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A MIXTURE OF NALBUPHINE, AZAPERONE, AND MEDETOMIDINE FOR IMMOBILIZING RINGTAILS (*BASSARISCUS ASTUTUS*)

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ABSTRACT: We evaluated a combination of nalbuphine HCl (40 mg/mL), azaperone tartrate (10 mg/mL), and medetomidine HCl (10 mg/mL), a combination known as NAM or NalMed-A, in 23 ringtails (*Bassariscus astutus*) during 29 handling events for a radio-collaring study in southern Oregon, US, from August 2020 to March 2022. The combination was delivered to ringtails by hand injection at 0.075 mL NAM per estimated 1 kg body mass. The mean (± standard deviation, SD) dosage calculated post hoc was 3.366 (±0.724) mg/kg nalbuphine, 0.841 (±0.181) mg/kg medetomidine, and 0.841 (±0.181) mg/kg azaperone. All captured ringtails were effectively immobilized with a mean (SD) induction time of 13.24 (±3.57) min. The medetomidine and nalbuphine components were antagonized with a combination of atipamezole and naltrexone HCl with a mean (SD) recovery time of 2.48 (±1.94) min. This combination appeared to be safe and effective for immobilizing ringtails with a low volume dose, smooth antagonism, and rapid recovery. In addition, NAM does not contain any drugs that are US Drug Enforcement scheduled, which makes it useful for immobilization procedures by wildlife professionals in the US.

Key words: azaperone, *Bassariscus astutus*, chemical immobilization, medetomidine, nalbuphine, NalMed-A, NAM, ringtails.

INTRODUCTION

Ringtails (*Bassariscus astutus*) are small, nocturnal, omnivorous procyonids that are widespread throughout the southwestern US and northern Mexico (Poglayen-Neuwall and Toweill 1988). Oregon, US, represents the most northern edge of their distribution, where their range is limited to the southwest corner of the state. Ringtails are listed as a state sensitive species within Oregon and may be vulnerable to habitat loss and fragmentation (Oregon Department of Fish and Wildlife 2016), but there is limited information on ringtail ecology in Oregon (Green et al. 2018), because of their restricted distribution, suspected low population density, and nocturnal behavior. The Oregon Department of Fish and Wildlife's Oregon Conservation Strategy has indicated an agency need for additional information on ringtails (Oregon Department of Fish and Wildlife 2016).

In August 2020 we initiated a radio-collar study to document southern Oregon ringtail population parameters. This research required handling and safe immobilization of ringtails; however, published chemical immobilization recommendations for the species are limited. Previously documented drug combinations for ringtail immobilization during capture and handling include ketamine hydrochloride 5–25 mg/kg (Callas 1987; Wyatt 1993; Myers 2010), ketamine 10 mg/kg and acepromazine 0.2 mg/kg (Jessup 1982), and tiletamine HCl and zolazepam HCl at 10 mg/kg of body weight (Harrison 2012; Kreeger and Arnemo 2018). The US Drug Enforcement Administration (DEA) regulates the possession and use of ketamine and tiletamine-zolazepam, making their use by nonveterinarian field personnel problematic. We sought a safe and effective chemical immobilization drug for handling ringtails that would provide the benefits of rapid sedation and reversibility with nonscheduled agents.

A combination of nalbuphine HCl (40 mg/mL), azaperone tartrate (10 mg/mL), and medetomidine HCl (10 mg/mL), referred to as NAM or NalMed-A (ZooPharm, Fort Collins, Colorado, USA), has been described as a safe, effective alternative for immobilization of wildlife (Wolfe et al. 2014). The benefits of NAM include smooth induction and recovery with rapid antagonism and no DEA regulatory scheduling (Wolfe et al. 2017). This combination has been used to safely immobilize a wide range of species including elk (*Cervus elaphus nelsoni*), American bison (*Bison bison*), black bears (*Ursus americanus*), and American beaver (*Castor canadensis*; Wolfe et al. 2014, 2016, 2017; Roug et al. 2019), but its use has not been documented in smaller mammals. We assessed the efficacy of NAM for the immobilization and handling of ringtails in the field.

MATERIALS AND METHODS

We captured ringtails between 1 August 2020 and 30 April 2022, in a 324 km^2 area of southern Oregon west of Medford (42°11′18″N, 122°57′53″W). The study area contained a mixture of federal lands (US Bureau of Land Management and United States Department of Agriculture Forest Service), private timberlands, and other private properties. The dominant vegetation was mixed conifer-hardwood forests, oak (*Quercus* spp.) savannas, and shrublands. All ringtails were captured in PVC traps with a guillotine-style closure and opening rear door that measured 15.2 cm × 61 cm (No. 6 Skunk-N-More Live Trap, Ztraps, Lake View, Iowa, USA). Traps were placed next to downed logs, approximately 10.2–61 cm in diameter, and baited with grape jelly. Other baits used included raisins, canned tuna fish, canned sardines, and wet cat food. We placed a total of 71 live traps at baited sites over 543 trap nights.

