J S Afr Vet Assoc. 2023;94:35-41
https://doi.org/10.36303/JSAVA.513

J S Afr Vet Assoc
ISSN 2224-9435 EISSN 1019-9128

ORIGINAL RESEARCH

Development of a novel immobilisation protocol for black-faced impala (*Aepyceros melampus ssp. petersi*) in Etosha National Park

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Black-faced impala (*Aepyceros melampus ssp. petersi)* are endemic to Namibia where conservation management involves immobilisation and translocation, and mortality with current protocols is common. Critically evaluated field immobilisation protocols are needed to maximise animal safety.

This prospective study was done in two phases: the first compared etorphine- and thiafentanil-based combinations, the second evaluated the influence of oxygen in impala receiving the thiafentanil-based combination. Animals (10 per group) received 50 mg ketamine (K) and 10 mg butorphanol (B), with either 2.0 mg etorphine (E) or 2.0 mg thiafentanil (T). A third group of ten impala were anaesthetised using TKB with supplemental nasal oxygen (O) at a rate of 5 L/minute. Behavioural, metabolic and physiological variables were assessed within five minutes of recumbency and at 10, 15, and 20 minutes post-recumbency. Statistical analyses for non-parametric data were performed to compare the treatment groups as well as time points; $p \leq 0.05$ considered significant.

Following darting, 7/10 EKB animals were standing when approached, compared to 2/20 in the thiafentanil treatment groups. Time to first effect was significantly higher for EKB (155 ± 105.7 seconds) compared to TKBO (61.5 ± 21.4 seconds). Time to sternal after darting was significantly higher with EKB (411.6 ± 174 seconds) compared to TKB (160.5 ± 85.4 seconds) and TKBO (166 ± 77.3 seconds).

This study builds on previous work investigating the effects of potent opioids on impala and is the first evaluating their use in a field setting. The thiafentanil combination had a faster onset and resulted in a smoother induction than the etorphine combination. Additionally, oxygenation improved in animals receiving oxygen supplementation.

Keywords: *Aepyceros melampus*, sedation, butorphanol, impala, potent opioid, etorphine, thiafentanil, translocation

Introduction

Black-faced impala (*Aepyceros melampus ssp. petersi*) are a rare sub-species distinct from common impala (*Aepyceros melampus*) (IUCN 2017). Historically they existed in Angola and Namibia, but currently there is no data on the population in Angola, and only an estimated 3 000–4 000 mature animals exist in Namibia, with a projected 10% decline over three generations (IUCN 2017). They are listed as vulnerable, with the major threats being poaching, drought, and hybridisation with the common impala (IUCN 2017). Management of black-faced impala includes translocation using anaesthetic drugs administered by remote dart, with limited opportunities for support. While immobilisation studies have been performed in common impala, most have been performed in captive or boma settings (Buck et al. 2017; Gerlach et al. 2017; Perrin et al. 2015; Zeiler & Meyer 2017a; Zeiler et al. 2015). Habituation is shown to reduce the stress response in other species following darting and manual capture (Meyer et al. 2008). This difference, and differences in environmental conditions between captive and field settings may have an impact on drug dose and animal responses. Hence, evaluation of the safety and efficacy of immobilisation protocols for black-faced impala in their natural (field) habitat is needed to minimise morbidity and mortality of this vulnerable species during translocation.

The most common protocols for immobilisation of impala involve a potent opioid (thiafentanil or etorphine) combined with another drug, commonly an alpha-2 agonist such as medetomidine (Pfitzer et al. 2019; Zeiler & Meyer 2017b). This combination is routinely used for a number of non-domestic hoof stock species and is generally well tolerated. However, common impala demonstrate markedly decreased respiratory rates and impaired oxygenation when under opioid anaesthesia (Grobler et al. 2001; Pfitzer et al. 2020; Pfitzer et al. 2021; Zeiler et al. 2015). Many strategies have been advocated to manage these respiratory issues and are often circumstance-dependent. One that is increasingly proposed is the use of the opioid agonist-antagonist butorphanol in combination with a potent opioid. Butorphanol is a kappa agonist and mu receptor antagonist, while thiafentanil is solely a mu receptor agonist, and etorphine is an agonist for mu-, kappa-, and delta opioid receptors (Ogutman et al. 1995). The use of butorphanol has shown demonstrable benefit to respiratory (e.g. respiratory rate) and metabolic variables as well as immobilisation quality in white rhinoceros (*Ceratotherium simum*) (Miller et al. 2013). Intravenous boluses of butorphanol have been used to rescue impala from apnoea following induction of immobilisation with a potent opioid, but to the authors' knowledge, there are no objective studies evaluating the impact of including butorphanol in the

