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FIELD ANESTHESIA OF FREE-RANGING NUTRIAS (*MYOCASTOR COYPUS*) FOR SURGICAL REPRODUCTION CONTROL

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ABSTRACT: The nutria (*Myocastor coypus*), a rodent native to South America, has been introduced and has established feral populations at numerous locations in North America, Europe, and Asia. As such, the nutria is subject to research and management programs, including investigation of surgical fertility-control techniques. We evaluated the efficacy of a mixture of ketamine and medetomidine, with additional use of isoflurane and reversal with atipamezole, to provide safe, reliable anesthesia for surgical procedures under field conditions. We anesthetized 40 free-ranging nutrias between December 2018 and March 2019, in Turin, Italy, to perform surgical reproduction control techniques. We administered a ketamine and medetomidine mixture (6 mg/kg and 140 μg/kg, respectively) after trapping the animals and weighing them in the cage traps. After induction, we reweighed the rodents and performed a brief clinical examination. The times of loss of palpebral and pedal reflexes were noted. After induction of anesthesia, heart rate, respiratory rate, and percentage of oxygen saturation were monitored and recorded. Isoflurane was delivered through a face mask to 27 nutrias (70%) to maintain an adequate depth of anesthesia. Upon completion of surgery and other procedures, atipamezole was administered to the animals at doses 2.5 higher than those of medetomidine (actual dose: 366±31 μg/kg). Induction times were short (3±2 min), with the animals completely immobilized. The heart rate and respiratory rate both decreased. After administration of atipamezole, recoveries were smooth and complete. There were two deaths after higher doses of atipamezole and longer surgeries. Carprofen (4 mg/kg) was administered subcutaneously for its analgesic effects. The animals were released at the end of all the procedures. Overall, the medetomidine and ketamine mixture, with supplemental isoflurane in most instances, provided a reliable anesthesia in free-ranging nutrias, adequate for performing surgical procedures under field conditions.

Key words: Atipamezole, immobilization, isoflurane, ketamine, medetomidine, *Myocastor coypus*, nutria, reproduction control.

INTRODUCTION

Because of the potentially severe effects on biodiversity, economy, ecosystem functionality, and public health (Bertolino and Genovesi 2007; Zhu et al. 2019; Bertolino et al. 2020), the nutria (*Myocastor coypus*), is considered a pest in many regions (Bertolino et al. 2011) and has been included in the first list of "invasive alien species of Union concern" by Commission Implementing Regulation (EU) no. 2016/1141.

This rodent is, therefore, the subject of many studies and research projects aimed primarily at outlining its distribution, habits, and effect on the environment; moreover, pursuant to regulation (EU) no. 1143/2014 and, in Italy, to D.L. 230/17, concerning management measures for invasive alien species, various containment programs for nutria have been proposed to reduce its population. Among these methods is the surgical control of the fertility of these rodents, which considers welfare and social acceptability (Dubois et al. 2017) by avoiding euthanasia.

In the territory of the Metropolitan City of Turin in Italy, a pilot project for the control of a population of nutria was implemented through an agreement between the Metropolitan City of Turin, the Servizio Tutela della Fauna e della Flora, and the Struttura Didattica Speciale Veterinaria of the University of Turin. The latter has entrusted its accomplishment to the CANC (Centro Animali Non Convenzionali), the exotic and wild animals' unit of the Ospedale Veterinario Universitario (Veterinary Teaching Hospital) of the Veterinary Science Department of the University of Turin. The project was carried out under field conditions, pursuant to D.L. 230/17.

There is only scarce literature detailing the anesthetic management of these animals, especially in similar circumstances. We decided to use a pharmacologic cocktail that could, albeit in part, be antagonized, thus, accelerating the recovery of the subjects.

