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Caroline C. Sauvé
Université de Montréal, caroline.sauve.6@umontreal.ca

Yves Rondenay
University of Montreal

Are R. Berentsen
USDA APHIS National Wildlife Research Center

Mel J. Rivera-Rodriguez
USDA Animal and Plant Health Inspection Service (APHIS)

Patrick A. Leighton
University of Montreal

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From the Field

Alfaxalone Successfully Immobilizes Small Indian Mongooses (*Urva auropunctata*): A Field Report

CAROLINE C. SAUVÉ,[1] *Faculté de médecine vétérinaire, Université de Montréal, 3200 Rue Sicotte, Saint-Hyacinthe, QC J2S 2M2, Canada*

YVES RONDENAY, *Faculté de médecine vétérinaire, Université de Montréal, 3200 Rue Sicotte, Saint-Hyacinthe, QC J2S 2M2, Canada*

ARE R. BERENTSEN, *United Sates Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA*

MEL J. RIVERA-RODRIGUEZ, *United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, 602 Duncan Drive, Auburn, AL 36849, USA*

PATRICK A. LEIGHTON, *Faculté de médecine vétérinaire, Université de Montréal, 3200 Rue Sicotte, Saint-Hyacinthe, QC J2S 2M2, Canada*

ABSTRACT We investigated intramuscular administration of alfaxalone (5.3–10.0 mg/kg) as an immobilizing agent in free-ranging small Indian mongooses (*Urva auropunctata*) on the island of St. Kitts, West Indies. From 5–11 January 2020, we successfully immobilized 10 of 11 mongooses. Alfaxalone provided rapid onset (median = 3 min) of immobilization. Mean duration of immobilization was 16.8 ± 3 min. Mean recovery time was 5.6 ± 1 min. There was no effect of dose administered on induction, anesthesia, or recovery times at the dose range used. We concluded that alfaxalone represents an effective alternative to dissociative agents for the immobilization of free-ranging mongooses. Further studies are necessary to quantify alfaxalone effects on mongoose vital rates and blood parameters.

KEY WORDS alfaxalone, chemical immobilization, small Indian mongoose, *Urva auropunctata*, wildlife immobilization.

Immobilization of free-ranging wildlife is carried out for a variety of management and conservation purposes such as disease surveillance, translocation, health monitoring, and other research objectives. However, immobilization of free-ranging wild animals can be challenging as it is often conducted in remote locations and with limited availability of vital-sign monitoring equipment. Upon immobilization, animal health status and body weight are generally unknown, suggesting that drugs used should have a wide safety margin, although rapid delivery of small volumes necessitates potent concentrated drugs (Fahlman 2008). Additionally, a drug with either an antagonist or short recovery time to full mobility may reduce potential predation following anesthesia.

The most commonly used anaesthetic drugs in wild carnivores are the dissociative anaesthetics ketamine and tiletamine combined with either a benzodiazepine (e.g., tiletamine-zolazepam) or an alpha-2-agonist (e.g., ketamine-xylazine or ketamine-medetomidine; Fahlman 2008, Kreeger and Arnemo 2018). Although common protocols have been demonstrated to be effective and relatively safe, dissociative anaesthetics are controlled substances in several countries, complicating use in international or transboundary studies. Moreover, in the context of frequent drug shortages, the veterinary field is encouraged to develop alternative protocols for routine procedures.

Alfaxalone is a synthetic neuroactive steroid that is approved for intravenous (IV) induction or maintenance of general anesthesia in cats and dogs (Rezende 2015). However, off-label use by the intramuscular (IM) route has been described in dogs, cats and rabbits (Huynh et al. 2015, Tamura et al. 2015*a*, *b*, Maney 2017, Cruz-Benedetti et al 2018). Recently, IM administration of alfaxalone was reported to rapidly induce immobilization in reptiles (Kischinovsky et al. 2013, Hansen and Bertelsen 2013) and captive marmosets (*Callithrix jacchus*; Bakker et al. 2013).

Interspecific variation in response to drugs is common; therefore it is important that research be carried out for each species of interest to identify the most suitable drugs and dosages (Pearson et al. 1968). The small Indian mongoose (*Urva auropunctata*) is an opportunistic carnivore native to Southern Asia and parts of the Middle East that was introduced to a number of Caribbean and Pacific islands during the late 19th and early 20th centuries (Hinton and Dunn 1967, Nellis and Everard 1983). There is increasing

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[1]*E-mail: caroline.sauve.6@umontreal.ca*

interest in mongoose immobilization for research and management purposes, as mongooses represents a substantial threat to several native species in tropical ecosystems (Berentsen et al. 2017). Moreover, the small Indian mongoose is the principal wildlife reservoir for canine rabies in several Caribbean islands, representing a serious public health concern (Seetahal et al. 2018).

