval, is via the intramuscular (IM) route through darting or pole syringe. These drug delivery techniques of injection reduce the risks of injury to both the handler and the serval, and reduces the amount of anxiety caused by physical restraint prior to injection. However, serval are lightly built with thin muscle masses, therefore, care must be taken to use low-impact darting systems with the capability to scale down the velocity of the dart delivered (Kock & Burroughs 2012). The volume of dart is also important to consider, as low volumes of drug also contributes to less kinetic energy (Kinetic energy = 1/2Mass x Velocity^2). Drug combinations give us the ability to produce a balanced anaesthesia, providing multiple beneficial general anaesthetic properties such as amnesia, muscle relaxation, unconsciousness and analgesia (Bednarski 2011). With the multiple properties associated with the use of drug combinations, dosages and subsequent drug volumes of individual anaesthetics necessary for adequate immobilisation are significantly reduced, which, has the added benefit of reducing physiological adverse effects associated with higher dosages of each anaesthetic drug when administered alone (Armitage-Chan 2008).

Currently the dissociative anaesthetic cyclohexylamines (ketamine and tiletamine) are the primary drugs of choices used for immobilising serval. Often, these drugs are combined with

sedatives that have muscle relaxant properties, such as the α_2-adrenergic receptor agonists (xylazine, medetomidine) or benzodiazepines agonists like midazolam or zolazepam (already combined with tiletamine in a product called Zoletil).

Ketamine

Ketamine (domestic cat dose 5-10 mg kg^{-1}), an arylcyclohexylamine derivative is a commonly used anaesthetic drug for a broad range of species, including domestic cats, and causes dose-dependent central nervous system depression (Kastner 2007; Kock & Burroughs 2012; Muir et al. 2013). Advantages of ketamine include excellent somatic analgesia, cardiovascular stability and minimal respiratory effects when administered within the recommended dose range (Kastner 2007; Armitage-Chan 2008). Cardiopulmonary effects become evident when administered at doses that are much higher than that needed for anaesthesia or analgesia (Armitage-Chan 2008). At excessive dosages adverse effects such as seizures have been described (Taylor et al. 1998; Muir et al. 2013). Dissociative anaesthetics like ketamine are suitable for IV and IM administration and have a relatively rapid onset of action; however, duration of action is usually short lived in felids, with effects wearing off after approximately 20-45 minutes (Kreeger 1996; Gunkel & Fortune 2007; Ramsay 2014). Some reflexes (pinnal and ocular reflexes) are retained during immobilisation with ketamine and some animals may demonstrate hyper-responsiveness to sound (Kastner 2007, Muir et al. 2013). It is therefore imperative to maintain a quiet environment to facilitate induction (Ramsay 2014). In dogs, ketamine is primarily metabolised by the liver into the main active metabolite, norketamine, which is excreted by the kidneys (Kastner 2007; Ko 2013; Muir et al. 2013). In domesticated cats, there is limited metabolism by the liver and ketamine is mostly excreted unchanged via the urinary system (Kastner 2007). This is possibly due to the fact that domestic cats lack the

P450 enzyme to metabolise ketamine and we speculate this may be similar in serval (Meyer & Fish 2008).

Despite the proposed safety and other advantageous qualities of ketamine, there are known adverse effects that include excessive salivation, spontaneous tonic-clonic spasms, muscle tremors and hypertonia (Plumb 2008; Muir et al. 2013). Dissociative anaesthetics should not be used alone in felids because of these undesirable adverse effects (Kastner 2007). Ketamine is therefore routinely administered in combination with sedatives (benzodiazepine agonists or α_2-adrenergic receptor agonists) which improves muscle relaxation and quality of recovery (Kastner 2007; Armitage-Chan 2008; Muir et al. 2013; Ramsay 2014).

There are no antagonist drugs for ketamine and recoveries in cats can be prolonged with ataxia and hyperexcitability (stormy) (Kastner 2007; Muir et al. 2013; Ramsay 2014). However, cats are reported to tolerate ketamine better than dogs (Ko 2013). Ketamine administered in combination with a sedative reduces the requirement for the amount of ketamine needed for anaesthesia and thereby improves the recovery characteristics (Ko 2013).

Butorphanol

Butorphanol (0.2-0.8 mg kg^{-1} domestic cat dosage) is a synthetic opioid with partial mixed kappa-agonist and mu-antagonist activity and is widely used for its sedative effects (Kerr 2007; Plumb 2008; Bush et al. 2012). Through binding kappa-receptors, butorphanol's agonistic properties in the central nervous system, provides visceral but poor somatic analgesia in cats (Kerr 2007; Ramsay 2008; Kock & Burroughs 2012). Respiratory depression, loss of thermoregulatory homeostasis, nausea and vomiting are common side effects encountered in cats that have received pure mu-receptor agonists (morphine, fentanyl,

hydromorphone). The severity of side effects largely depend on which opioid is used and the subsequent dose that was administered. Butorphanol is a mixed agonist-antagonist that produces less severe cardiopulmonary side effects compared to other pure mu-receptor agonists and vomiting after administration in cats is rarely described (Ramsay 2008; Bush et al. 2012). The associated respiratory depression and analgesic effects are dose-dependent and is characterised by a 'ceiling effect', where further dose increases do not result in increases in respiratory depression or analgesia (Kerr 2007; Bush et al. 2012).

Butorphanol has synergistic effects when administered in combination with α_2-adrenergic receptor agonists by improving the overall sedative effects in domestic cats (Papastefanou et al. 2015). Butorphanol properties are characterised by a 'ceiling effect', with increasing doses, there is no reciprocal increase in analgesic properties and respiratory depression (Kerr 2007; Sawyer 2008; Bush et al. 2012). Large cats appear to be more sensitive to butorphanol (Ramsay 2008). The drug effects of butorphanol are antagonizable through the administration of naltrexone, proving advantageous should there be an adverse anaesthetic reaction and is beneficial for rapid and complete recovery (Ramsay 2008).

Midazolam

The two most common benzodiazepine agonists used in chemical capture of free-ranging felids are diazepam and midazolam (Ramsay 2014). Midazolam is considered more potent and a better sedative compared to diazepam (Kock & Burroughs 2012). Midazolam (0.2-0.3 mg kg^{-1} domestic cat dosage) is a benzodiazepine agonist belonging to the imidazol compound group that facilitates the action of gamma-aminobutyric acid causing depression of the limbic system resulting in sedative effects and muscle relaxation (Murrell 2007; Meyer & Fish 2008; Ko 2013). In humans, midazolam is known to cause amnesia and it is uncertain if it has a

similar effect on free-ranging felids (Meyer & Fish 2008; Ramsay 2014). If this is true, amnesic properties can be beneficial as it may facilitate future captures if free-ranging animals are required to be captured again (Kock & Burroughs 2012; Ramsay 2014). Benzodiazepine agonists duration of action is short lived and they have no analgesic properties (Ko 2013). Benzodiazepine agonists have no effect on the peripheral nervous system and only produce neural inhibition affecting the central nervous system function, as a result, there are limited systemic side effects associated with its use (Kock & Burroughs 2012).

Due to its water-soluble properties, midazolam can be mixed with other anaesthetic drugs and is readily absorbed when administered intramuscularly (Ko 2013; Ramsay 2014). Midazolam can also be administered orally to carnivores using tainted bait, which is often done prior to immobilisation and results in good sedation (Kock & Burroughs 2012). However, if benzodiazepine agonists are administered intramuscularly or intravenously alone, it can result in unpredictable sedation in healthy domestic cats, instead it may produce paradoxical excitement or aggression so it is therefore recommended to be given in combination with other sedatives (Ilkiw et al. 1996; Murrell 2007; Kanda & Hikasa 2008; Bednarski 2011; Ko 2013). The drug effects of midazolam can be reversed by administering an antagonist, flumazenil, however, its expense precludes its routine use (Bednarski 2011; Kock & Burroughs 2012).

Medetomidine

Medetomidine (0.005-0.12 mg kg^{-1} domestic cat dosage), a selective α_2-adrenergic receptor agonist from the imidazoline derivative group of drugs and is commonly used for its effective sedative, muscle relaxation and analgesic properties which, significantly improves handling of animals (Murrell 2007; Sawyer 2008; Ko 2013). The sedative and analgesic properties of

medetomidine are dose-dependent, and with increasing doses there is a resultant increase in intensity of these properties (Murrell 2007; Ko 2013). However, a plateau effect is reached, where further dose increases result in an increase in the duration of affect and not intensity (Murrell 2007). Medetomidine reduces the dose required of drugs used for induction of general anaesthesia (Murrell 2007; Sawyer 2008).

Medetomidine produces a number of undesirable cardiovascular effects which include: intense vasoconstriction, hypertension and reflex bradycardia in cats (Sinclair 2003; Murrell 2007; Ko 2013). The effects on blood pressure are biphasic, with an initial increase followed by a normotensive or mild hypotensive state (Murrell 2007). The resultant bradycardia occurs in two phases; the initial phase results in a decrease in heart rate mediated by the baroreceptor reflex in response to the increase in blood pressure and the second phase is due to a reduction in sympathetic tone as a result of decreased noradrenaline (Murrell 2007). These effects are thought to be contributing factors in the cause of a decrease in cardiac output and reduced blood flow to many organs which negatively impacts oxygen delivery (Sinclair 2003; Murrell 2007). The advantage, despite the decrease in cardiac output, is that α_2-adrenergic receptor agonists, like medetomidine, also reduces tissue oxygen demand and consumption as well as maintaining perfusion to certain vital organs, therefore mitigating the decreased tissue perfusion (Gregoretti et al. 1992; Murrell & Hellebrekers 2005). In addition to the adverse cardiovascular effects, medetomidine commonly causes emesis, increased urine production, transient hyperglycaemia and myoclonic twitching (Sawyer 2008, Murrell 2007, Ko 2013).

Following intramuscular injection, medetomidine is rapidly and completely absorbed (Sinclair 2003; Meyer & Fish 2008). Medetomidine has a rapid onset of action and duration of

effect can last approximately two hours, which proves useful when performing non-surgical procedures (Ko 2013). The clinical effects of medetomidine can be pharmacologically antagonised administering atipamezole at two-and-a-half times that of the administered medetomidine dose intramuscularly in domestic cats (Vaha-Vahe 1990).