induction dart of impala (Zeiler & Meyer 2017b). The impact of medetomidine is also not fully elucidated but given the periodic challenges of obtaining this drug in certain parts of southern Africa, authors elected to evaluate a modified opioid-based protocol with a view to improving immobilisation safety in impala in their natural habitat.

The aim of this study was to compare selected behavioural, metabolic, and physiological responses of black-faced impala to etorphine or thiafentanil in combination with butorphanol and ketamine under field conditions. Additionally, the impact of supplemental oxygen in impala receiving the thiafentanil combination was assessed. It was hypothesised that the induction time and immobilisation quality would differ between the two opioids, and that supplemental oxygen would positively influence blood oxygenation and other physiological variables.

Materials and methods

This study utilised free-ranging black-faced impala living within Etosha National Park in Namibia. Thirty adult female impala weighing an average of 50 kg (range 43–60 kg) in presumed good health based on visual evaluation were selected for study. The study was done in two phases. During the first phase, animals received either an etorphine- or thiafentanil-based drug protocol in a randomised manner. During the second phase the influence of nasal oxygen on physiological variables in animals receiving the thiafentanil-based protocol was assessed.

During phase one all animals received 50 mg ketamine (K, Ketamine HCl, 200 mg/ml, Intersana, Namibia), 10 mg butorphanol (B, Dolorex, 10 mg/ml, MSD Animal Health, South Africa), and either 2.0 mg etorphine (E, Captivon, 9.8 mg/ml, Wildlife Pharmaceuticals [Pty] LTD, South Africa) or 2.0 mg thiafentanil (T, Thianil, 10 mg/ml, Wildlife Pharmaceuticals [Pty] Ltd, South Africa). Ten female impala were included in each group. They were darted in the triceps muscle from a vehicle at various watering holes within the park, using a gauged projector (X-Caliber; Pneu-Dart Inc., Pennsylvania, United States [US]) combined with 1 ml P-type Pneu-Darts with 1.9 cm barbed needles (Pneu-Dart Inc., Pennsylvania, US). The average ambient temperature was 29.0 ^{0}C (range 21–37 ^{0}C), and the mean barometric pressure was 670.4 mmHg (range 669–672 mmHg).

Once darted, animals were followed by car or on foot until they were recumbent, then carried into the shade, blindfolded and placed in sternal recumbency to facilitate breathing, and with the head up and nose pointing downwards to minimise aspiration in case of regurgitation. The dart was removed, and the wound inspected and treated with a wound spray (Terramycin Wound Spray, Pfizer Laboratories [Pty] Ltd, South Africa). Animals were also marked with paint to avoid recapture.

Monitoring and sample collection began once the animal was placed in sternal recumbency and continued at five-minute intervals for 20 minutes. Respiratory rate was assessed visually, and heart rate assessed via auscultation with a stethoscope (Litmann Master Cardiology, 3M™, Minnesota, USA) over a period of 15 seconds. Rectal temperature was measured using a handheld digital thermometer (iProven Dt-R1221AWG Medical Thermometer, Oregon, USA). Peripheral oxyhaemoglobin saturation (SpO_2) was measured using a pulse oximeter (2500A Vet Pulse Oximeter, Nonin Medical Inc, Minnesota, USA) with a transflectance sensor placed inside the vulva. The environmental temperature was measured by the Pantek Technologies LLC Fisher Scientific Ambient Temperature Thermometer (Pantek Technologies LLC, New Jersey, USA) and the barometric pressure

Table I: Immobilisation (recorded just after induction), muscle relaxation during recumbency and recovery quality scoring guidelines; recovery and induction scores are adapted from Pon et al. (2016)