Mongooses are typically immobilized by IM injection of tiletamine-zolazepam (5 mg/kg; e.g., Johnson et al. 2016) or a 50:1 mixture of ketamine:xylazine (Choudhary et al. 2013). The objective of our study was to assess whether alfaxalone, administered IM, could be a suitable alternative to dissociative agents for undertaking minor procedures such as morphological measurements and blood collection in free-ranging small Indian mongooses.

STUDY AREA

We conducted our study from 5–11 January 2020, on the island of St. Kitts, West Indies. The study site was a 0.5-km^2 plot of subtropical dry forest dominated by small trees and shrubs including river tamarind (*Leucaena leucocephala*), *Croton* spp. and *Acacia* spp., with an understory of herbaceous plants such as buttonsage (*Lantana involucrata*), bull nettle (*Cnodoscolus urens*) and Guinea grass (*Panicum maximum*; Lindsay and Horwith 1999). The climate was tropical marine, with an average annual temperature of 27.8°C with little seasonal variation (CARICOM et al. 1993). Average annual rainfall was 1,625 mm, most of which occurs from August to November (CARICOM et al. 1993). St. Kitts is free from terrestrial rabies and no cases have been reported in mongooses or any other mammalian species (Seetahal et al. 2018).

METHODS

We live-captured mongooses using cage traps (Tomahawk Live Trap, Hazelhurst, WI, USA) baited with canned tuna. Traps were baited daily in the morning and checked within 24 hours. Upon capture, we transferred mongooses into a conical canvas bag where they were physically restrained. We immobilized captured mongooses via intramuscular injection of alfaxalone (Alfaxan, Vetoquinol B.V., Breda, NL; 10 mg/mL) at a targeted dose of 6–12 mg/kg. The dose range was derived from intramuscular doses of 5 and 12 mg/kg reported for successful sedation in cats and marmosets, respectively (Bakker et al. 2013; Rodrigo-Mocholi et al. 2018) and considering that wildlife species usually require higher doses than domestic animals due to the stress associated with handling (Fahlman 2008). Because animal weight could only be determined after immobilization, the handler estimated the dose volume based on animal size, and calculated the actual dose administered *a posteriori*. All capture, handling and immobilization procedures were approved by the Animal Use Ethics Committee of University of Montreal (CÉUA 19-Rech-1945).

After immobilization we collected morphological data including sex, weight (±25 g; Ohaus 8004-MN spring scale, Parsippany, NJ) and nose-tail length (±1 mm; soft tape measure) and inserted a Passive Integrated Transponder (PIT) tag (Biomark APT12 FDX_B, Boise, Idaho, USA) via subcutaneous injection for individual animal identification. We recorded induction, immobilization, and recovery times. We defined induction time as the time elapsed between injection and the loss of postural tonicity. Immobilization time was defined as the time elapsed between loss of postural tonicity and first return of muscle tonicity (e.g., limb movement or rigidity). Recovery time represented the time elapsed between resumption of muscle tonicity and regained ambulatory function. We collected up to 1.0 mL of blood by venipuncture of the cranial vena cava as described for ferrets (Briscoe and Syring 2004). Given the remote and rugged terrain of the study site, vital-sign monitoring equipment was unavailable during immobilization, thus, specific vital parameters (e.g., blood pressure, oxygen saturation) could not be measured. However, animals were regularly observed for changes in respiration and ocular position, an indicator of depth of anesthesia, and verified every minute until recovery.

We assessed the effects of dose on immobilization duration using robust linear models using the MASS and sfsmisc packages in R (Venables and Ripley 2002, R Core Team 2019, Maechler 2020). Induction, immobilization and recovery times were response variables and dose administered was the fixed effect. Data was tested for normality using the Shapiro-Wilk test and we present results as means ± standard errors (SE) for normally-distributed variables, and as median values otherwise. Statistical significance was determined at $\alpha = 0.05$.