The use of xylazine and ketamine (Bó et al. 1994) in nutria allowed short-term sedations in previous studies (Guichón and Cassini 2005; Fratini et al. 2015; Passos Nunes et al. 2019). Despite the lower cost of xylazine, medetomidine was used in this study because it has a specific antagonist, atipamezole. The use of combinations of medetomidine and ketamine for the immobilization of nondomestic mammals was reviewed by Jalanka and Roeken (1990) and was applied by Popescu et al. (2010) for a surgical procedure in two nutrias.

Isoflurane has been used for anesthetic purposes in nutria in two studies, for short procedures (Johnson et al. 2014) but without any description of the consequences on vital parameters after induction. However, isoflurane is commonly used in rodents (Yarto-Jaramillo 2015).

We investigated a combination of medetomidine and ketamine plus supplemental isoflurane, as required, for surgical anesthesia of nutrias under field conditions.

The project received ethical approval by the Departmental Executive Committee of the Veterinary Science Department of University of Turin (report N.2, 30/1/2018).

MATERIALS AND METHODS

We anesthetized 40 free-ranging nutrias, 15 males and 25 females, to inhibit fertility, preferably through laparoscopic vasectomy or salpingectomy, as part of a project performed under field conditions between December 2018 and March 2019, in two areas (45°03′N, 7°41′E and 45°04′N, 7°43′E) in the city of Turin, Italy. We captured the animals with cage traps (live animal trap, SGD Group Srl., Treviso, Italy) set near the colony of nutrias and baited with fruit and vegetables, checked regularly by trained technicians. Once trapped, the rodents were kept for at least 2 h in a sheltered gazebo (FleXtents PRO, Dancover A/S, Hillerød, Denmark) with stable temperature, to reduce stress levels and to improve the effectiveness of the anesthesia; the cages were covered with blankets to reduce visual contact with the operators.

In the order of capture, we immobilized the nutrias with a mixture of ketamine (Ketavet 100®, 100 mg/mL, Intervet Productions Srl., Aprilia, Italy) and medetomidine (Sedator®, 1.0 mg/mL, Dechra Veterinary Products Srl., Turin, Italy), at preestablished doses of 6 mg/kg and 140 μg/kg, respectively, administered into the thigh muscles through the metal meshes of the cages using a handheld syringe; we prepared the drugs on the basis of the weight of the previously calibrated cages containing the animals.

After immobilization, animals were pulled out of the cages and weighed more accurately and, then, underwent a brief clinical examination and abdominal trichotomy for the surgery. Induction time (T0: recumbency and no response to handling) and time of loss of palpebral and pedal reflexes were recorded. The rectal temperature (Digi-Vet SC 12, Kruuse A/S, Langeskov, Denmark), heart rate (using a stethoscope), and respiratory rate (counting chest movements) were also measured.

We used a specifically equipped field-mobile unit (m-OVU: mobile unit of the Ospedale Veterinario Universitario) to perform surgery. Nutrias were placed in dorsal recumbency, and those who could undergo minimally invasive laparoscopic surgery (adults and nonpregnant females) were placed in Trendelenburg position (i.e., head-down, by tilting the surgical table approximately 30 degrees). Before beginning the surgery, we administered lukewarm fluids subcutaneously: 30 mL/kg of a 1:1 mixture of Ringer's lactate and 5% glucose (both Baxter SpA, Rome, Italy), and 4 mg/kg carprofen subcutaneously (Rimadyl® Iniettabile, 50 mg/mL, Zoetis Italia Srl., Rome, Italy), as part of the analgesic management.