Drug Combinations

When considering dosages it is important to note that aggressive, excited and agitated animals require higher doses, alternatively, tame or calm animals require lower doses (Bednarski 2011; Kock & Burroughs 2012). Balanced anaesthesia is achieved by the administration of two or more drugs at their lowest dose to produce desirable general anaesthetic affects and provide a stable physiological state (Bednarski 2011). Cyclohexylamines, opioid agonists and α_2-adrenergic receptor agonists used alone or in various combinations, can be administered intramuscularly and have been widely used for immobilisation in domestic felids (Tamura et al. 2015).

A quick, calm, stress free and complete recovery is important for patients when considering the ideal anaesthetic combination (Bednarski 2011). Antagonism of butorphanol, midazolam and medetomidine is possible with the use of naltrexone, flumazenil and atipamezole, respectively (Murrell 2007; Bednarski 2011; Kock & Burroughs 2012). Unfortunately, there are no antagonist drugs for ketamine which could result in lasting unfavourable effects during recovery once all other drugs are antagonised (Kastner 2007; Muir et al. 2013; Ramsay 2014). Butorphanol-midazolam-medetomidine combination has the advantage of being fully antagonisable, which is essential in wildlife capture and faster and complete recoveries (Bednarski 2011; Ramsay 2014). To prevent stormy recoveries associated with ketamine, it has been advocated to antagonise the clinical effects of medetomidine using atipamezole at

least 45-50 minutes after administrating the immobilising drug combination. This advocation is a general recommendation in domestic cats to allow sufficient time for ketamine to be metabolised and excreted (Kreeger 1996; Gunkel & Fortune 2007; Kastner 2007; Kock & Burroughs 2012; Ramsay 2014).

When deciding on a drug combination, ketamine is considered a drug of choice for immobilising free-ranging felids (Ramsay 2014). Ketamine-medetomidine combination is regularly used for minor surgical procedures in domestic cats. A combination of ketamine and medetomidine administered intramuscularly effectively anaesthetises domestic cats undergoing ovariectomy at 5.0 mg kg^{-1} and 0.08 mg kg^{-1}, respectively (Verstegen et al. 1989). They noted that the common bradycardic effects of medetomidine was compensated by the indirect stimulating chronotropic effect of ketamine (stimulates noradrenaline release). Beneficially, as a result of its indirect sympathomimetic effects through increased noradrenaline release at synaptic junctions, administration of ketamine can reduce bradycardic effects associated with medetomidine use with its resultant positive effects on heart rate and cardiac output (Kastner 2007, Murrell 2007, Meyer & Fish 2008, Sawyer 2008). The unique cardiovascular stimulatory effects of ketamine are beneficial during the phase 2 bradycardia in response to medetomidine, when the bradycardia is as a result to a decrease in sympathetic tone (Murrell 2007). During the phase 1 effects of medetomidine, these cardiovascular effects can have detrimental effects on the heart by increasing the myocardial workload and oxygen consumption (Kastner 2007). Patas monkeys (*Erythrocebus patas*) were effectively immobilised using hand-injection technique with ketamine (3.0 mg kg^{-1}), butorphanol (0.4 mg kg^{-1}) and medetomidine (0.04 mg kg^{-1}) intramuscularly (Kalema-Zikusoka et al. 2003). Similarly, Rockhill et al. (2011) successfully and reliably immobilised bobcats (*Lynx*

rufus) using KBM administered intramuscularly at 4.0, 0.4 and 0.04 mg kg^{-1}, respectively. Langan et al. (2000) immobilised captive serval using ketamine, butorphanol and medetomidine at 1.0 ±0.2, 0.2 ±0.03 and 0.047 ±0.010 mg kg^{-1}, respectively. The combination produced a calm and rapid induction and recovery; however, during general anaesthetic, the serval experienced significant decreases in heart and respiratory rates after administration of the combination. Particular attention to heart rate, mean arterial pressure and haemoglobin saturation with oxygen is important in animals receiving this combination to ensure adequate perfusion of vital organs. Respiratory rate, expiratory tidal volume and exhaled carbon dioxide should be closely monitored to ensure adequate ventilation. In general, immobilisation with ketamine in combination with an α_2-adrenergic receptor agonist may be prolonged with additional ketamine boluses, but there is a danger of undesirable excitatory effects during recovery (Ramsay 2014).

Ilkiw et al. (1996) discovered that on its own, even at high dosages, midazolam (5 mg kg^{-1}) does not produce significant sedation in healthy domestic cats. Similarly, Kanda and Hikasa (2008) noticed that duration of lateral recumbency was enhanced when midazolam and medetomidine were used in combination opposed to when they were used on their own. It was also noted that the neurohormonal adverse effects of medetomidine, such as hyperglycaemia and reduction of noradrenaline, when given alone were significantly reduced when it was used at the same dose in combination with midazolam. Benzodiazepines also provide cardioprotective abilities through slowing the heart rate and increasing oxygen delivery, thereby reducing the hearts oxygen demand (Meyer & Fish 2008).

Eggers et al. (2016) compared the cardiopulmonary effects and efficacy of two different doses of butorphanol, midazolam and medetomidine in captive and free-ranging black-footed

cats (*Felis nigripes*). The drug combination, BMM, produced a quick induction, reliable sedation and a calm recovery. Free-ranging black-footed cats pre-emptively received higher BMM doses (0.4, 0.2 and 0.1 mg kg^{-1}, respectively) compared to those used for the captive cats (0.2, 0.1 and 0.05 mg kg^{-1}, respectively). Captive cheetah (*Acinonyx jubatus*) required 0.2 ±0.02, 0.15 ±0.02 and 0.035 ±0.004 mg kg^{-1} of butorphanol, midazolam and medetomidine, respectively which provided a fast induction, stable sedation and rapid recovery (Lafortune et al. 2005). Free-ranging lions (*Panthera leo*) were darted intramuscularly and effectively sedated with BMM at 0.3, 0.2 and 0.05 mg kg^{-1}, respectively (Wenger et al. 2010). Similar to KBM, patas monkeys were successfully immobilised using butorphanol 0.4 mg kg^{-1}, midazolam 0.3 mg kg^{-1} and medetomidine 0.04 mg kg^{-1} intramuscularly (Kalema-Zikusoka et al. 2003).

Due to their larger muscle mass, Kock & Burroughs (2012) mentions that serval and caracal may be darted; however, other small felids should be physically restrained before administering the drug by injection to induce general anaesthesia. Typically, to immobilise and induce general anaesthesia, the free-ranging felids such as caracal (*Caracal carcal*), African wild cat (*Felis silvestris*) and black-footed cat are injected with either a ketamine-Zoletil or Zoletil-xylazine combination (Kock & Burroughs 2012).

Both ketamine-butorphanol-medetomidine and butorphanol-midazolam-medetomidine have proven to be effective in immobilising free-ranging and captive animals. For the purpose of this study we used similar strategies suggested by Langan et al. (2000), Kalema-Zikusoka et al. (2003) and Rockhill et al. (2011) for immobilising captive serval, patas monkeys and bobcats with KBM, respectively, and to Eggers et al. (2016), Lafortune et al. (2005), Wenger et al. (2010) and Kalema-Zikusoka et al. (2003) for immobilising black-footed cats, captive

cheetah, lions and patas monkeys with BMM, respectively. Similar to Eggers at al. (2016), whereby higher doses were used for free-ranging black-footed cats to that of captive black-footed cats, we also used much higher doses to dart free-ranging serval to those used by Langan et al. (2000) in captive serval.

Shortfall in Literature

To my knowledge, there is no literature and investigations on drug combinations for effective and reliable immobilisation and their respective cardiopulmonary affects in free-ranging serval. There is very little research done on serval in general, possibly due to the difficulty in studying these felids. Furthermore, investigations into serval behaviour, territory (home ranges) and diet are relevant, especially due to their adaption to specific habitats. In order to study home ranges and diets, the serval must be immobilised to allow fitting of tracking collars and collection of various samples. Therefore, there is a gap in literature on different chemical capture drug combinations for effective immobilisation of serval.

The quality, duration and effect of immobilisation and general anaesthesia with ketamine-butorphanol-medetomidine or butorphanol-midazolam-medetomidine have not been compared in serval. It is in my opinion that developing immobilisation protocols for serval will improve welfare and ethical standards in handling these animals during routine management and other research procedures.

Aims and Objectives

Aims:

The aim was to identify a reliable and effective drug combination to immobilise free-ranging caged-trapped serval that produces a rapid, calm and uneventful induction into immobilisation and provide a calm recovery.

Objectives:

The study aim was achieved by comparing the efficacy, quality and cardiopulmonary effects of ketamine-butorphanol-medetomidine to butorphanol-midazolam-medetomidine induction, immobilisation and recovery of cage trapped serval.

Once the drugs were administered, the efficacy, quality and cardiopulmonary effect of the induction, immobilisation and recovery were compared by assessing the following variables:

1. Time to first effect
2. Time to head down (head resting on floor and relaxed)
3. Time to handling
4. Quality and efficacy of induction
5. Cardiopulmonary effects (heart rate, respiratory rate, temp, SpO_2, arterial blood pressure, arterial blood gases, $P_E'CO_2$)
6. Quality of anaesthesia/immobilisation
7. Time to first intervention
8. Quality of recovery
9. Time to sternal
10. Time to standing

Hypothesis

Quality and efficacy of immobilisation hypothesis

H0: The times and quality of induction, immobilisation and recovery of serval administered with ketamine butorphanol medetomidine will be no different to butorphanol midazolam medetomidine.

H1: The times and quality of induction, immobilisation and recovery of serval administered with ketamine butorphanol and medetomidine combination will be significantly different to butorphanol midazolam and medetomidine combination.

Cardiopulmonary hypothesis

H0: The cardiopulmonary effects of ketamine butorphanol and medetomidine combination will be no different to butorphanol midazolam and medetomidine combination.

H1: The cardiopulmonary effects of ketamine butorphanol and medetomidine combination will be significantly different to butorphanol midazolam and medetomidine combination.

Each variable of interest will be tested for significance to determine if there is enough evidence to reject the stated null hypothesis and favour the alternative hypothesis which states that there will be a difference between the two drug combinations for the variable of interest.

Benefits Arising from the Study

So little is known about these elusive cats and as a result we are not well equipped in helping conserve this species be it through increasing human-wildlife interaction or preventing rapidly declining populations. By researching immobilisation drug combinations, we can make field-

ready recommendations to effectively capture these cats. These recommendations will provide us with future opportunities to study these free-ranging cats by collaring and tracking them, learning more about their home ranges, distributions, social dynamics and hunting patterns. Serval have been observed to adapt well to disturbed environments. One study suggests that certain pockets of unused disturbed land in industrial areas have had a positive impact on serval density (Loock 2018). Factors such as decreased interspecific competition, abundance of food and protection from prosecution from livestock farmers are proposed to be possible contributing factors, however, further studies are required to provide us with a better understanding to the methods and determining factors for their adaptation. Currently serval populations are listed as least threatened; however, with the exponential increase in global human population with resultant urbanisation, habitat destruction and poaching, there is a distinct possibility we will witness a decline and possible extinction of serval in our lifetime. Further studies will better equip us for future conservation efforts.