After capture we transported ringtails to a processing location, typically <50 m from the capture location. We determined sex of a trapped individual using visual cues such as head width (Stangl et al. 2014) and estimated body weight before immobilization using the mean body weights from male and female adult ringtails previously captured in Oregon. We delivered NAM at approximately 0.075 mL per estimated 1 kg bodyweight (3 mg/kg nalbuphine, 0.75 mg/kg azaperone, 0.75 mg/kg medetomidine) using a 1.0 mL syringe fitted with a 2.54 cm, 28-gauge needle via hand injection into the closest thigh muscle while individuals were restrained within the PVC trap. Ringtails were then transferred to a holding cage and placed in a dark, quiet location. We checked immobilized individuals approximately every 3–5 min to ensure safe induction and record the time that ringtails became nonresponsive to stimuli. When the animal was nonresponsive on approach, we weighed it and placed it on an insulated platform. To protect against hypothermia in cold ambient temperatures we placed ringtails on an adjustable heating pad to maintain a body temperature of approximately 37.6 C (Poglayen-Neuwall and Toweill 1988), and to prevent hyperthermia if ambient temperature was above 35 C and body temperature was increasing, we cooled ringtails with an isopropyl alcohol-water mixture applied to the pinnae and groin.

We initiated vital rate monitoring immediately after the animal was nonresponsive and recorded respiration and temperature every 5–10 min during the handling process. We measured respiration by visually assessing chest movements (breaths/minute) and monitored temperature with a rectal digital thermometer (5610, Dynarex, Orangeburg, New York, USA). We measured heart rate and oxygen saturation with a pulse oximeter (340V, Mediaid, Torrance, California, USA). We did not monitor heart rate or oxygen saturation during recapture events, in which we attempted to minimize handling time. To prevent hypoxemia, we gave immobilized ringtail supplemental oxygen at 0.5–2.0 L/min via a small mammal facemask (JorVet, Loveland, Colorado, USA).

Based on information in the literature for this species, as well as body weights from ringtails previously captured in Oregon (Poglayen-Neuwall and Toweill 1988), we considered females >700 g and males >800 g to be adults. These individuals were fitted with either a VHF (16 g M1545 or M1740, Advanced Telemetry System, Isanti, Minnesota, USA) or GPS (40–42 g Quantum 4000, Telemetry Solutions, Concord, California, USA) radio collar. We assessed each captured animal for body condition, age, and presence of ectoparasites; collected hair samples for genetic analysis; measured body weight (g), length (cm), tail length (cm), and girth (cm); and marked each ringtail with a passive integrated transponder (MiniHPT10, Biomark, Boise, Idaho, USA).

After procedures were complete, nalbuphine and medetomidine were antagonized with an intramuscular injection of naltrexone HCl (50 mg/mL, ZooPharm) and atipamezole (25 mg/mL, ZooPharm), delivered at a range of 2.23–5.21 mg naltrexone per milligram of nalbuphine (delivered at a single volume of 0.25 mL/animal), and 5 mg atipamezole per milligram of medetomidine. We transferred ringtails into a holding cage for a minimum of 15 min after administering the antagonists and released them when they became alert (scratching, head up, and standing without swaying) and capable of finding suitable cover from predators and environmental elements.

We defined induction time as the time elapsed between initial NAM injection and the time when the individual became unresponsive to stimuli, and we scored subsequent sedation in five categories based on response to stimuli during handling: (1) animal could not be handled, (2) can be handled but attempts to move away, (3) does not move away but reacts or vocalizes when handled, (4) only slight movement when handled, or (5) no response to stimulus. We wanted to ensure that immobilized ringtails were in a sedation category of $\geq$4 to prevent further stress during the capture process. We recorded recovery time, to the nearest minute, from antagonist injection to lifting of head and time to normal activity as lapse time from the initial antagonist injection to release. To account for repeated measurements from the same individual we used mixed-effect models to estimate mean temperature and respiration. Using statistical software R (version 3.0.2; R Core Team 2021) and package *lme4*, we fit intercept-only models to estimate means with individual ringtails as the random effect and the vital rate as the dependent variable (Bates et al. 2015).