We connected each patient to a multiparametric monitoring system (Infinity Delta®, Dräger Italia SpA, Corsico, Italy) to measure vital parameters through electrocardiography and pulse oximetry; the pulse oximeter probe was placed on the pinna. The measured data (heart rate [HR] and oxygen saturation [Spo_2]), together with the respiratory rate (RR), were recorded 5 min after induction (T_5) then every 10 min (T_{10} and T_{20}); each animal was given pure oxygen through a face mask (anesthetic face mask for cats Ø 85 mm, Jørgen Kruuse A/S, Langeskov, Denmark). When needed, as indicated by a 20% increase in HR, 2–2.5% isoflurane (IsoFlo 100%, Zoetis) was dispensed using a vaporizer (Datex Ohmeda Tec 5, Soma Technology Inc., Bloomfield, Connecticut, USA) and delivered through a rebreathing system until the end of the surgery. For the rodents undergoing minimally invasive surgery (n=6), manual-assisted ventilation at 12 breaths/min was required, using a reservoir bag (rebreathing bag 3.0 L, Jørgen Kruuse).

At the end of the surgery, we implanted a transponder tag (Therachip®, Bioforlife Italia Srl., Milan, Italy) subcutaneously in the left prescapular region, and an ear tag: pink on the right ear for females and blue on the left ear for males. Given the impracticability of treating the animals after release, we administered prophylactic antibiotics: 0.2 mL/kg of a mixture of benzylpenicillin benzathine and dihydrostreptomycin sulfate (Rubrocillina Forte Veterinaria, 250,000 IU/mL+100 mg/mL, Intervet Productions Srl., Aprilia, Italy), subcutaneously in the right prescapular region.

Upon completion of the procedures, we moved the nutria back into the gazebo, took biometric measurements, and collected blood samples and oral and anal swabs. We administered atipamezole hydrochloride (Sedastop, 5 mg/mL, Ecuphar Italia Srl., Milan, Italy) to each nutria, intramuscularly in the quadriceps, at a dosage 2.5 times higher than that of medetomidine; in the first six animals, the dosage was five times higher than that of the medetomidine.

We placed each animal inside a cage and evaluated its respiratory rate and the reappearance of the palpebral and pedal reflexes, checked through the meshes of the cage until complete recovery (all reflexes present and spontaneous movements after atipamezole administration). We, then, covered the cages with blankets and offered water and food (vegetables and fruits) to the animals. The food was left in the cages until release, which took place in the evening in the same place as capture. Animals were monitored for 3–5 d after release. Neutered animals were recognized through the presence of the ear tag. Both direct observation and video recordings were performed to detect any particular behavior or the presence of dead nutria.

We used program R (version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria) to perform the statistical analyses, using a significance level of $P<0.05$. For all parameters, median and interquartile ranges (IQRs) are reported. Differences in recovery time between animals that received isoflurane and animals that did not receive isoflurane were assessed using Mann-Whitney U-test. We used a repeated-measures analysis of variance test with Bonferroni post hoc test to analyze the HR and the RR at different time points from T_0 to T_{20}.

RESULTS

The weight of the animals, the rectal temperature at induction, the actual dosages of the drugs, the induction time, the times of loss and reappearance of palpebral and pedal reflexes, the time to complete recovery, the time to spontaneous feeding, the duration of anesthesia (from administration of ketamine/medetomidine mixture to administration of atipamezole), and the duration of surgery are shown in Table 1.

We carried out minimally invasive surgery (salpingectomy or vasectomy) on six nutrias (15%), traditional surgery (hysterectomy) on 34 (85%). To maintain an adequate anesthetic depth, 27 subjects (70%) needed isoflurane, delivered at a concentration between 2% and 2.5%. There was no significant difference in time to recovery ($P>0.05$) comparing animals that received isoflurane (median, 10 min; IQR 7.25–11.75 min) and animals that did not receive isoflurane (median, 12 min; IQR 10–15 min).

The overall dose of atipamezole was 408±123 µg/kg (mean±SD; range, 330–790 µg/kg); in detail, for the first six animals, treated with a dose five times higher than that of the medetomidine, the mean dose of atipamezole was 703±121 µg/kg (range, 528–790 µg/kg). For the remaining animals, treated with a dose 2.5 times higher than that of medetomidine, the mean dose of atipamezole was 366±31 µg/kg (range, 330–480 µg/kg).