Materials and Methods

Pilot Study

A preliminary study was carried out before the commencement of the main study. The main objective and aim was to investigate effective drug dosages and combinations to effectively and safely immobilise serval. This investigation and its findings allowed us to successfully carry out our main study. The preliminary, observational investigation was approved by the animal ethics committee of the University of Pretoria (V101-17) and a capture permit (#7282; Mpumalanga Tourism and Parks Agency) was obtained prior to the commencement of the study.

Ten free-ranging serval were cage-trapped on the Sasol Synfuels property in Secunda, Mpumalanga, South Africa (26° 33'S, 29° 10'E), over a five day period. On arrival at the cage trap, the serval was observed from a 10 meter distance and their body weight was estimated for calculation of the drug volumes. The serval received one of the randomly assigned drug combinations, as follows:

KBM-5: Ketamine 5.0 mg kg^{-1} (Ketonil 200 mg mL^{-1}; Wildlife Pharmaceuticals); Butorphanol 0.2 mg kg^{-1} (Butonil 50 mg mL^{-1}; Wildlife Pharmaceuticals); Medetomidine 0.08 mg kg^{-1} (Metonil 40 mg mL^{-1}; Wildlife Pharmaceuticals)

KBM-8: Ketamine 8.0 mg kg^{-1}; Butorphanol 0.2 mg kg^{-1}; Medetomidine 0.08 mg kg^{-1}

ZM: Zoletil 5.0 mg kg^{-1} (Zoletil 50 mg mL^{-1}; Virbac); Medetomidine 0.065 mg kg^{-1}

AM: Alfaxalone 0.5 mg kg^{-1} (Alfaxan 10 mg mL^{-1}; Jurox); Medetomidine 0.05 mg kg^{-1}

ABM: Alfaxalone 2.0 mg kg^{-1}; Butorphanol 0.2 mg kg^{-1}; Medetomidine 0.08 mg kg^{-1}

The KBM-5 group comprised of 3 serval, the KBM-8 group comprised of 3 serval, the ZM comprised of 1 serval, the AM comprised of 1 serval and the ABM comprised of 2 serval. The initial dose rates of KBM-5 were unexpectedly inadequate to reliably chemically capture cage-trapped free-ranging serval and often two darts were required to achieve a satisfactory immobilisation, whereas the KBM-8 doses were reliable and induced the fastest times to first handling. The median time to first handling for KBM-5 was 2420 seconds (40.3 minutes), much longer compared to KBM-8 of 900 seconds (15 minutes). The KBM-8 combination was the most reliable, requiring less darts per serval and shorter times to first handling. Three darts were used in one serval receiving the KBM-8 combination; however, the first two darts did not discharge and therefore were not included in the total dose calculations.

The ZM combination produced a reliable calm capture in one serval with acceptable first effect, recumbency, head down and first handling times. The AM combination was not reliable and a second dart was required (total dose alfaxalone 1.0 mg kg^{-1}; medetomidine 0.10 mg kg^{-1}) to achieve recumbency. Times to first effect, recumbency, head down and first handling were prolonged and unsatisfactory. Once handled and just prior to placement of the intravenous cannula, the serval spontaneously recovered and launched into a speedy and successful escape attempt and was not captured again. Therefore, no actual weight was obtained and the protocol was adjusted to the ABM combination. The ABM combination was successful and provided an ability to handle the serval reliably, but the times to events were longer (median time to first handling 1275 seconds (21.2 minutes)) than the KBM-8 combination.

Our preliminary findings suggested that a high dose ketamine-butorphanol-medetomidine required further investigation in terms of its practicality and reliability in cage-trapped free-ranging serval capture. Zoletil-medetomidine combination also produced an effective and reliable immobilisation, but because of the expense of Zoletil in South Africa, we decided to do a comparative investigation with high dose butorphanol-midazolam-medetomidine instead. However, Zoletil-medetomidine combination still warrants further investigation.

Main Study

The investigation was approved by the animal ethics committee of the University of Pretoria (V108-17) and a capture permit (#7282; Mpumalanga Tourism and Parks Agency) was obtained prior to the commencement of the study.

Experimental design

A prospective, blinded comparative drug trial where thirty serval were randomly assigned (six balanced randomization blocks where 4 several were allocated in order of immobilisation per block; www.randomization.com) to either ketamine-butorphanol-medetomidine (*n* = 15) or butorphanol-midazolam-medetomidine (*n* = 15) drug combination for immobilisation.

Experimental procedure

Pre-immobilisation phase

Picture 1. Picture demonstrating a serval (after drug administration) trapped in a cage trap (loaded onto a vehicle) covered with thick camouflaged netting.

Free-ranging serval on the Sasol Synfuels property in Secunda, Mpumalanga, South Africa (26° 33'S, 29° 10'E), were opportunistically used in the trial based on the ability to cage trap them over a two-week period. The total number of serval enrolled into the study was kept as low as possible to ensure a statistically relevant and scientific outcome (reduction). Cage traps (2 x 0.8 x 0.8 meters; 30 mm square wire mesh enveloped around a metal 15 mm square tubing frame) were purpose-built with a pressure plate triggered guillotine gate (Picture 1). Cages were

assembled to ensure there were no wires or other structures that may have inflicted harm to the serval when trapped. Based on historic population density information, up to fifteen cage traps were placed at strategic locations around the property. Location and layout of the cage traps were important aspects to consider and were determined by the Sasol capture team based on extensive knowledge of serval home ranges, habitat preferences and density studies previously carried out by the team using camera traps and GPS collar data. The cage traps were baited with a freshly caught and killed guinea fowl (*Numida meleagris)* and covered with thick camouflaged netting to hide the trap and provide shelter from the elements. Serval in search for food entered the trap to investigate the bait. The guinea fowl, suspended from the roof of the cage by their legs using wire, were positioned above the pressure plate in order to attract the serval far enough into the trap to activate the pressure plate. When an unsuspecting serval walked into the trap to investigate the bait, it stood on the pressure plate thereby activating it, which caused the guillotine door to drop thus trapping the serval inside the cage. Daily trapping success averaged 20% (range 0 to 33%). A centrally located procedure room on the Sasol property was used to set up all the monitoring and radiographic equipment. Cage traps were monitored by the capture team every four to eight hours. If a serval was trapped, the capture team loaded the cage with the trapped serval onto a vehicle (pickup truck) and brought it to the procedure room where it was immobilised. Once the cages were offloaded, the capture team would then go out to collect the next trapped serval if more than one serval was trapped on a given day. In the event that more than one serval was trapped on any given day, the order in which the serval were collected was determined by the duration the serval had been trapped, distance of trap from the procedure room and stress level and health of the serval. The serval were immobilised for dental examination,

sampling (haematology and biochemistry), biometric measurements and ecto- and endoparasite collection as part of ongoing dental and ecology studies.

On arrival to the procedure room area, the loaded vehicle was parked 15-20 meters away. Then, the serval was observed from a 10 meter distance and their body weight was estimated for calculation of the drug volumes. According to Kock & Burroughs (2012) the average adult weight of serval is 7-13 kg. The serval received one of the randomly assigned drug combinations, in a balanced sequential order where the primary investigator was always blinded to the combination, as follows:

> **KBM**: Ketamine 8.0 mg kg^{-1} (Ketonil 200 mg mL^{-1}; Wildlife Pharmaceuticals; RSA); Butorphanol 0.4 mg kg^{-1} (Butonil 50 mg mL^{-1}; Wildlife Pharmaceuticals); Medetomidine 0.08 mg kg^{-1} (Metonil 40 mg mL^{-1}; Wildlife Pharmaceuticals)
>
> **BMM:** Butorphanol 0.4 mg kg^{-1}; Midazolam 0.3 mg kg^{-1} (Dazonil 50 mg mL^{-1}; Wildlife Pharmaceuticals; RSA); Medetomidine 0.08 mg kg^{-1}

The primary investigator was blinded and therefore unaware of which drug combination was used on the serval. Before darting, the general health, demeanour and stress levels of the serval was assessed as the cage was approached by the primary investigator. Each serval was assigned a stress score (Table 1) based on their degree of fight or flight response when approached, their response to the cage trap, trauma wounds sustained from failed escape attempts, exposure to noxious stimuli and stressors (loud noises, human presence, excessive handling prior to darting) and reaction to human presence. Serval are free-ranging felids and should display aggressive behaviour to any approaching threat, such as a human, any acquiescent behaviour was noted as an indication that the serval could potentially be ailing.

Table 1. Simple descriptive scoring system used to categorise the stress response the serval exhibited prior to being darted with either ketamine-butorphanol-medetomidine or butorphanol-midazolam-medetomidine drug combinations.

Stress Score		
Score	**Description**	**Classification**
0	Serval is relaxed in the cage, unperturbed by human presence, with no capture related injuries or escape attempts	Calm
1	Serval is calm in the cage, but is agitated when approached. Serval paces around the cage in search for escape routes. No capture related injuries sustained.	Mildly stressed
2	Serval is slightly agitated by the cage trap. Intermittent pacing. When approached, serval is notably agitated with increasing signs of pacing and escape attempts. Minor capture related injuries.	Moderately stressed
3	Multiple escape attempts and pacing with/without human presence. Vocalisation. Moderate to severe self inflicted wounds sustained from attempts at escaping. Prolonged capture.	Markedly stressed

Immobilisation phase

The immobilisation team would slowly approach the cage trap with the serval inside. The primary investigator waited for the serval to move to the opposite end of the cage providing a clear view before projecting the dart. The drug combination was administered into the gluteal muscle group via a 3 ml blow dart (2.0 x 25 mm plain needle) projected from a commercial blow pipe (Dan-Inject 0.125 m blowpipe system; Dan-Inject) (Picture 2). The dart was not filled to full capacity, but rather pressurised using compressed air from an air-filled syringe to push the plunger forward into position, once the dart was charged with the drug combination. Once the dart was placed and fully discharged a stopwatch was started (Time

Picture 2. Picture demonstrating a bounced dart (bottom dart) that failed to deliver and a dart that completely delivered the drugs intramuscularly into a serval using a blow dart delivery system

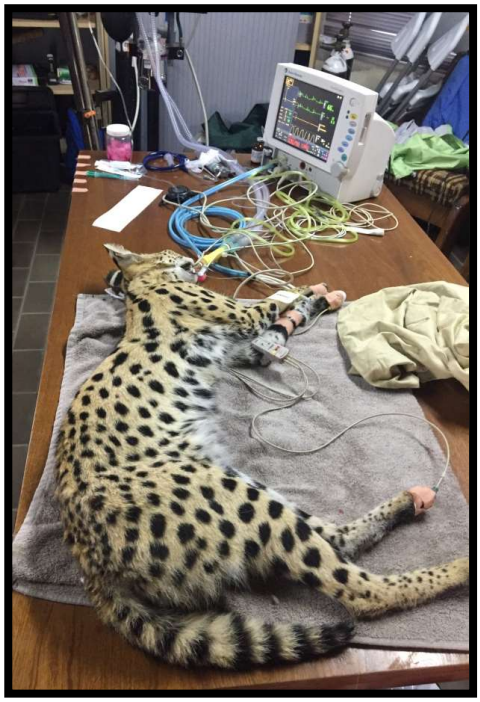
zero; T0) to record times to events: 1) first effect (ataxia, vacant staring into distance, head bobbing), 2) recumbency (sternal or lateral), 3) head down (chin placed on floor of cage and neck relaxed), and 4) first handling (opening cage to handle). Based on the above data, each serval was assigned an induction score (Table 2) to classify the efficacy of the drug combination to swiftly and effectively induce immobilisation. If the dart did not discharge or if dart bounced, a new dart was drawn up and the serval was re-darted.