RESULTS

We evaluated alfaxalone in 11 mongooses at doses ranging from 5.3 to 10.0 mg/kg, as calculated following administration once animals were immobilized and weighed. Alfaxalone resulted in successful immobilization of 10 of 11 mongooses. One mongoose demonstrated light sedation but retained muscle tone and voluntary movement until it walked away 20 mins post-injection (Table 1). The 10 immobilized mongooses displayed ventromedial rotation of the eyeball throughout the immobilization phase, indicating an appropriate plane of anesthesia (Stage III, light to medium planes; Soma 1971). Median induction time was 3 min (range = 2–11 min, $n = 10$). Mean (SE) immobilization time was 16.8 (3) min (range = 4–38 min), which was sufficient for all morphological measurements and biological sample (blood) collection to be performed. All immobilized animals fully recovered, with a mean (SE) recovery time of 5.6 (1) min (range = 0–14 min). Animal handling did not result in any detectable injury, and no adverse effect of alfaxalone administration was observed during the procedure or upon animal release. One mongoose was recaptured 2 days following alfaxalone administration and displayed no signs of injury or altered behaviour.

There was no effect of the dose administered on induction, immobilization or recovery times (Fig. 1) over the range of dosages used in our study. Median induction times were lower for males (2 min, $n = 4$) than females (3 min, $n = 6$). Likewise, mean immobilization and recovery times were all

Table 1. Individual characteristics, dose administered and induction, immobilization and recovery times for 11 free-ranging small Indian mongooses immobilized by intramuscular injection of alfaxalone (10 mg/mL) on the island of St. Kitts in January 2020.

Date	Sex	Weight (g)	Dose volume (mL)	Dose administered (mg/kg)	Induction time (min)	Immobilization time (min)	Recovery time (min)
05 Jan 2020	M	550	0.4	7.27	3	18	8
07 Jan 2020	F	375	0.2	5.33	NA	NA	NA
07 Jan 2020	F	500	0.3	6.00	3	20	14
08 Jan 2020	F	375	0.25	6.67	4	17	6
08 Jan 2020	F	425	0.25	5.88	3	10	7
09 Jan 2020	M	475	0.3	6.32	2	17	7
09 Jan 2020	M	550	0.3	5.45	2	16	0
10 Jan 2020	M	425	0.25	5.88	2	7	1
10 Jan 2020	F	450	0.3	6.67	11	4	6
11 Jan 2020	F	300	0.3	10.00	2	21	7
11 Jan 2020	F	425	0.3	7.06	3	38	0

lower for males ($n = 4$) than females ($n = 6$). Sex-specific differences were not statistically significant (Table 2); however, sample size limited statistical power, thus, an effect of mongoose sex on immobilization and recovery times cannot be excluded.

DISCUSSION

Our study suggests that alfaxalone represents a potential alternative to dissociative agents for the chemical immobilization of wild small Indian mongooses. Alfaxalone resulted in successful immobilization of 10 of 11 of the animals tested. Alfaxalone has benefits compared with classic mongoose immobilization protocols (i.e., not a controlled substance in some countries, efficient as a single agent). However, unlike benzodiazepines and alpha-2-agonists present in drug combinations commonly used for wildlife immobilization, alfaxalone is not reversible. When used intravenously to induce anesthesia, alfaxalone is typically administered to effect. To effect administration is not possible when a drug is injected intramuscularly because of the increased delay of action associated with this route. It is therefore important to identify and use minimal effective doses. The lowest dose used in our study (5.3 mg/kg) appeared to be insufficient as it only induced partial immobilization, whereas doses at 5.45 mg/kg and up to 10.0 mg/kg successfully induced immobilization. Our dose range therefore represents a starting point to guide eventual dose-response studies in mongooses, which are necessary to determine species specific optimal alfaxalone doses. In addition, dose volumes (0.5–1.2 mL/kg) must be taken into account when considering the IM use of alfaxalone. It is generally considered that IM administration should be limited to animals weighing less than 10 kg (Nieuwendijk 2011). Alfaxalone can also be used in combination with other drugs such as benzodiazepines and opioids to reduce dose volumes required (e.g. Lee et al. 2015).