Two deaths occurred after 528 and 370 min from induction and 432 and 263 min after administration of the antagonist, respectively; both had received the higher dose of atipamezole.

Values for HR, RR, and $\text{Sp}O_2$ after the time of administration of the anesthetic agents, are shown in Table 2. We found statistically significant differences over time for both HR and RR. For HR, differences were obtained between T_0 and T_{10} (P=0.0008) and between T_0 and T_{20} (P=0.002). For RR, differences were obtained between T_0 and T_{10} ($P=9.5\times10^{-5}$) and between T_0 and T_{20} ($P=7.8\times10^{-5}$).

TABLE 1. Weight, rectal temperature at induction, dosages of the drugs (ketamine, medetomidine, and atipamezole), induction time, times of loss and reappearance of palpebral and pedal reflexes, time of complete recovery and of spontaneous feeding, and durations of anesthesia (time from administration of ketamine/medetomidine mixture to administration of atipamezole) and surgery in 40 nutrias (*Myocastor coypus*) immobilized with a mixture of ketamine and medetomidine mixture, with supplemental isoflurane in 27 (70%) subjects to maintain an adequate depth of anesthesia. The procedures were performed under field conditions between December 2018 and March 2019 in the city of Turin, Italy.

Variable	Mean	SD	Minimum	Maximum
Weight (kg)	4.97	1.94	0.85	9.5
Temperature (C)	35.8	0.7	34.6	36.7
Ketamine (mg/kg)	6.3	0.4	5.7	7.3
Medetomidine (μg/kg)	147	9	134	170
Atipamezole (μg/kg)	408	123	330	790
Induction time (min)	3	2	0	8
Loss of palpebral reflex (min)	5	2	2	10
Loss of pedal reflex (min)	4	2	0	10
Reappearance of palpebral reflex (min)	2	2	0	7
Reappearance of pedal reflex (min)	5	4	1	14
Complete recovery (min)	11	8	2	46
Spontaneous feeding (min)	20	10	7	53
Duration of anesthesia (min)	50	18	25	107
Duration of surgery (min)	21	11	11	71

DISCUSSION

The population-control project in the territory of the city of Turin was designed to avoid lethal removal of the animals, both to avoid going against public opinion and to delay immigration of nutrias from other population nuclei. This surgical reproduction-control project gave us the opportunity to study a protocol for field anesthesia, widening the knowledge on nutria anesthesia, which is very limited by the lack of literature.

The combination of ketamine and medetomidine, with supplemental isoflurane in most instances, provided reliable anesthesia of nutrias, allowing performance of both traditional and minimally invasive surgical procedures under field conditions. Furthermore, the use of atipamezole provided a quick and complete recovery, allowing the animals to be released within the day.

TABLE 2. Descriptive analysis of some physiologic variables at different time points (T0, T5, T10, and T20) of 40 nutrias (*Myocastor coypus*) after anesthesia performed with a combination of ketamine (6.3±0.4 mg/kg) and medetomidine (147±9 μg/kg) under field conditions between December 2018 and March 2019 in the city of Turin, Italy.

Variable[a]	Time from induction,[b] mean±SD (range)			
	T_0	T_5	T_{10}	T_{20}
HR (beats/min)	133±19 (80–182)	133±28 (40–240)	126±14 (92–163)	124±15 (80–150)
RR (breaths/min)	50±11 (28–72)	45±16 (16–80)	40±12 (12–64)	37±15 (12–64)
Spo_2 (%)	—[c]	94±4 (88–100)	96±4 (84–100)	95±5 (81–100)

[a] HR = heart rate; RR = respiratory rate; Spo_2 = oxygen saturation.

[b] T_0 = time at induction (recumbency and no response to handling); T_5 = time at 5 min after induction; T_{10} = time at 10 min after induction; T_{20} = time at 20 min after induction.

[c] First measured at T_5. See "Materials and Methods" section for additional information.