RESULTS

A total of 34 handling events using NAM resulted in a successful immobilization and release. We captured 27 adults (21 males, six females), and seven individuals were recaptured (six males, one female). Body weights of captured ringtails ranged from 827 g to 1,170 g (mean 1,002.0 g, SD 91.5 g) for males and 712 g to 942 g (mean 814.4 g; SD 75.1 g) for females. Based on published data using NAM for immobilization, we delivered a dose of 0.054 mL/kg (2.18 mg/kg nalbuphine, 0.54 mg/kg azaperone, and 0.54 mg/kg medetomidine) to our first captured ringtail, but adequate sedation was not achieved within 20 min; therefore, a supplemental dose of NAM at 0.05 mL/kg was administered, and a sedation score of 4 was achieved within 9 min. Based on this initial handling event, we delivered a dose of 0.075 mL/kg (3 mg/kg nalbuphine, 0.75 mg/kg azaperone, 0.75 mg/kg medetomidine) to all subsequently captured ringtails.

We excluded five captures from the analysis. In four captures the animal received a partial initial dose and required a supplemental dose after 20 min (one-half of original dose); these were excluded due to uncertainty regarding the total administered dose. We also excluded our initial animal, due to time elapsed between doses. The resulting 29 capture events (Table 1) received a mean$\pm$SD sedation score of 4.07$\pm$0.88. Sedation scores for 23 capture events were $\geq$4, with scores of 2 and 3 recorded for six immobilization events; no sedation score $<$2 was recorded. The mean$\pm$SD volume of NAM delivered was 0.079$\pm$0.011 mL with a mean dose of 0.084$\pm$0.018 mL/kg. This dose equates to a mean$\pm$SD dosage of 3.37$\pm$0.724 mg/kg nalbuphine, 0.84$\pm$0.181 mg/kg azaperone, and 0.84$\pm$0.181 mg/kg medetomidine.

There was no significant difference in the NAM dose (mL/kg) delivered to males vs. females (Kruskal-Wallis χ^2=0.357, df=1, P=0.058). The lowest delivered dose that resulted in adequate sedation was 0.06 mL/kg (2.4 mg/kg nalbuphine, 0.6 mg/kg azaperone, 0.6 mg/kg medetomidine), which resulted in complete sedation 5 min after the dose was administered. Mean$\pm$SD induction time was 13.24$\pm$3.57 min and ranged 5–19 min before animals could be safely handled.

At least two respiratory rates and body temperature measurements were recorded for 23 ringtails during the handling process. The mean$\pm$SD respiratory rate was 23.8$\pm$7.11 breaths/minute (95% confidence limits 21.73–25.88, range=12–44). We observed respiratory depression for 9% of capture events and increased supplemental oxygen to 2 L/minute. We observed an increase in mean$\pm$SD respiratory rate from our initial recording immediately

TABLE 1. Dose of nalbuphine HCl 40 mg/mL, azaperone tartrate 10 mg/mL, and medetomidine HCl 10 mg/mL (NAM), delivered to 23 ringtails (*Bassariscus astutus*) during 29 capture events in Oregon, US, August 2020 to March 2022.

Variable	*n*	Mean	Median	SD	Range
NAM (mL/kg)	29	0.08	0.08	0.02	0.06–0.14
Nalbuphine (mg/kg)	29	3.37	3.24	0.72	2.4–5.6
Medetomidine (mg/kg)	29	0.84	0.81	0.18	0.6–1.4
Azaperone (mg/kg)	29	0.84	0.81	0.18	0.6–1.4
Induction (min)[a]	29	13.24	13	3.57	5–19
Sedation score[b]	29	4.07	4	0.88	2–5
Initial respiration (breaths/min)	23	22.3	20	7.14	12–44
Final respiration (breaths/min)	23	25.3	24	6.89	14–36
Initial temperature (C)	23	37.34	37.3	0.89	35.4–39.3
Final temperature (C)	23	37.02	37.2	1.02	34–39
Recovery time (min)[c]	28	2.48	2	1.94	0–8

[a] Time (min) from initial NAM injection until individual was unresponsive to stimuli.
[b] Sedation score categories based on response to stimuli during handling: (1) animal could not be handled, (2) can be handled but attempts to move away, (3) does not move away but reacts or vocalizes when handled, (4) only slight movement when handled, (5) no response to stimulus.
[c] Time (min) from antagonist injection to head up.

after handling, from 22.3±7.14 breaths/min to the final recorded respiratory rate of 25.3±6.89 breaths/min. The mean±SD body temperature for immobilized ringtails was 37.2±0.96 C (95% confidence limits=36.83–37.59, range=34–39.3). We observed a slight reduction in mean±SD body temperature from our initial temperature recorded immediately upon handling 37.3±0.89 C to the final recorded temperature 37±1.02 C. Pulse oximeter readings including heart rate and oxygen saturation were obtained for 14.7% of captures, with a mean±SD oxygen saturation of 93±5.89% (range=82–98, n=5). We recorded a mean±SD heart rate of 109±32.18 beats/minute (range=73–172, n=5).