Table 2. Simple descriptive scoring system used to classify the quality and efficacy of induction into anaesthesia/immobilisation of serval using ketamine-butorphanol-medetomidine and butorphanol-midazolam-medetomidine drug combinations.

Efficacy of Induction Score		
Score	**Description**	**Classification**
0	Fully conscious, demonstrating signs of aggression towards people approaching, no signs of ataxia or drug effect.	No effect
1	Very slow onset of sedation, noticeable response to auricle stimulation (auricle reflex) or tail tug at 15 minutes or longer after drug administration. Vocalisation and some defensive/aggressive displays, mild signs of ataxia (stumbling)	Poor
2	Slow onset of signs of sedation, slight response to auricle stimulation (auricle reflex) or tail tug at 10-15 minutes after drug administration. Minimal vocalisation, no sign of aggressive behaviour, animal in lateral/sternal recumbency.	Moderate
3	Rapid calm transition from awake to anaesthesia/immobilisation, no response to auricle stimulation (auricle reflex) or tail tug at 5-10 minutes after drug administration. No vocalisation or signs of aggression, animal in lateral/sternal recumbency	Good
4	Rapid calm onset of signs of anaesthesia/immobilisation, no response to auricle stimulation (auricle reflex) or tail tug in 5 minutes or less after drug administration. No vocalisation or signs of aggression, animal in lateral/sternal recumbency	Excellent

Once darted, the serval was closely monitored at a distance so as not to cause undue stress which could prolong initial drug effect. After head down, the immobilisation team waited two minutes before approaching the cage trap. The serval was removed from the cage trap when

there was no response to a tail tug and auricular reflex (satisfactory state of immobilisation). If the serval was deemed inadequately immobilised and unsafe to handle after 15 minutes from dart placement, then they were administered an intramuscular bolus of ketamine (1 mg kg^{-1}) and a waiting period of five minutes was allowed before attempting handling. If there was no drug effect observed at 15 minutes, it was assumed that the serval did not receive the drug combination and a new dart was drawn up and the serval was darted again. The time of the second dart placement was assumed as T0.

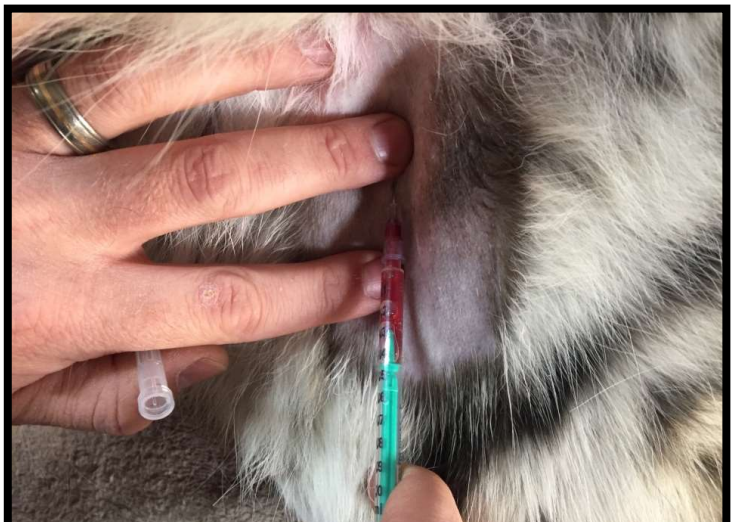


Picture 3. Picture demonstrating a serval connected to a multiparameter monitoring machine.

Once removed from the cage trap, the serval was placed into a hessian sack and weighed using an electronic hanging scale. After it was weighed, the serval was removed from the hessian sack and transferred to a work bench and placed on a blanket. The cephalic vein was aseptically cannulated (22 gauge, 25 mm; Jelco) and secured in place using 25mm adhesive bandage tape (Elastoplast). The servals larynx was desensitised using 0.5ml local anaesthetic (Lignocaine 2%) before they were orotracheally intubated with a cuffed polyvinyl chloride endotracheal tube using a laryngoscope. If orotracheal intubation was not possible because of increased jaw tone and head avoidance then the serval received an intravenous bolus of ketamine (0.5 mg kg^{-1}) before reattempting intubation. An isotonic crystalloid (Lactated Ringer's solution; Fresenius kabi) was infused at 10 ml kg^{-1} hr via a manual administration set 20 drop per millilitre connected to the cephalic catheter. If the serval were clinically dehydrated (sunken eyes, prolonged skin tenting, delayed capillary refill time, tachycardia,

low blood pressure) intermittent boluses of 30 ml kg^{-1} was administered. The serval were then instrumented (Picture 3) (electrocardiograph pads secured to paw pads, oesophageal temperature probe advanced to the 3rd intercostals space; pulse oximetry probe placed on tongue; oscillometric cuff placed on antebrachium of the thoracic limb with the cuff width 40-50% of the circumference of the limb) and connected to a multiparameter monitoring machine (Cardiocap 5; Datex; Finland) to measure physiological data (heart rate, respiratory rate, end tidal carbon dioxide [$P_E'CO_2$], oesophageal temperature, peripheral oxygen-haemoglobin saturation [SpO_2], non-invasive arterial blood pressure [NIBP] and expiratory tidal volume). The serval were connected to a circle breathing system and supplied with oxygen at a fixed flow rate of 2 L $minute^{-1}$. Once instrumented, the general condition of the cat was assessed in terms of body condition scoring. Each serval was assigned a body condition score (Score 1-5).

The serval was monitored continuously, but clinical data relevant for this study were collected immediately after instrumentation and then at five minutes intervals. The serval were either covered with blankets and warmed drip bags or their jugular, axilla and groin dampened with alcohol to maintain body temperature within normal limits (>36.5 and <38.5°C). Twenty minutes after instrumentation arterial blood was sampled from the femoral artery using a needle (24-gauge, 1-inch) and preheparinised syringe and needle for gas analysis



Picture 4. Picture demonstrating the technique used to sample arterial blood from the femoral artery using a needle and preheparinised syringe for gas analysis.

(Picture 4). The arterial blood sample was analysed using a patient side, portable, self-calibrating, blood gas analyser (interpreted at 37°C; EPOC Reader Blood Analysis and self-calibrating BGEM3 test cards; Epocal; Canada). The quality and depth of the anaesthesia/immobilisation was subjectively assessed (Table 3) after tracheal intubation based on response to external stimuli (tail tug, pedal reflex, medial and lateral palpebral reflex, auricle reflex) to determine the end-point of the drug combination effect. When the serval showed signs of arousal, they were administered a rescue intervention as either a single bolus of ketamine (0.5 mg kg^{-1}) and/or the isoflurane vaporiser was increased to deliver 2%. The time was recorded from dart placement to intervention as the time to first intervention. The immobilisation time was the time from the dart placement until all equipment was disconnected.

Table 3. Simple descriptive scoring system used to classify the quality and efficacy of anaesthesia/immobilisation of serval using ketamine-butorphanol-medetomidine and butorphanol-midazolam-medetomidine drug combinations.

Quality of Anaesthesia/Immobilisation Score		
Score	**Description**	**Classification**
1	Animal is unsafe to handle, vocalisation and shows, signs of aggression, all reflexes present	Limited effect
2	All reflexes still present (may show signs of hyper-reflexia) and involuntary limb movements.	Sedation
3	Muscle relaxation, no limb movement, delayed pedal and anal reflex.	Light -Moderate surgical anaesthesia
4	All reflexes absent (pedal reflex, laryngeal reflex, anal reflex), mild cardiopulmonary depression, mydriasis	Deep surgical anaesthesia
5	All reflexes absent, mydriasis, severe respiratory depression/apnoea, severe bradycardia, requires rescue intervention	Excessively deep anaesthesia effect

Recovery phase

A final health check was done on each anaesthetised serval to ensure they were healthy before release and any injuries sustained during the physical and chemical capture (self-inflicted trauma sustained from failed escape attempts), injuries sustained prior to capture or

any gross dental pathology (fractured teeth) were attended to. The recovery phase begun once either 1) the end-point of the drug combination effect was reached, or 2) if data for the dental and ecology studies were still required, then the serval was maintained under general anaesthesia for the duration of the examination using isoflurane in oxygen until all necessary data was collected. The venous cannula was removed and all equipment disconnected from the serval. The isoflurane and oxygen were turned off, the cuff of the endotracheal tube was deflated and the serval were extubated before moving them.