Score	Immobilisation description	Muscle relaxation description	Recovery description
1	No sedation apparent.	Not able to extend leg, firm resistance felt.	Very violent, self-inflicted injury, prolonged struggling or unable to stand 2 hr after the end of anaesthesia.
2	Standing sedation only. Low head carriage, ataxia, animal moves away when approached.	Able to extend leg but animal pulls back.	Excitement when recumbent, persistent unsuccessful attempts to stand, severe ataxia, and may fall again once standing. Aimless walking, high risk of self-inflicted injury.
3	Standing sedation only. Low head carriage, ataxia, animal does not move away when approached and can be touched.	Able to extend leg with mild resistance, animal pulling back only slightly.	Some staggering and ataxia, a few unsuccessful attempts to stand, ataxic immediately after standing up.
4	Sternal recumbency with head up, will attempt to rise when stimulated.	Little resistance, animal not pulling back when leg extended.	Slight ataxia and staggering, stood at first or second attempt, no serious instability.
5	Sternal recumbency with head up, will not attempt to rise when stimulated.	No resistance, leg remains fully extended when pulled.	No ataxia, no struggling, stood up at first attempt as if fully conscious.
6	Sternal recumbency with head down, lifts head on stimulation.		
7	Sternal recumbency with head down, does not lift head on stimulation, can be rolled into lateral recumbency, and maintains this position.		
8	Lateral recumbency. Animal induces into lateral recumbency and maintains this position.		

was measured by the iSTAT portable blood gas analyser (iSTAT Portable Clinical Analyzer, Abbott Laboratories, Illinois, US). This machine was also used to analyse arterial blood samples that were anaerobically collected from the auricular artery. A 0.5–1.0 ml sample was collected using a heparin-rinsed 1.0 ml syringe with a 25 gauge needle. The sample was immediately transferred into an iSTAT CG8 cartridge (iSTAT, Abbott Laboratories, Illinois, USA) for analysis of pHa, PaO_2 (partial pressure of oxygen in arterial blood), $PaCO_2$ (partial pressure of carbon dioxide in arterial blood), glucose and haemoglobin. Bicarbonate concentration (HCO_3) and base excess (BE) were calculated. Once the blood gas sample was aliquoted into the cartridge, lactate measurement was performed on a drop of blood using a Lactate Plus analyser (nova biomedical, Massachusetts, USA).

Time to first effect, recumbency and time from reversal to standing were measured with a wristwatch (Armitron Sport Digital Chronograph Resin Watch, Armitron Sport, China).

Quality of immobilisation was scored just after induction using the scoring established in white-tailed deer (*Odocoileus virginianus*) (Table I) (Pon et al. 2016). Muscle relaxation was assessed at each time point using a standardised five-point system by lightly pulling on the animal's left rear leg (Table I). The following variables were also evaluated at each time point: jaw tone, palpebral reflex, eye position, response to ear flick, response to needle prick at coronary band, response to venepuncture.

After the last arterial sample was taken, impala were weighed (Mellerware Berlin Mechanical bathroom scale, Creative Housewares [Pty] Ltd, South Africa) and then 20 mg naltrexone (Trexonil 50 mg/ml, Wildlife Pharmaceuticals [Pty] Ltd, South Africa) was administered IV in the jugular vein. Recovery quality was scored using a five-point system (Table I, adapted from Pon et al. 2016). Time to standing after administration of naltrexone was recorded. Once standing, impala were released from manual restraint and joined their herd.

To compare the effects of supplemental oxygen in black-faced impala, a third group of 10 impala were immobilised using the thiafentanil protocol as described previously. Additionally, once animals were sternal, they received intranasal oxygen insufflation (O) at a rate of 5 L/minute using a portable oxygen cannister containing 100% oxygen and rubber tubing placed in the nose to the level of the medial canthus of the eye. Behavioural and physiological variables were assessed as described previously. Oxygen administration was discontinued once naltrexone was given.

Data analysis

The continuous data were evaluated for assumption of normality (Shapiro–Wilk statistics) prior to using mixed models to analyse the data. Least square means were calculated to compare the treatments. Tukey's test was used to adjust for multiple comparisons and adjusted *p*-values were reported. If normality was not met or when the data was a score, a non-parametric Kruskal–Wallis test was used to compare the treatments at each time point. Friedman's test with post-hoc test was used to analyse the data after adjusting for repeated measures across time points, followed by pair-wise comparisons. The data collected only at one time point was analysed using ANOVA if normality was met and using Kruskal–Wallis test when normality was not met, followed by pair-wise comparisons. A *p*-value of 0.05 was used to evaluate statistical significance. SAS v9.4 (SAS Institute Inc., Cary, NC) was used for all statistical analyses.