Mean handling time was 30.55 (range=22–39) min from initial dose of NAM to injection of antagonist. The only observed physical response to stimuli during handling included twitching of the ears and extremities. All ringtails remained sedated for the entire period until antagonist was administered. Mean±SD recovery time was 2.48±1.94 min and ranged from 0 to 8 min after administration of intramuscular antagonists. Some individuals reacted to the stimulus of the antagonist injection and were alert, with head up and occasionally vocalizing in <1 min. During recovery some individuals shook their head from side to side, which we assumed was in response to the collar; however, we observed no attempts by ringtails to remove the radio collar during the final stages of recovery. Mean time to normal activity was 45 min and ranged from 15 to 108 min after administering the antagonists. Two ringtails (R06 and R09) were given additional time to recover, due to environmental conditions at the time of release.

DISCUSSION

All ringtails handled in our study were effectively immobilized with NAM, but mean induction time varied among individuals and was longer than has been reported for other drugs and drug combinations used to immobilize ringtails. For example, injections of 5–15 mg/kg of ketamine hydrochloride immobilized ringtails within 4 min (Callas 1987; Wyatt 1993). Given the relatively sedate behavior of ringtails in the PVC traps we used, we expected similar induction times between individuals administered NAM. However, differences in induction times might be a result of factors such as the injection site, individual responses to traps, ambient

temperature, and reaction to stimuli when being transported to processing location. We documented ringtail immobilization with doses as low as 0.06 mL/kg (2.4 mg/kg nalbuphine, 0.6 mg/kg medetomidine, 0.6 mg/kg azaperone), although the mean dose delivered was 0.084 mL/kg (3.36 mg/kg nalbuphine, 0.84 mg/kg medetomidine, 0.84 mg/kg azaperone). Weight and sex of individuals were difficult to determine prior to handling, particularly in the winter months when individuals had thick pelage. Given the range of body weights we observed within sexes, using a separate handling cage for calculating body weights may be more appropriate than relying on sexually dimorphic characteristics and visual estimates. We attempted to estimate weight conservatively and use the lowest initial dosage possible, while also limiting handling time and the need for subsequent restraint and secondary injection.

Vital rate data for ringtails during capture events are poorly documented, making comparisons between our capture data and previous immobilization chemicals difficult. Successful pulse oximeter readings were limited in our study; this was likely to be associated with decreased blood flow to the extremities where we attached the device, probably caused by medetomidine-induced peripheral vasoconstriction in combination with cold ambient temperatures. We detected a decrease in body temperatures during handling, even with the use of an external heat source. Because of their small body size, protecting ringtails against hypothermia requires protection against environmental elements while being held in the trap, plus monitoring and provision of a heat source as required during handling and recovery. We observed respiratory depression in 9% of our capture events and oxygen saturation rates as low as 82%; supplemental oxygen is recommended during all captures to avoid hypoxemia (Roug et al. 2019). We monitored all captured ringtails for survival using radiocollar mortality beacon, 24 h post capture and at 7-d intervals for the life of the collar battery. We recorded no mortalities within 24 h of capture, and only one within 14 d, suggesting that the methods we used for safe handling were effective and produced no lingering adverse effects.

The dose of NAM recommended varies by species, with American bison (0.008 mL/kg), black bear (0.011–0.022 mL/kg), and beaver (0.02–0.06 mL/kg) all successfully immobilized with low doses (Wolfe et al. 2016, 2017; Roug et al. 2019). In contrast, our first attempted dose of 0.054 mL/kg did not adequately immobilize this species. After adjusting the dose to 0.075 mL/kg (3 mg/kg nalbuphine, 0.75 mg/kg azaperone, 0.75 mg/kg medetomidine), we were safely able to handle all subsequently captured individuals. Previous chemical immobilization drugs used for ringtails required similarly higher dosages; for example, tiletamine-zolazepam delivered at 10 mg/kg has been used to immobilize ringtails (Harrison 2012), while black bears can be safely immobilized with 7 mg/kg (Kreeger and Arnemo 2018).

We found that NAM provided similar advantages for immobilizing ringtails as reported for large mammals, including effective sedation with low volume dose, smooth antagonism, rapid recovery, and absence of DEA drug regulatory scheduling (Wolfe et al. 2017). In our study, NAM provided safe and effective immobilization for ringtails, but further evaluation is needed for use in other small mammals and mesocarnivores. Future dosage recommendations should take into consideration species-specific behavior, basal metabolic rate, safe handling procedures, and previous chemical immobilization drug dosages for the species.

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