The serval was returned to the cage trap with their necks fully extended ensuring patency of their airway. Once safely in the cage trap, the guillotine door was held approximately 10 cm ajar to allow the primary investigator to administer the antagonist drugs intramuscularly into the quadriceps muscle groups. The medetomidine and butorphanol, were pharmacologically antagonised by administering intramuscular atipamezole (5 mg kg^{-1} medetomidine; Atipamezole 20 mg mL^{-1}; Wildlife Pharmaceuticals; RSA) and naltrexone (2 mg kg^{-1} butorphanol; Trexonil 40 mg mL^{-1}; Wildlife Pharmaceuticals; RSA), respectively. After the antagonist was administered the serval was allowed to recover in the cage trap whilst being closely monitored, from 10 meters away, until recovered. The primary investigator carefully noted any signs of ataxia and recorded recovery times. The quality of recovery was assessed and each serval was assigned a recovery score (Table 4) based on; 1) time taken for the serval to recover from a recumbent unconscious state to a fully conscious, 2) time to standing and 3) clinical signs and severity of ataxia. A stopwatch was started immediately after administering the antagonist drugs to record time to sternal and time to standing. Time to standing was considered to be the recovery time. Once fully recovered from all drug effects, the serval were then loaded back onto the vehicles (pick up) and driven back to the original

capture location where they were released away from nearby human activity, hazardous objects (fences, vehicles, equipment, powerlines and roads) and water sources (rivers, marshes and service dams). The empty cage traps were re-baited with a fresh guinea fowl and taken to a different location determined by the capture team.

Table 4. Simple descriptive scoring system used to classify the quality and efficacy of recovery of serval after administration of antagonist(s) after immobilisation with ketamine-butorphanol-medetomidine and butorphanol-midazolam-medetomidine drug combinations.

Recovery Score		
Score	**Description**	**Classification**
1	Lateral recumbency for prolonged period of time to standing (> 15 minutes). Limited signs of attempting to right itself, tail flicking noticed	Poor
2	Stormy recovery, lateral recumbency for prolonged period of time to standing (10-15 minutes), obvious signs of ataxia once standing.	Moderate
3	Serval remains in lateral recumbency for a short period of time to standing (5-10 minutes), limited signs of ataxia after the serval stands	Good
4	Serval remains in lateral recumbency for very short period of time to standing (< 5 minutes), no to limited signs of ataxia after the serval stands	Excellent

Data Analysis

Data were assessed for normality by plotting histograms and evaluating descriptive statistics and applying the Anderson-Darling test for normality. All time to events, physiological and blood gas data were normally distributed and reported as mean (standard deviation or minimum, maximum). Total drug doses were calculated based on adding all placed darts that discharged per kg body mass, bounced darts were not included. Single point data (weight, times to events, blood gas values, number of darts used, body condition score) were compared using a student t-test. Physiological data collected over time were compared between groups using a general linear mixed model (Interactions: time, treatment, treatment x time; Fixed variables: time, treatment; Random variable: serval). Significant values were compared using Bonferroni correction for multiple pairwise comparisons. Spearman's rank

correlation coefficient was used to estimate the association between stress score (an ordinal scale) and number of darts used, time to first handling, blood glucose concentration and first rectal temperature, independent of drug combination used (Warner 2010). Physiological variable data were averaged at 10-minute intervals and tabulated over time. Data were analysed using commercial software (MiniTab version 18.1) and results interpreted at the 5% level of significance.

Results

A total of 21 serval were captured, of which, two individuals were recaptured 3 times on separate occasions. For the recaptured serval, only data for one KBM and one BMM captures were used in the analysis and data from the third capture was excluded. Eleven (5 females, 6 males) and twelve (10 females, 2 males) serval received the KBM and BMM combinations, respectively. The mean weight for KBM group was 10.7± 0.3 kg and no different to that of the BMM group 9.6 ± 0.3 kg (T = -1.11, p = 0.281) and all had a visually assessed body condition of 3 out of 5 (T = 1.42, p = 0.17). Some serval required more than one dart attempt due to either a bounced dart (n = 2) or non-discharge (n = 5), but these events were no different between the groups (T = 0.95 p = 0.356). Five serval sustained minor capture related injuries (most commonly skin abrasions to the head) as a result of repeated escape attempts from the cage trap.

The final calculated dose based on actual body weights were 8.7 (7.0 – 11.7), 0.4 (0.4 – 0.6) and 0.09 (0.07 – 0.12) mg kg^{-1} for ketamine, butorphanol and medetomidine, respectively in the KBM combination; and 0.5 (0.4 – 0.6), 0.4 (0.3 – 0.4) and 0.09 (0.07 – 0.11) mg kg^{-1} for butorphanol, midazolam and medetomidine, respectively in the BMM combination.

Times to handling, time to intervention and recovery times are reported in table 5. The mean time to first effect were similar for both combinations (T = 0.02, p = 0.984). The mean time to head down (T = 2.4, p = 0.026) and handling (T = 2.29, p = 0.033) times were significantly shorter in KBM compared to BMM. The quality of induction was scored as 2, regardless of the combination used. Two serval that received BMM required ketamine rescue (additional ketamine bolus to deepen sedation to facilitate handling). One required a bolus prior to handling; the other had regained consciousness after being handled which subsequently had to be recaptured using a capture net. None of the serval darted with KBM required ketamine rescue before handling. Collectively, stress score was strongly and positively correlated to blood glucose concentrations (r^2 = 0.788; p = 0.001) and temperature (r^2 = 0.634; p = 0.015).

Table 5. Mean, range and comparison of induction, intervention and recovery times in seconds for ketamine-butorphanol-medetomidine (KBM) and butorphanol-midazolam-medetomidine (BMM) in free-ranging serval cats.

		KBM		**BMM**	
Parameter	Unit	Mean	(Min-Max)	Mean	(Min-Max)
Time to first effect [a]	Seconds	270	(130 – 570)	271	(160 – 350)
	Minutes	4.5	(2.2 – 9.5)	4.5	(2.7 – 5.8)
Time to head down [a]	Seconds	442*	(300 – 800)	613*	(360 - 990)
	Minutes	7.4*	(5.0 – 13.3)	10.2*	(6.0 – 16.5)
Time to handling [a]	Seconds	611*	(360 – 900)	800*	(540 – 1320)
	Minutes	10.2*	(6.0 – 15.0)	13.3*	(9.0 – 22.0)
Time to first intervention [a]	Seconds	2214	(1850 – 3820)	2583	(1500 – 4350)
	Minutes	36.9	(30.8 – 63.7)	43.1	(25 – 72.5)
Total Immobilisation time [b]	Seconds	2820	(1900 – 4090)	3027	(1570 – 4420)
	Minutes	47.0	(31.6 – 68.2)	50.5	(26.2 – 73.6)
Time to sternal [c]	Seconds	472	(160 – 1020)	357	(60 – 930)
	Minutes	7.9	(2.7 – 17.0)	6.0	(1.0 – 15.5)
Time to standing [c]	Seconds	616	(260 – 1170)	397	(110 -1050)
	Minutes	10.3	(4.3 – 19.5)	6.6	(1.8 – 17.5)

[a] Significant finding (p <0.05)
[a] Time from first dart placement
[b] Time from dart placement until antagonist administration
[c] Time from antagonist administration

One serval from each combination coughed during tracheal intubation but neither require rescue intervention. Both combinations produced a reliable and adequate depth of anaesthesia and quality was subjectively scored as 3. Immobilisation was objectively scored at the end of the immobilisation based on observations and data collected during the immobilisation phase. The mean time to first intervention was 2215 seconds (36.9 minutes) for KBM and no different to 2583 seconds (43.1 minutes) for BMM (T = 0.55, p = 0.588). Serval required isoflurane rescue intervention to maintain general anaesthesia until dental and parasite and morphometric data was completed at 60 minutes, regardless of combination used. Eight of the eleven serval and nine of the twelve serval that received KBM and BMM were started on isoflurane, respectively. Eight and seven of the serval immobilised with BMM and KBM, respectively had no reflexes present at the start of general anaesthesia. Two serval immobilised with KBM had apneustic breathing patterns. Two serval immobilised with BMM had pronounced muscle fasciculation's. The medial palpebral reflex was most commonly found to be the first detectable reflex indicating the serval was returning to a lighter plane of anaesthesia.

Recovery was uneventful in both combinations, with BMM subjectively assessed to have a superior recovery (score 3) to that of KBM (score 2). After intramuscular administration of the antagonist drugs, time to sternal (T = -1.20, p = 0.243) and standing (T = -2.03 p = 0.056) were no different between the combinations. However, BMM recovery had less ataxia and was regarded as calmer and more co-ordinated (score 1) when compared to KBM (score 2).

Physiological data over time is reported in table 6. The heart rate was no different between combinations. The arterial blood pressure was significantly higher in KBM compare to BMM for the initial 40 minutes. There was a failure to obtain blood pressure results for the initial

15 minutes of anaesthesia in serval darted with KBM. At 50 minutes, arterial blood pressure decreased in both combinations. The respiratory rate was significantly slower ($F = 165.67$, $p < 0.001$) and the expiratory tidal volumes were significantly larger ($F = 5.3$, $p = 0.025$) in KBM compared to BMM. The $PE'CO_2$ was significantly higher ($F = 12.4$, $p = 0.001$), but clinically irrelevant in KBM compared to BMM. The temperature ($F = 0.07$, $p = 0.789$) and pulse oximetry ($F = 2.69$, $p = 0.103$) were no different between the combinations but only temperature decreased significantly over time in both combinations ($F = 2.64$, $p = 0.003$).

Table 6. Comparison of physiological parameters for ketamine-butorphanol-medetomidine (KBM) and butorphanol-midazolam-medetomidine (BMM) in free-ranging serval cats.