Results

Time to first effect, time to sternal recumbency after darting, and quality of immobilisation are presented in Table II. Time to first effect was significantly longer for EKB compared to TKBO, and time to sternal after darting was significantly longer with EKB compared to TKB and TKBO. Quality of immobilisation just after induction was significantly better with TKB and TKBO compared to EKB.

Table II: Time (seconds) to first effect and sternal recumbency after darting, and quality of immobilisation just after induction, in black-faced impala (*Aepyceros melampus ssp. petersi*) administered 2.0 mg etorphine, 50 mg ketamine, and 10 mg butorphanol, (treatment EKB) or 2.0 mg thiafentanil, 50 mg ketamine, and 10 mg butorphanol (treatment TKB) or 2.0 mg thiafentanil, 50 mg ketamine, and 10 mg butorphanol with supplemental oxygen (treatment TKBO)

Variable	Treatment EKB	Treatment TKB	Treatment TKBO
Time to first effect (sec)	110 (65–395)[a]	70 (45–215)[a,b]	60 (20–90)[b]
Time to sternal recumbency after darting (sec)	388 (101–735)[a]	125 (75–330)[b]	150 (20–280)[b]
Quality of immobilisation just after induction	3 (2–8)[a]	6 (3–8)[b]	8 (3–8)[b]

Data are presented as median, minimum and maximum; higher score for quality of immobilisation just after induction indicates a deeper level of immobilisation, less reactive to stimuli; values with the same superscripted letter [a,b] are not significantly different from each other for a given variable

Table III: Muscle relaxation scores and significance between timepoints within a treatment group in black-faced impala (*Aepyceros melampus ssp. petersi*) administered 2.0 mg etorphine, 50 mg ketamine, and 10 mg butorphanol, (treatment EKB) or 2.0 mg thiafentanil, 50 mg ketamine, and 10 mg butorphanol (treatment TKB) or 2.0 mg thiafentanil, 50 mg ketamine, and 10 mg butorphanol with supplemental oxygen (treatment TKBO)

Muscle relaxation score post positioning in sternal recumbency				
Minutes	5	10	15	20
Treatment EKB	4 (2–5)	4 (2–5)	4 (2–5)	3.5 (2–5)
Treatment TKB	4 (4–5)[a]	4 (3–4)[a,b]	4 (2–5)[a,b]	3.5 (2–4)[b]
Treatment TKBO	5 (4–5)	4.5 (4–5)	4 (3–5)	4.5 (3–5)

A higher score indicates more muscle relaxation; data are presented as median, minimum and maximum; values with the same superscripted letter [a,b] are not significantly different from each other for a given treatment

Table IV: Heart rate, pHa, PaO_2, and lactate values in black-faced impala (*Aepyceros melampus ssp. petersi*) administered 2.0 mg etorphine, 50 mg ketamine, and 10 mg butorphanol, (treatment EKB) or 2.0 mg thiafentanil, 50 mg ketamine, and 10 mg butorphanol (treatment TKB) or 2.0 mg thiafentanil, 50 mg ketamine, and 10 mg butorphanol with supplemental oxygen (treatment TKBO)