No formal comparisons in induction, immobilization and recovery times were attempted between classic dissociative anaesthetics and alfaxalone in our study. However, it is our impression based on previous field observations that alfaxalone generally resulted in similar durations of the induction and immobilization phases, but a shorter recovery phase, than anaesthesia with tiletamine-zolazepam. Although there was a delay between return of muscle tonicity and recovery of reflexes in all 10 mongooses successfully immobilized with alfaxalone in our study, the later phase of recovery was

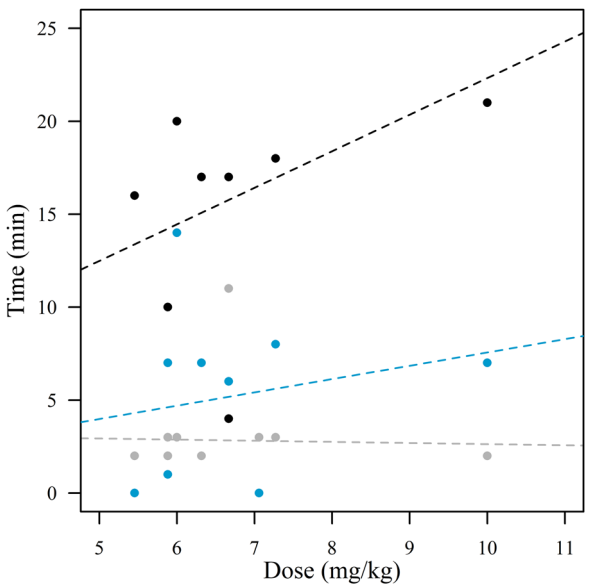

Figure 1. Alfaxalone dose (mg/kg) administered to free-ranging small Indian mongooses on the island of St. Kitts in January 2020, related to induction (grey circles), immobilization (black circles), and recovery (blue circles) times (min). No significant effect was found using linear regression for induction ($t_8 = -0.23$, $P = 0.82$), immobilization ($t_8 = 0.96$, $P = 0.34$), or recovery times ($t_8 = 0.61$, $P = 0.55$), represented by grey, black and blue dashed lines, respectively.

Table 2. Mean (±1 SE) immobilization and recovery times associated with the intramuscular injection of alfaxalone in female ($n = 6$) and male ($n = 4$) Indian mongooses on the island of St. Kitts in January 2020. The *t*-statistics, degrees of freedom (DF) and *P*-values shown are from Welch two sample *t*-test on means.

Duration (min)	Females	Males	*t*-value	DF	*P*-value
Immobilization time	18.3 ± 3.7	11.6 ± 1.6	0.71	7.28	0.50
Recovery time	6.67 ± 1.8	4.00 ± 2.0	0.98	7.01	0.36

rapid, with animals typically opening their eyes, regaining their righting reflex, and walking away within a few seconds. Our results were consistent with what has been reported in marmosets, in that alfaxalone (12 mg/kg) resulted in equivalent immobilization durations, but lower recovery times, than ketamine alone (100 mg/kg) or ketamine (25 mg/kg) combined with medetomidine (0.5 mg/kg) (Bakker et al. 2013).

The single mongoose that was not successfully immobilized received the lowest dose of alfaxalone administered in our study (5.3 mg/kg); however, other mongooses that received similar doses (5.45 and 5.89 mg/kg; Table 1) were successfully immobilized. We believe the failure of alfaxalone to immobilize this individual illustrates interindividual variation in response to the drug at the lower limit of the dose range used in our study. Injection of a second dose was not attempted because the mongoose was not sufficiently sedated to be weighed in order to calculate the required additional dose. In the dose range that successfully resulted in immobilization (5.45–10 mg/kg), induction, immobilization, and recovery times were not related to the dose administered, suggesting that alfaxalone clearance is rapid and not saturated at these doses in mongooses. Similarly, in dogs alfaxalone plasma clearance and volume of distribution did not differ between IV doses of 2 and 10 mg/kg (Ferré et al. 2006).

Our study represents an assessment of the efficacy of alfaxalone in immobilization of small Indian mongooses. Our study was opportunistic in nature and took place during a concurrent mongoose population density study. The study took place in remote and rugged field location accessible only on foot, prohibiting the transportation of equipment required to monitor vital parameters that would typically be performed in a more controlled laboratory setting. Nonetheless, basic parameters such as respiration, pulse (apex beat) and ocular position were monitored at 1 to 5-min intervals, and no adverse effects on these parameters were observed. Therefore, additional studies conducted in a controlled environment comparing immobilization time for alfaxalone and the dissociative anaesthetics regularly used to immobilize mongooses are needed to optimize alfaxalone doses for use in mongooses. Likewise, alfaxalone effects on mongoose vital rates and blood parameters (e.g., indicators of muscle damage; Bakker et al. 2013) should be investigated. Additionally, increasing sample size would allow investigation of sex-specific optimal doses. Although captive settings are ideal for such studies, one must be aware that free-ranging animals may require higher doses than their captive relatives due to capture and handling induced stress (Fahlman 2008).

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