Parameter	Unit	Combination	0 minutes		5 - 15 minutes		20 - 30 minutes		35 - 45 minutes		50 - 60 minutes	
			Mean	(Min-Max)	Mean	(Min-Max)	Mean	(Min-Max)	Mean	(Min-Max)	Mean	(Min-Max)
Heart Rate	Beats per min	KBM	75	(48-93)	72	(51-89)	72	(54-89)	73	(49-86)	70	(51-83)
		BMM	70	(43-128)	71	(47.-117)	70	(49-120)	69	(47-109)	69	(55-94)
Sys BP	mmHg	KBM	NR	NR	NR	NR	162*	(152-175)	161*	(145-172)	141	(141-141)
		BMM	163	(155-171)	156	(145-166)	143*	(131-152)	138*	(110-158)	144	(119-159)
Dia BP	mmHg	KBM	NR	NR	130*	NR	142*	(114-138)	112	(103-118)	94	(94-94)
		BMM	121	(110-131)	105*	(97-115)	98*	(84-114)	88	(68-105)	87	(68-98)
Mean BP	mmHg	KBM	NR	NR	144*	NR	137*	(128-151)	130*	(117-141)	112	(112-112)
		BMM	138	(126-149)	123*	(112-136)	117*	(101-132)	109*	(89-126)	107	(89-116)
Resp Rate	Breaths per min	KBM	13	(5-18)	14	(7-27)	15	(9-26)	16	(11-27)	19	(16-26)
		BMM	31	(4-42)	29	(14-38)	25	(14-33)	23	(14-29)	23	(15-27)
$PE'CO_2$	mmHg	KBM	44	(35-56)	46*	(34-58)	48*	(39-61)	48*	(40-59)	44	(39-49)
		BMM	41	(28-50)	44*	(35-49)	45*	(37-50)	45*	(37-50)	46	(44-48)
Exp Vt	mL	KBM	169*	(98-240)	193*	(117-283)	127*	(110-150)	119	(95-143)	87	(87-87)
		BMM	67*	(65-78)	132*	(65-290)	87*	(75-99)	102	(98-110)	95	(85-104)
SpO_2	%	KBM	95	(93-99)	96	(89-99)	97	(95-100)	99	(97-100)	96	(96-96)
		BMM	96	(87-99)	98	(95-100)	98	(95-99)	98	(94-100)	97	(94-100)
Temp	Degrees Celsius	KBM	39.3	(37.8-39.7)	38.8	(37.2-40.1)	38.5	(36.7-39.8)	38.3	(36.8-39.8)	38.5	(37.7-39.3)
		BMM	39.4	(38.2-41)	38.8	(38.6-40.3)	38.4	(37.3-39.3)	38.4	(37-39.3)	38.3	(37.6-38.9)

* - significant finding ($p < 0.05$), Sys BP – systolic arterial pressure, Dia BP – diastolic arterial pressure, Mean BP – mean arterial pressure, $PE'CO_2$– end tidal carbon dioxide, SpO_2 – oxygen saturation, Temp - temperature

Figure 1. Boxplot and whiskers of heart rate (**a.**) and line graph of mean arterial pressure (**b.**) against anaesthetic time in serval (*Leptailurus serval*) that received either butorphanol-midazolam-medetomidine (BMM) or ketamine-butorphanol-medetomidine (KBM) intramuscularly by blow dart technique.

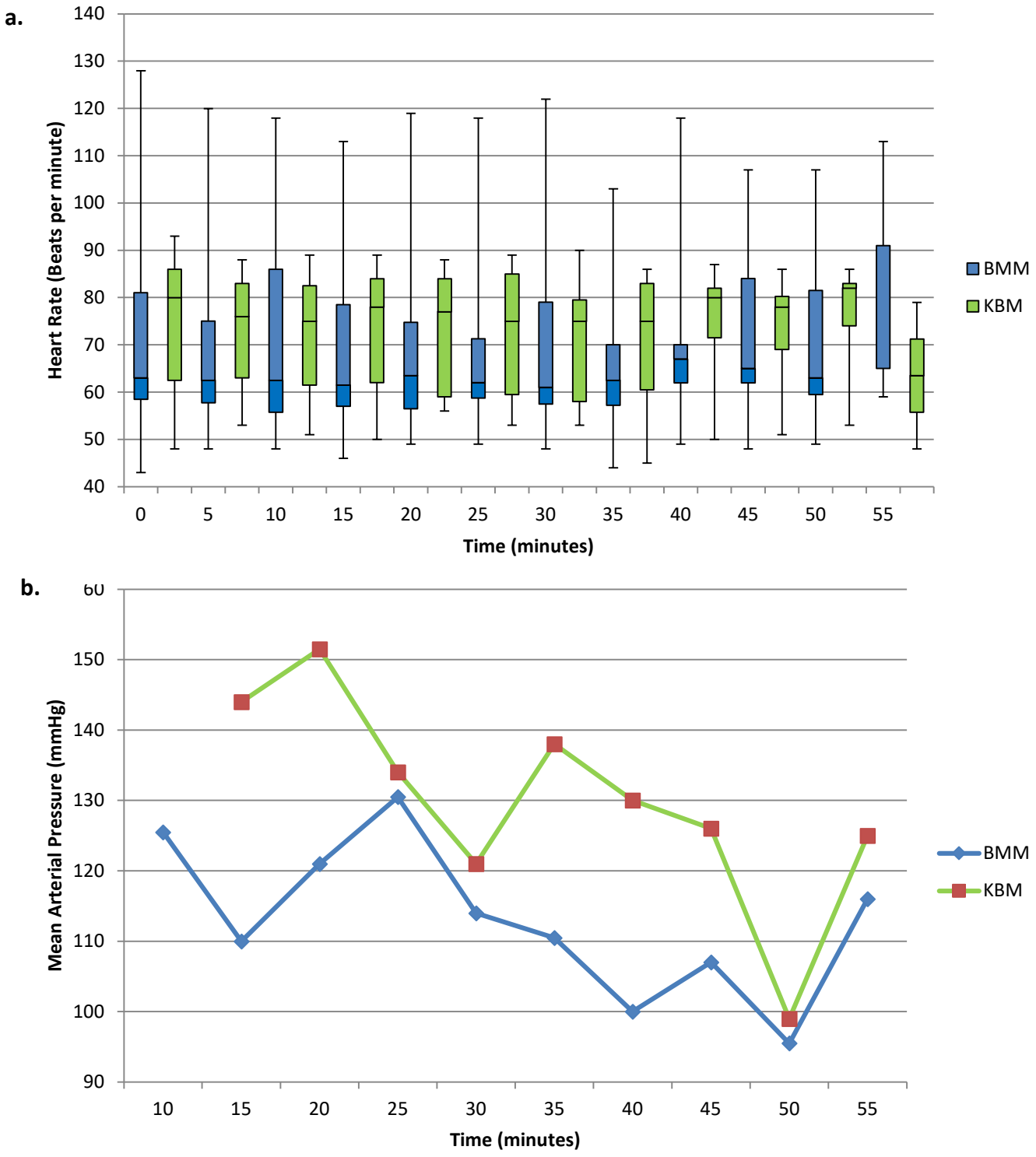


Figure 2. Boxplot and whiskers of respiratory against anaesthetic time in serval (*Leptailurus serval*) that received either butorphanol-midazolam-medetomidine (BMM) or ketamine-butorphanol-medetomidine (KBM) intramuscularly by blow dart technique.

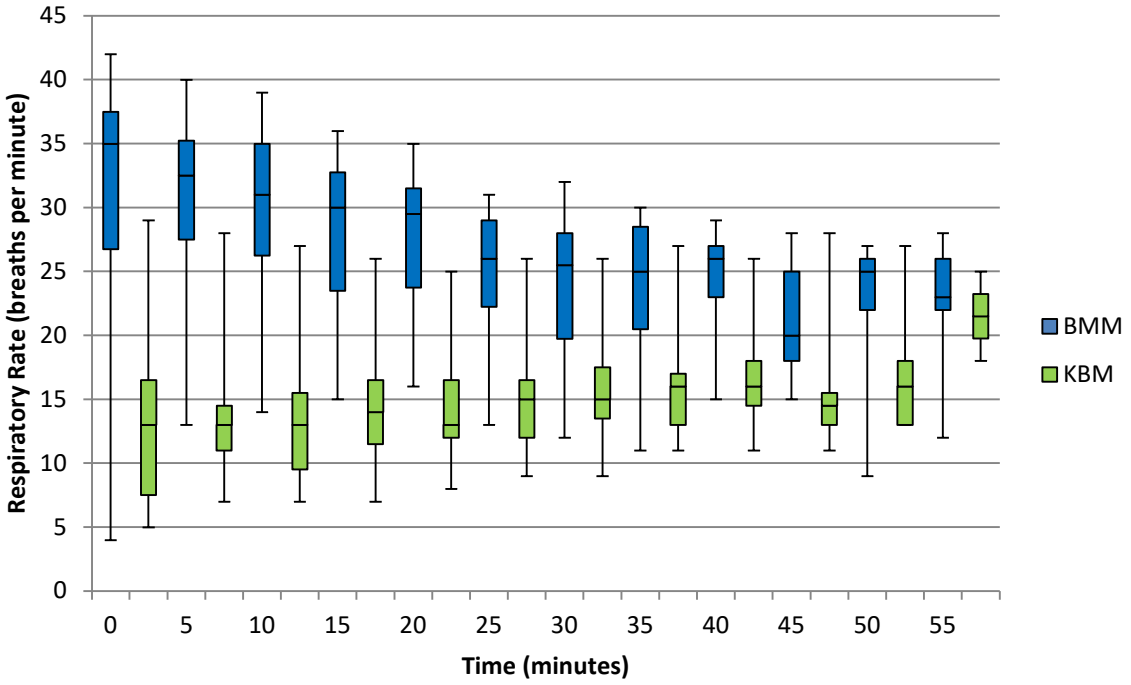
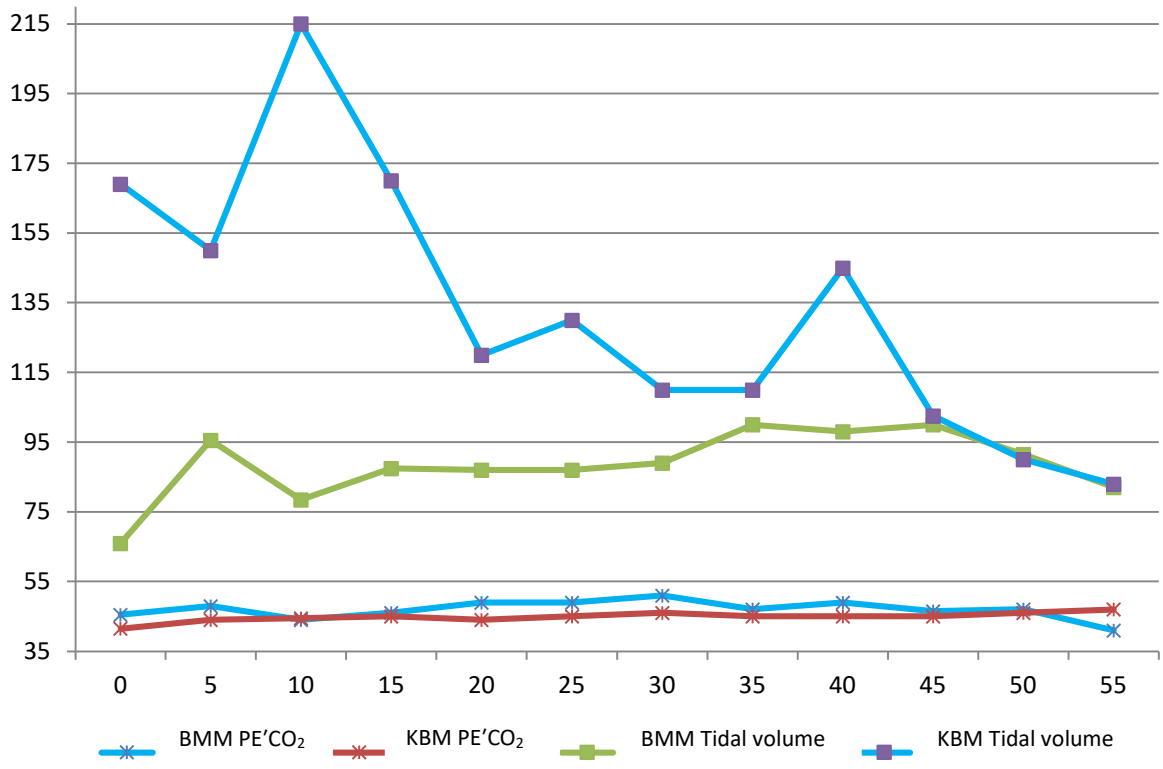


Figure 3. Line graph plotting mean expired tidal volume (mL) and end tidal carbon dioxide (mmHg) values against anaesthetic time (minutes) in serval (*Leptailurus serval*) that received either butorphanol-midazolam-medetomidine (BMM) or ketamine-butorphanol-medetomidine (KBM) intramuscularly by blow dart technique.