		Minutes post-positioning in sternal recumbency			
Variable	**Treatment**	**5**	**10**	**15**	**20**
Heart rate (beats/min)	EKB	98.8 ± 14.1[a]	96.5 ± 19.3[a]	100.2 ± 19.0[a]	101.4 ± 20.0
	TKB	138.8 ± 16.3[b]	129 ± 12.5[b]	132.6 ± 17.4[b]	123 ± 15.8
	TKBO	142.6 ± 29.3[c]	125.4 ± 28.5[a,b]	114.4 ± 22.0[a,b]	107.6 ± 20.6
pHa (units)	EKB	7.36 ± 0.1	7.39 ± 0.1	7.39 ± 0.0	7.40 ± 0.1
	TKB	7.31 ± 0.1	7.36 ± 0.07	7.35 ± 0.1	7.39 ± 0.1
	TKBO	7.36 ± 0.0	7.38 ± 0.1	7.39 ± 0.0	7.39 ± 0.1
PaO_2 (mm Hg)	EKB	46.2 ± 9.4	52.6 ± 4.5[a,b]	48.2 ± 5.5[a,b]	48.8 ± 7.8[a,b]
	TKB	36.5 ± 8.3	44.7 ± 5.6[a]	42.9 ± 5.4[a]	46.5 ± 8.1[a]
	TKBO	34.0 ± 8.1	62.2 ± 9.8[b]	59.8 ± 11.1[b]	66.3 ±12.8[b]
$PaCO_2$ (mm Hg)	EKB	49.6 ± 2.8	46.6 ± 3.5	48.7 ± 3.8	48.8 ± 9.4
	TKB	53.4 ± 3.9	50.5 ± 3.0	53.6 ± 5.7	49.0 ± 6.9
	TKBO	55.8 ± 4.4	54.1 ± 7.1	52.9 ± 5.4	55.0 ± 8.2
Lactate (mmol/L)	EKB	3.41 ± 3.8	2.72 ± 2.8	2.57 ± 2.1	2.38 ± 1.6
	TKB	5.55 ± 5.1	4.26 ± 4.2	3.83 ± 3.2	3.31 ± 3.3
	TKBO	2.84 ± 1.3	1.73 ± 0.9	1.39 ± 0.6	1.19 ± 0.6

Values with the same superscripted letter [a,b] are not significantly different from each other at a given time point between treatments

Muscle relaxation scores are presented in Table III. Animals became less relaxed over time with all three protocols, but this change was only significant for the TKB group with a significantly lower relaxation score at 20 vs 5 minutes. Impala in the TKBO group were more relaxed than the TKB groups; this was statistically significant at the 10 minute time point.

Heart rate, pHa, PaO_2, $PaCO_2$ and lactate values over time are presented in Table IV. There was a significant interaction of treatment and time for heart rate. Mean heart rate was significantly slower with EKB than TKB at t = 5, 10 and 15, and also slower than TKBO at t = 5. Heart rate significantly decreased over time with TKBO. pHa was significantly lower at t = 5 than t = 20 for the TKB treatment group compared to the other two groups. pHa was not significantly different at any other time point for any of the three protocols. The PaO_2 in TKB was significantly lower than for TKBO at t = 10, t = 15 and t = 20. For the TKBO treatment group, PaO_2 was significantly lower at t = 5 than t = 10, 15, or 20. Lactate decreased over time for both TKB and TKBO and was significantly higher at t = 5 than at all other time points. There was no statistical difference in lactate between the time points with EKB.

There were no differences in HCO_3., BE, glucose, Hb, $PaCO_2$, SpO_2, jaw tone, palpebral reflex, eye position, response to ear flick, response to needle prick on the coronary band, response to venepuncture, respiratory rate, rectal temperature, recovery quality, or time from reversal to standing between treatments.

Discussion

In summary, time to first effect was significantly longer for EKB compared to TKBO, and time to sternal after darting was significantly longer with EKB compared to both thiafentanil protocols. Also, the quality of immobilisation just after induction was significantly better with both thiafentanil protocols compared to the etorphine protocol. While there was no difference in respiratory rates between the treatment groups, mean heart rate was significantly slower with EKB than TKB at t = 5, 10 and 15. Providing nasal oxygen to the TKBO treatment group resulted in significantly higher PaO2 at three of the four time points compared to the TKB treatment group, and also resulted in a statistically significant increase in oxygenation within the TKBO treatment group over time.

Animals in all three treatment protocols were adequately immobilised and allowed handling until administration of naltrexone. Additionally, no mortality was observed during the study. Time to first effect and time to sternal recumbency after darting are consistent with those reported in common impala (*Aepyceros melampus*), despite using a lower dose of potent opioids in this study (Meyer et al. 2008; Pfitzer et al. 2021; Zeiler & Meyer 2017b). The synergistic effect of ketamine likely facilitated the immobilising effects of the potent opioid, without compromising animal safety or effectiveness of the drug protocol. Time to recumbency was quicker with the thiafentanil combination compared to the etorphine protocol, as previously reported in common impala (Meyer et al. 2008; Pfitzer et al. 2021). A rapid induction is important to minimise 'roam' time during which an animal might cause themselves harm, so while both etorphine and thiafentanil (in combination with ketamine and butorphanol) resulted in recumbency, the faster onset with thiafentanil has potential benefit. Thiafentanil provided more consistency and may minimise complications (e.g. trauma, hyperthermia) associated with prolonged roaming post-drug administration.