The doses used in our study were chosen based on those provided in the literature (Jalanka and Roeken 1990) but increasing the doses slightly to allow for the performance of invasive procedures: ketamine: 6 vs. 5 mg/kg, and medetomidine: 140 vs. 100 µg/kg. The actual doses given were close to the preestablished doses, with some degree of variability (ketamine: 5.7–7.3 mg/kg; medetomidine: 134–170 µg/kg) because the initial weight evaluation on caged nutria was inaccurate.

Induction times were similar to those reported in literature (Jalanka and Roeken 1990; Popescu et al. 2010) and short enough to be used on wild animals. The attention paid to the preanesthetic management of the nutrias resulted in a smooth induction phase without phenomena of incomplete pharmacologic effect or even excitatory response; both of which are possible and common using α_2-agonists in excited animals (Lamont and Grimm 2014; Rankin 2015).

After the coadministration of ketamine and medetomidine, the HR decreased. This was probably because of the action of the medetomidine, although partially offset by the use of ketamine and, in some cases, of isoflurane, which have a positive chronotropic effect (Berry 2015; Rankin 2015). The HR showed a continuous decrease over time, although falling only slightly below the 140–160 beats/min range considered physiologic in nutrias (Ferrante and Opdyke 1969) but with higher values than those reported in the study of Popescu et al. (2010), which used a similar protocol in two nutrias leading to more-severe bradycardia (64 beats/min). The decrease in HR could have also been associated with higher values because of the handling affecting the initial measurements. The wide range of variation highlights the great individual variability for this parameter (Table 2). It is also known that the HR of the nutria decreases strongly, and in few seconds, down to 15–20 beats/min, with concomitant peripheral vasoconstriction, when submerged in water. This reduces the myocardial oxygen demand, with a good tolerance of these hemodynamic changes (Ferrante and Opdyke 1969; McKean 1982; Sergina et al. 2015). These adaptations are involved in the dive response, an autonomic reflex consisting of bradycardia, a reduction in cardiac output, and peripheral vasoconstriction to conserve endogenous oxygen stores when diving (Houser 2018). It is possible that the effects caused by α_2-agonists, being similar to these physiologic adaptations to submersion, do not have negative consequences on nutrias.

The trend of RR recorded in our study showed a decrease comparable to that reported by Popescu et al. (2010). The depressant effect on the respiratory system function is probably due to the action of ketamine and, in this study, also of isoflurane; nevertheless, after the use of these agents, the tidal volume usually increases to maintain an adequate respiratory minute volume (Berry 2015; Steffey et al. 2015).

Oxygenation of patients does not appear to have been significantly affected by the reduction in RR; the mean Spo_2 remained >95% (Table 2), a value generally considered adequate to avoid hypoxia. This was probably due to the supplemental delivery of pure oxygen to all animals. At the first evaluation after connection of the pulse oximeter probe (T5), when the subjects had only recently started receiving oxygen, the average Spo_2 was slightly <95%. This could be related to a period of poor oxygenation and respiratory depression because of the injectable agents, whereas the presurgical procedures were being performed immediately after the induction. Furthermore, peripheral vasoconstriction caused by medetomidine may lead to erroneously low Spo2 (Flecknell 2016; Mama 2019).

Some animals undergoing minimally invasive surgery, with iatrogenic pneumoperitoneum created to perform laparoscopy, required manually assisted ventilation at the established respiratory rate of 12 breaths/min. The correct size of the face masks applied on nutrias, obligate nasal breathers, allowed the execution of this technique, which is usually only implemented with endotracheal intubation (Mama and de Rezende 2015; Scott et al. 2020).