The arterial blood gas analyses (arterial blood was sampled 20 minutes after instrumentation) were unremarkable and no different between the combinations (Table 7). The $PaCO_2$ was very closely matched to the $PE'CO_2$ based on visual inspection.

Table 7. Comparison of blood gas analysis for ketamine-butorphanol-medetomidine (KBM) and butorphanol-midazolam-medetomidine (BMM) in free-ranging serval cats.

	KBM		**BMM**	
Variable	Mean	(Min-Max)	Mean	(Min-Max)
pH	7.32	(7.25 - 7.41)	7.32	(7.25-7.38)
$paCO_2$	43	(34 - 52)	44	(38-51)
paO_2	318	(235 - 424)	414	(191-511)
$cHCO_3^-$	22	(19 - 27)	22	(16.6-26.7)
BE(ecf)	-4.00	(-8.80 – 1.00)	-3.62	(-10.70 – 0.8)
Sodium (Na)	146	(117 - 153)	151	(146-159)
Potassium (K)	3.4	(2.5 - 4.6)	3.4	(3.0-3.7)
Calcium (Ca)	1.0	(0.53 - 1.21)	1.2	(1.02-1.37)
Chloride (Cl)	118	(111 - 123)	117	(112-126)
$cTCO_2$	24	(19.8 - 28.6)	24	(17.7-28.3)
Anion gap	10	(9 - 12)	11	(7-14)
Anion gap K	13.6	(12 - 15)	14.5	(11-18)
Haematocrit (Hct)	0.34	(0.27 - 0.39)	0.32	(0.29-0.37)
Haemoglobin (cHb)	7.15	(5.6 - 8.3)	6.78	(6.2 – 7.9)
BE (b)	-4.02	(-8.4 – 0.1)	-3.56	(-10.0 – 0.3)
Glucose	8.0	(5.9 – 10.4)	11.1	(7.5 – 20.0)
Lactate	2.4	(1.1 – 3.9)	2.6	(1.0 – 4.8)
Creatinine	106	(59 - 142)	92	(74 - 116)

$PaCO_2$: arterial partial pressure carbon dioxide; PaO_2: arterial partial pressure oxygen; $cHCO_3^-$: bicarbonate, BE: base excess

The null hypothesis for quality and efficacy of anaesthesia was not rejected because there was no significant difference in the times and quality of induction, general anaesthesia and recovery of serval administered with KBM to those administered with BMM. The null hypothesis for the cardiopulmonary effects was rejected and the alternative hypothesis adopted because there were significant differences between the cardiopulmonary effects on serval administered with KBM to those administered with BMM.

Discussion

Free-ranging cage trapped serval immobilised using either ketamine-butorphanol-medetomidine or butorphanol-midazolam-medetomidine produced predictable and reliable immobilisation with stable cardiopulmonary affects. KBM had a notably shorter time to first handling compared to BMM. The stress score correlated to blood glucose concentrations and temperature. The systolic blood pressure, respiratory rate, end tidal carbon dioxide and tidal volume were different between the combinations. Despite no difference in recovery times, there was less ataxia noted during BMM recovery.

Drug combinations that provide rapid induction, stable immobilisation and that are reversible are ideal for free-ranging felids that require short-duration non-invasive procedures (Rockhill et al. 2011). We found that KBM provided a quicker and more favourable onset of light plane anaesthesia. Rapid induction times are important for animal and handler safety alike. Two serval darted with BMM required an additional ketamine bolus before they could be safely handled. Similarly, Eggers et al. (2016) found that eight out of twenty-three black-footed cats that received BMM required additional boluses of BMM before they could be safely handled. When dealing with free-ranging animals, where the threat of injury to animal and personnel are high, combinations that do not require additional boluses are advantageous for safety (Wenger et al. 2010). In the interest of safety for personnel and animals alike, free-ranging animals should always be approached with caution and assessed, by response to stimuli (tail tug, auricular reflex, palpebral reflex), for adequate sedation before handling.

Kock & Burroughs (2012) described that the ideal time to first handling in chemically captured free-ranging animals is under eight minutes. BMM has been noted to produce a

reliable induction and immobilisation in cheetah, black-footed cats, lions and patas monkeys (Kalema-Zikusoka 2003; Lafortune et al. 2005; Wenger et al. 2010; Eggers et al. 2016). Similarly, KBM has also been found to produce an effective and reliable induction and general anaesthesia in bobcats, captive serval and patas monkeys (Langan et al. 2000; Kalema-Zikusoka et al. 2003; Rockhil et al. 2011). Faster induction times were observed in cheetah, black-footed cats, lions and patas monkeys and that were darted with BMM compared to our findings (Kalema-Zikusoka et al. 2003; Lafortune et al. 2005; Wenger et al. 2010; Eggers et al. 2016). A possible explanation for the slower induction times seen in the serval can be explained by the exposure to stressors prior to darting. Stegmann and Jago (2006) noted the potential for a delayed induction in animals that undergo periods of stress, excitation and physical exertion. Furthermore, previously stressed animals may require higher drug doses for induction (Gunkel & Lafortune 2007). It is therefore important to minimise noise and provide shelter from other environmental stressors to facilitate a rapid induction (Rockhill et al. 2011; Ramsay 2014). Our serval were subject to stressors such as transportation while trapped in a cage and exposure to people within their fight-flight zone outside the procedure room area prior to darting. Captive serval required 1.0 ±0.2, 0.2 ±0.03 and 0.047 ±0.010 mg kg^{-1} of ketamine, butorphanol and medetomidine, respectively which were significantly lower doses compared to what we found to be effective in free-ranging serval (Langan et al. 2000). The difference in effective drug dose suggests that animals that are habituated with their surroundings, human presence and capture techniques undergo less stress during capture and therefore require lower doses.

In addition to safety, a quick onset of immobilisation aids to decreasing the distance of wandering after being darted (Kock & Burroughs 2012) and mitigates prolonged exposure to

stress and struggling associated with frequent escape attempts, thereby, helping to prevent possibilities of injury during escape attempts (Nielsen & Woolf 2002) and ameliorating increases in body temperature due to excessive movement (Stegmann & Jago 2006; Kock & Burroughs 2012) and stress (Meyer & Fish. 2008).

'Stress response' is a term used to describe the animal's normal endocrine systems response to any noxious stimuli (Johnson & Norman 2007). It is assumed that body temperature in free-ranging felids is to be of a similar range to that of domesticated cats (Gunkel & Lafortune 2007). It was observed that serval exposed to excessive amounts of stress (transportation, prolonged handling of the cage trap, excessive human presence, excessive noise, repeated failure of dart attempts, repeated failed escape attempts, and injuries sustained from escape attempts) showed significantly higher blood glucose levels and temperature. These results indicated that serval exposed to excessive environmental stimuli experienced a significant 'stress response' with a resultant 'stress-induced hyperthermia and hyperglycaemia'. Hyperthermia is thought to result from high environmental temperatures, stressful and prolonged inductions to general anaesthesia (Ko & Krimins 2014), muscle heat production and from certain drug reactions such as ketamine side effects that cause muscular hypertonicity (Kock & Burroughs 2012) and reduced heat loss due to vasoconstrictive effects of medetomidine (Murrell 2007). However, acute rises in body temperature during immobilisation are more closely related to the stress response exhibited by an animal and not because of drug effects, environmental temperature and muscular activity (Meyer & Fish 2008). Free-ranging felids lose heat through panting (Ko & Krimins 2014), which they are unable to do under general anaesthesia. Hyperthermia, especially if body temperatures exceed 41°C, should be treated immediately and the most effective and practical field method

could be total body water-dousing and rubbing the hair to ensure the water makes contact with the skin (Sawicka et al. 2015). Other less practical field methods could include applying alcohol to shaved areas to facilitate evaporative cooling, bolusing fluids, reversal of α_2-adrenergic receptor agonist, cold water enemas and moving the patient to an area with a low ambient temperature (Kock & Burroughs 2012; Ko & Krimins 2014). Furthermore, struggling and exposure to stressors have been reported to result in stress induced hyperglycaemia, observed in our serval that had a higher stress score (Rand et al. 2002). Medetomidine can induce a transient hyperglycaemia (Murrell 2007); however, both drug combinations received the same dose of medetomidine and in both groups we detected a significant increase in blood glucose levels in those that were more stressed, therefore, suggesting there is a relationship between hyperglycaemia and stress. Kanda and Hikasa (2008) discovered that midazolam can negate neurohormonal adverse effects of medetomidine, such as hyperglycaemia. Therefore, the addition of midazolam to combinations with medetomidine, may aid in reducing medetomidine induced hyperglycaemia and its compounding effect on stress induced hyperglycaemia.