Additionally, the quality of immobilisation following darting was better with thiafentanil than etorphine, with 7/10 of the

etorphine treatment group still standing when approached, compared to 2/20 in the two thiafentanil treatment groups (one with TKB and one with TKBO). Animals that were still standing when approached became recumbent once they were freed from branches or other obstacles in the environment. While the distance travelled after darting was not measured, subjectively the animals in the etorphine treatment group appeared to travel farther before reaching sternal recumbency, and often only stopped their forward motion when they became tangled in an obstacle such as a grove of trees.

Muscle relaxation decreased over time with each of the protocols, but animals were able to be safely handled for the 30 minute data collection period. Muscle rigidity is a known side-effect of potent opioids and in addition to drug-induced respiratory depression may contribute to hypoxaemia (Zeiler & Meyer 2017b). Apnoea following drug administration was demonstrated in 33% of impala immobilised with thiafentanil and azaperone in another study (Meyer et al. 2008), and they are a species known to be more sensitive to respiratory depression caused by potent opioids (Pfitzer et al. 2020). None of the animals in this study demonstrated either profound muscle rigidity or apnoea, and there was no difference in muscle relaxation or respiratory rate between animals receiving either of the two potent opioids. The addition of butorphanol in the immobilisation darts likely ameliorated the muscle rigidity and respiratory depression of the potent opioids as has been shown in other species (Buss et al. 2018).

Lactate was highest at the initial measurement for both TKB and TKBO, which also corresponded with the lowest pHa at initial measurement. This lactic acidosis likely resulted from the animals running following darting, and quickly normalised once animals were recumbent.

There was a significant difference in heart rate between the two potent opioid protocols, with animals immobilised with the etorphine protocol having slower heart rates than those immobilised with the thiafentanil protocol in the current study. Despite the use of adjunctive medications in the current study, values for heart rate are broadly comparable to those reported for only etorphine and thiafentanil in common impala (Pfitzer et al. 2020). The difference in heart rates between the potent opioids observed in this study may be due to differences in receptor affinities between the two drugs. Thiafentanil is solely a mu receptor agonist, while etorphine is an agonist for mu-, kappa-, and delta opioid receptors. Mu agonists have minimal effects on heart rate, compared to kappa-agonists, which have been demonstrated to cause bradycardia (Ogutman et al. 1995). All protocols evaluated in this study included butorphanol which is a kappa agonist, so it is possible that the combined kappa activity of etorphine and butorphanol contributed to the slower heart rate in EKB. While the resting heart rate of black-faced impala is unknown, the values of the animals in this study were within the reference range for sheep, which are ruminants of similar size (Izwan et al. 2018); the significance of the difference in heart rate between the two opioid-based protocols is not known. While opioids may differentially activate the sympathetic nervous system to cause tachycardia, a prior report (Pfitzer et al. 2020) evaluating thiafentanil and etorphine in the absence of other medications did not show a statistical difference in heart rate and in fact the initial value tended to be higher in the etorphine as compared to the thiafentanil group. There were no statistical differences between PaO_2 and $PaCO_2$ among the groups that might have impacted heart rate.

PaO_2 values increased over time for all three treatment groups and as expected were the highest for the group supplemented with nasal oxygen. With oxygen supplementation, animals were no longer clinically hypoxaemic with PaO_2 values greater than 60 mmHg at 10 and 20 minutes during recumbency (Paterson et al. 2009). Nasal insufflation of oxygen has been associated with decreased muscle rigidity and movement when $PaO_2 \geq 70$ mm Hg in elk (Paterson et al. 2009). While the PaO_2 values in the impala of this study did not reach these levels, muscle relaxation was significantly reduced for TKB compared to TKBO at t = 10.