The need to deliver isoflurane or to administer additional boluses of ketamine, as described by Popescu et al. (2010), suggests that the combination of ketamine and medetomidine alone at the proposed dosages is not always sufficient for the maintenance of an adequate anesthetic depth in case of prolonged surgical procedures. However, we decided to avoid the administration of higher doses of ketamine to limit the risk of a prolonged effect that could have compromised rapid release of the animals after surgery. Isoflurane was administered to animals when an adequate depth of anesthesia needed to be maintained because of its fast metabolism and elimination. However, isoflurane was not delivered to all subjects; a certain degree of individual variability should be taken into account when anesthetizing nutrias. In our study, the use of isoflurane never caused the onset of apnea, a common situation in other hystricomorph rodents, such as *Cavia porcellus*, the guinea pig (Yarto-Jaramillo 2015). Additionally, we found no significant difference in the recovery times when comparing animals that received isoflurane and those that did not receive isoflurane. The use of this inhalant anesthetic, therefore, did not appear to adversely affect the recovery.

The duration of the pharmacologic restraint was long enough to allow partial or complete elimination of ketamine. The metabolism of this dissociative anesthetic and the initial relatively low dose of ketamine, possible because of its synergistic effect with medetomidine in a multimodal anesthesia approach, enabled a smooth recovery without any of the potential adverse effects of cyclohexamines, such as ataxia, hyperreflexia, sensitivity to touch, and increased motor activity, common in this phase (Berry 2015).

After administration of the atipamezole, the nutrias recovered rapidly (complete recovery, 11±8 min) in times comparable to those reported by Jalanka and Roeken (1990) (5–11 min), although in that study the antagonist was administered 40–60 min after induction and without performing invasive procedures. Much longer times (about 90 min) were reported in the study of Popescu et al. (2010), in which the use of repeated boluses of ketamine to maintain anesthesia for ovariohysterectomy resulted in a prolonged recovery, despite the antagonism of medetomidine.

In our study, there were two deaths caused by cardiorespiratory arrest after doses of atipamezole five times higher than those of medetomidine, a dosage reported in literature (Jalanka and Roeken 1990; Popescu et al. 2010; Hahn 2019); no deaths occurred when half the dose was used, based on the dosage ranges reported in the literature for other rodents (Flecknell et al. 2015). The death of the two nutrias could be related to the possible, although rare, adverse effects of atipamezole, such as hypotension and tachycardia (Rankin 2015). They could have also resulted from the duration of the anesthesia, which, in these two subjects, was greater than in the others. Furthermore, these two nutrias underwent minimally invasive laparoscopic surgery (vasectomy and salpingectomy, respectively). They were held for a few hours between capture and induction (4 h for the female and 6 h for the male). It is probable that the prolonged detention was stressful.

Thus, medetomidine could have been already partially eliminated, exacerbating the effects of atipamezole. A necropsy of the animals was performed, and no macroscopic lesions were reported. Use of atipamezole at a dose 2.5 times that of the medetomidine dose (366±31 μg/kg) did not seem to have produced a less-effective recovery than the higher dose (703±121 μg/kg).

Intraoperative pain control was obtained with preemptive analgesia, including synergistic analgesic action of ketamine and medetomidine (Berry 2015; Rankin 2015) with carprofen, at the same dosage (4 mg/kg) reported by Popescu et al. (2010), although with a single administration, because of the management program requiring release of the nutrias within the day. Butorphanol has also been reported as an analgesic drug in nutrias (Johnson et al. 2014) but was rejected after consideration of the risks of respiratory depression linked to the use of opioids, which is difficult to manage when working in the field. The stability of the monitored parameters of nociception during the intraoperative procedures indicated the reliability of the adopted protocol for pain control.

Administration of the antagonist will have eliminated the residual analgesic action of medetomidine, and the complete elimination of ketamine also subtracted the analgesia related to it. However, from the monitoring checks performed on the animals in the days after the surgery, no nutria showed any particular behavior that in some way could be indicative of pain.

Overall, the combination of medetomidine and ketamine, with supplementary isoflurane as required, provided a good-quality immobilization and anesthesia in free-ranging nutrias, adequate for performing surgical procedures under field conditions, whereas the use of atipamezole speeded recovery, allowing prompt release of the animals.

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