Both combinations produced a stable and reliable immobilisation in which the serval were sufficiently sedated to allow safe handling and permit the performance of non-surgical procedures. Similar lengths of duration of sedation were seen in free-ranging lions, black-footed cats, and patas monkeys anaesthetised using BMM combination with longer durations seen in cheetah (Kalema-Zikusoka et al. 2003; Lafortune et al. 2005; Wenger et al. 2010; Eggers et al. 2016). BMM produced a longer anaesthetic in a comparative study in patas monkeys when compared to KBM (Kalema-Zikusoka et al. 2003). Although both BMM and KBM average times to rescue intervention were similar, subjectively, BMM was assessed to

have a mildly deeper sedation effect and serval appeared to be less easily aroused when compared to KBM. Rockhill et al. (2011) reported that KBM produced a shorter duration of general anaesthesia in bobcats, when compared to our study, and it was noticed that the bobcats were sensitive to noises and movement in the environment. Similarly, serval that received KBM showed earlier increases in their strength and frequency of responses to pinnal, pedal and palpebral reflexes and were found to be more easily aroused when compared to serval that received BMM. It is well known that with the use of ketamine, the pinnal and ocular reflexes are still present in the animal's dissociative state (Kastner 2007). The reason for this is because duration of action of ketamine is usually short lived in felids, with effects wearing off after approximately 20-45 minutes (Kreeger 1996; Gunkel & Fortune 2007; Ramsay 2014). Ketamine also affects the uptake of noradrenaline by inhibiting the noradrenaline transport system with a resultant increase in noradrenaline concentrations (Hara et al. 2002; Kitagawa et al. 2003). Noradrenaline has an affinity for adrenergic receptors (Kock & Burroughs 2012) and competes with α_2-adrenergic receptor agonists resulting in a decrease in the depth of sedation and the animal being more sensitive to environmental stimuli.

The arterial blood pressure was significantly higher in KBM compared to BMM for the initial 40 minutes. Similarly, patas monkeys that received KBM showed higher blood pressure readings than those that received BMM (Kalema-Zikusoka et al. 2003). Black-footed cats that received BMM demonstrated normotensive blood pressure readings (Eggers et al. 2016). Ketamine related increases in noradrenaline has stimulatory effects on the cardiovascular system by increasing sympathetic tone with resultant increases in heart rate, blood pressure and cardiac output (Hara et al. 2002; Kastner 2007; Ko 2013). Hypertension is also a common

side effect of α_2-adrenergic receptor agonists (Gunkel & Lafortune 2007), together with the stimulatory effects of ketamine, could possibly explain why we identified higher blood arterial pressure in the KBM group. There was a complete failure rate to obtain blood pressure results for the initial 15 minutes of anaesthesia in serval darted with KBM. Stegmann and Jago (2006) had similar difficulties obtaining non-invasive blood pressure readings in cheetah that were immobilised with ketamine and medetomidine. Our initial blood pressure values for KBM may have been too high and therefore exceeded the capacity of the oscillometric blood pressure monitor to read those values, which could potentially be detrimental to the health of the animal, particularly in cardiac diseased animals. It was observed that blood pressure values decreased when isoflurane rescue was initiated. Isoflurane causes a dose dependent cardiovascular depression with resultant vasodilation and hypotension (Matthews 2007; Fornes 2010). For adequate perfusion, mean arterial blood pressure in free-ranging felids should be maintained between 60 and 110 mmHg which was the case in our serval (Gunkel & Lafortune 2007).

Bradycardia is described to be a heart rate between 25 and 60 beats per minute, depending on the species and body size; and in cats of this size, we considered bradycardia as a heart rate of less than 60 beats per minute (Gunkel & Lafortune 2007). With both combinations, the heart rate in serval were considered above what would be considered bradycardic. Similarly, there was no bradycardia noted in captive serval that received KBM and black-footed cats that received BMM (Langan et al. 2000; Eggers et al. 2016). Verstegen & Petcho (1993) found that statistically significant bradycardia was caused in dogs that were immobilised with BMM. Similarly, bradycardia was observed in a significant portion of lion that received BMM and it was also reported in patas monkeys (Kalema-Zikusoka et al. 2003;

Wenger et al. 2010). Bradycardia was observed in patas monkeys that received KBM (Kalema-Zikusoka et al. 2003).

Notable differences in respiratory rate and tidal volumes was identified between KBM and BMM. Respiratory depression was seen with KBM with normal respiratory rates in BMM. Verstegen & Petcho (1993) and Eggers et al. (2016) both noted that there were no significant respiratory effects in dogs and black-footed cats that were immobilised with BMM, respectively. Two cats immobilised with KBM also showed apneustic breathing patterns. Ketamine produces a dose-dependent respiratory depression and is known to cause apneustic breathing patterns (Meyer & Fish 2008; Lamont & Grimm 2014). Acceptable ranges for end tidal carbon dioxide in free-ranging felids is said to be between 30 to 50 mmHg (Gunkel & Lafortune 2007). Both groups were found to be in the acceptable range for end tidal carbon dioxide; however, showed significant differences in tidal volume. KBM produced deeper, slower breaths compared to shallower, faster breathing in BMM. Despite their differences, both groups were considered to be adequately ventilated (based on $PE'CO_2$ and $PaCO_2$) with normal pulse oximetry values. Pulse oximetry is calibrated for human use, caution should be used when interpreting readings in free-ranging felids as it is not considered a reliable predictor of ventilation status and is known to produce falsely low readings (Langan et al. 2000; Lafortune et al. 2005; Gunkel & Lafortune 2007).

During recovery, BMM was subjectively assessed to have a better and less ataxic recovery than KBM. Kalema-Zikusoka et al. (2003) noted a similar pattern of recovery in Patas monkeys with a longer and more ataxic recovery with KBM compared to BMM. Felids recovering from ketamine may be stormy and display ataxic behaviour (West et al. 2014). Ketamine cannot be antagonised, therefore, sufficient time is necessary for the liver to metabolise and the kidneys

to excrete the drug and its metabolites, norketamine, to avoid stormy recoveries (Kastner 2007; Kock & Burroughs 2012). A calmer recovery in BMM can be explained by the lasting placated action of midazolam, which was not antagonised in our study. Benzodiazepines agonists can be antagonised by administration of flumazenil, but due to costs, has limited use in wildlife (Kock & Burroughs 2012). In captive scenarios the sedative effects of midazolam have beneficial effects during recovery; however, it is advantageous to be antagonised in free-ranging conditions (Gunkel & Lafortune 2007). Eggers et al. (2016) noticed that black-footed cats that did not receive flumazenil were oblivious of their surroundings and were at risk of injury or predation. Animals immobilised with BMM that are at risk of predation or injury should be recovered in a sheltered, controlled environment safe from predators and only released when all drug effects have worn off or should be fully reversed at time of antagonist administration (Wenger et al. 2010; Kock & Burroughs 2012).

The major limitation of our study was the overall monitoring of the cage traps. Accessibility to the property was restricted to certain hours during the day, which limited continual access to the cages. The monitoring could have been improved by using camera traps for continual monitoring or using cage traps that activate a signal when an animal has been trapped. These interventions would have assisted us in obtaining accurate duration of cage confinement. However, when collecting the trapped serval in the field, they appeared calm, ate the bait and were often found resting without demonstrating anxiety, until the cage was approached. The transport of the serval to the procedure room was another limitation whereby we exposed them to additional stressors which likely influenced our induction times. To reduce exposure to stress from travel, serval can be darted at the trapping site. This posed another logistical concern of setting up, darting the serval, capturing data and packing up after each

serval at each capture site. This is time consuming and would result in serval being trapped for longer periods of time. Furthermore, despite these limitations, we had no mortalities and only minor morbidities during the study.

Conclusion

In conclusion, ketamine-butorphanol-medetomidine and butorphanol-midazolam-medetomidine drug combinations can be used to reliably and effectively chemically capture free-ranging cage-trapped serval. Serval that demonstrate stress and anxiety must be monitored for capture induced hyperthermia and treated immediately to avoid capture related complications. Both drug combinations provided adequate general anaesthesia that lasted at least 35 minutes. Overall, KBM provided the most reliable induction into anaesthesia and BMM provided the calmest recovery. Neither combination was overall preferred; however, BMM is full-antagonizable and could be advantageous in free-ranging conditions where serval must be released immediately after recovery.

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Addendum

Data collection forms

SERVAL (*Leptailurus serval*) IMMOBILISATION DATA CAPTURE SHEET

PRIMARY INVESTIGATOR:

Dr CJ Blignaut

chrisbliganut90@gmail.com

072 424 9051

SERVAL ID: _____ **DATE:_____**

ANAESTHETIC COMBINATION:

1. **KBM** ☐
2. **BMM** ☐

DOSAGES:

Ketamine: _____ **Antisedan: _____**

Butorphanol: _ **Naltrexone: _____**

BODY CONDITION SCORE: ☐

Medetomidine: _____

Estimated weight: ☐

Midazolam: _____

Actual weight: ☐

Male/Female: _____

Estimated age: ☐

PHYSIOLOGICAL PARAMETERS:

TIME (MIN)	RESP RATE	HEART RATE	ARTERIAL BP	TEMP	SpO_2	END TIDAL CO_2	RESPONSE EXT STIM	Exp Tidal Volume (ml)	ABG/VBG
0									
5									
10									
15									
20									
25									
30									
35									
40									
45									
50									
55									
60									
65									
70									
75									

80									
85									
90									
100									
110									
120									

STRESS SCORE

Score: 0 1 2 3

EFFICACY OF INDUCTION SCORE

Score: 0 1 2 3 4 **Dart Time:**

Time from darting to first effect _____ min
Time from darting to recumbency _____ min
Time from darting to head down _____ min
Time from darting to first handling _ min
Remarks:

QUALITY OF GENERAL ANAESTHESIA SCORE

Score: 1 2 3 4 5 **ISO STOP:**

Remarks: **Extubation time:**

Time (Min)	Response to external stimuli (Y/N)	Amount of top up required (Ketamine)	
		Time (Min)	Amount (ml)

Injuries:

RECOVERY SCORE

Score: 1 2 3 4

Injection time: _____

Time from reversal to first movement _____min

Time from reversal to standing _____min

Signs of ataxia: None / Mild / Moderate / Severe

Remarks:

_____

_____

_____

Publications arising from the study

Christiaan J Blignaut, Gerhard Steenkamp, Daan Loock, Roxanne Emslie & Gareth E Zeiler, Ketamine-butorphanol-medetomidine versus butorphanol-midazolam-medetomidine immobilisation of serval (*Leptailurus serval*). *Veterinary Anaesthesia and Analgesia* (Under review: October 2019)

Animal ethics approval certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	Ketamine-butorphanol-medetomidine versus butorphanol-midazolam-medetomidine immobilisation of serval (*Leptailurus serval*)
PROJECT NUMBER	V108-17
RESEARCHER/PRINCIPAL INVESTIGATOR	Dr. CJ Blignaut

STUDENT NUMBER (where applicable)	U 29003352
DISSERTATION/THESIS SUBMITTED FOR	MSc

ANIMAL SPECIES	Serval (*Leptailurus serval*)	
NUMBER OF SAMPLES	30	
Approval period to use animals for research/testing purposes		October 2017 – October 2018
SUPERVISOR	Prof. G Zeiler	

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	30 October 2017
CHAIRMAN: UP Animal Ethics Committee	Signature	

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