Many reasons have been postulated for low PaO_2 values following administration of potent opioids, including decreased respiratory rate, decreased tidal volume, ventilation/perfusion mismatch, diffusion impairment and pulmonary hypertension (Buss & Meltzer 2001; Meyer et al. 2015). While nasal oxygen alone cannot correct all these variables, it can help mitigate severe hypoxemia as demonstrated and may have benefit to animals that are recumbent for long periods of time. While the fraction of inspired oxygen was not measured, insufflation of oxygen should improve PaO_2 values more substantively if the decrease is related to hypoventilation or ventilation perfusion mismatching which suggests causes other than these. In domesticated sheep both postural changes in the absence of drugs and pulmonary parenchymal changes following medications, most notably alpha-2 adrenergic agonists, are reported to contribute to hypoxaemia (Celly et al. 1999). Etorphine-induced pulmonary hypertension is well documented on domestic goats (Meyer et al. 2015). There is no comparable data in non-domesticated small ruminants and limited study with other drugs to make additional comparisons. Novel therapeutics such as the administration of the R-enantiomer of 8-hydroxy-2-(di-n-propylamino) tetralin, a serotonergic ligand, have been shown to improve PaO_2 in common impala, but these drugs are not readily available to most veterinarians globally (Pfitzer et al. 2019). However, nasal oxygen is a relatively easy method to correct hypoxaemia in the field and should be considered when feasible.

Implications

One of the goals of this project was to find a protocol that produces enough restraint for routine sample collection and transport of black-faced impala, but allows support staff with limited medical training to safely manage the animals. Both the etorphine and thiafentanil protocols evaluated here provided conditions allowing animals to be transported in sternal recumbency, without extreme muscle rigidity or apnoea. All animals demonstrated some degree of hypoxaemia, which was improved but not resolved with the addition of nasal oxygen. Nasal oxygen is easily applied in a field setting and can reduce opioid-induced hypoxaemia and reduce the risk of poor

outcomes in this vulnerable species, and therefore should be applied during prolonged immobilisations.

Study limitations

The small sample size within each protocol is acknowledged as a limitation of this study. However, as this study was conducted in the field it was not feasible to increase the sample size. The initial treatments were randomised, and the animals were marked to avoid recapture but the observers were not blinded. The weights of the animals included in the study were estimated prior to darting, and only confirmed once data collection had begun. (A dose of 2 mg of both potent opioids was selected based on a review of literature from common impala in which similar doses for both drugs have been used [Pfitzer et al. 2021; Zeiler & Meyer 2017b]). Additionally, this facilitated equivalency in dart volumes, but authors recognise that these doses may not be equipotent in black-faced impala. For logistical reasons, only females were included in this study. Males may have slightly different drug- and stress-related responses than females, however this has not been confirmed (Collins et al. 2016). Lastly, measurement of blood pressure would allow better interpretation of the cardiovascular effects of the drug combinations, but was not feasible in this field setting.

Conclusion

This study builds on previous work investigating the effects of potent opioid-based drug combinations on impala, but it is the first study evaluating their effects in the animal's natural habitat, and in the subspecies black-faced impala. The results confirm that at these doses the thiafentanil-based protocol has a faster onset and results in a smoother transition to recumbency than the etorphine-based protocol, which is advantageous in free-roaming animals. The degree of drug-induced effects was appropriate for sample collection, and handling and monitoring by a field assistant without medical training. The results here also demonstrated that the severe hypoxaemia caused by potent opioids in impala can be treated with the use of nasal oxygen insufflation. All of the protocols tested in this study resulted in severe hypoxaemia, so nasal oxygen insufflation should be used whenever possible. While both potent opioid protocols tested here achieved the goal of a workable level of field immobilisation in black-faced impala, the more rapid onset and improved relaxation induced by the thiafentanil protocol may offer an advantage in field settings.

Acknowledgements

The study was supported by the staff of Etosha National Park, especially Pierre du Preez and staff from the Etosha Ecological Institute. The authors wish to thank Greg Lewbart for the generous loan of his equipment in the field.

Conflict of interest

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Funding soucre

This work was supported by the North Carolina Zoological Society, the Namibian Ministry of Environment, Forestry and Tourism, and the Chicago Zoological Society Chicago Board of Trade Endangered Species Fund.

Ethical approval

The authors declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.

Prior to the commencement of the study, ethical approval was obtained from the North Carolina Zoo Institutional Animal Care and Use Committee and by Namibian National Commission on Research Science & Technology, Authorization No AN20190525. All institutional and national guidelines for the care and use of laboratory animals were followed.

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