FIELD USE OF ISOFLURANE AS AN INHALANT ANESTHETIC IN THE AMERICAN MARTEN (*MARTES AMERICANA*)

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ABSTRACT: We evaluated the effectiveness and practicality of using isoflurane as an inhalation anesthetic with oxygen as a gas carrier for American martens (*Martes americana*) in a field setting. Sixty-eight martens were trapped in the Waswanipi Cree Model Forest (Québec, Canada) from October to November 2005 and anesthetized with isoflurane in 100% oxygen (1 l/min) using a face mask. Induction setting of isoflurane was 3% for all animals. Mean (±SD) length of induction was 1.8±1.2 min. Maintenance isoflurane settings ranged from 1% to 4%. Procedures lasted an average of 16.4±7.1 min and were uneventful. Length of recovery, defined as the interval between the end of the procedure and animal release, was short (6.3±2.8 min), and well below reported lengths of recovery using injectable anesthetics (≥70 min). As compared to open drop administration of isoflurane described in previous studies, the use of an anesthesia machine prevents the risk of potential fatal anesthetic overdose. We conclude that among anesthesia techniques currently available, isoflurane with oxygen as a gas carrier is a safe and useful field anesthetic in martens, when issues with equipment portability can be overcome.

Key words: American marten, anesthesia, field study, inhalant, isoflurane, *Martes americana*, restraint cone.

INTRODUCTION

The choice of an anesthetic agent in wild mammals depends on numerous factors, such as field logistic constraints, equipment cost, and safety for both animals and staff. Chemical immobilization with injectable agents requires little equipment and is relatively simple to administer. In addition, newer drugs are associated with fewer adverse effects than older agents. As a result, injectable drugs are frequently used for the anesthesia of wild mammals in the field. However, recoveries can be prolonged, control of anesthesia levels is limited, and most injectable drugs are associated with cardiovascular and respiratory side effects. Inhalant anesthesia reduces the hazards of general anesthesia and ensures a rapid postoperative recovery of vital functions (Calvey and Williams, 2001).

American marten (*Martes americana*) has been recognized throughout its range as being sensitive to anthropogenic forest disturbances such as commercial forestry (e.g., Potvin et al., 2000). In this context, marten population maintenance is often recommended as an indicator for forest habitat integrity (Thompson, 1991). In addition to this, the marten is an important species for fur trapping by local and native peoples.

Different anesthetic protocols have been reported for the field anesthesia of this mustelid, either using injectable drugs (Belant, 1992; Kreeger, 1999; Belant, 2005) or inhaled agents (Herman et al., 1982; Potvin et al., 2004). However, to the best of our knowledge, field use of isoflurane using a portable anesthesia machine with a precision vaporizer previously has not been described for martens. The objective of our study was to assess the efficiency, practicality, and usefulness of isoflurane inhaled anesthetic with oxygen as a gas carrier for American martens in a field setting and compare that protocol with other published methods for this species.

FIGURE 1.   Restraint cone used to immobilize martens for mask induction with isoflurane. A. The fabric cone is connected to the trap to allow transfer of the animal. B. Mask induction of the animal in the restraint cone is greatly facilitated by the safe access to its snout via the opening in the extremity of the cone.

MATERIALS AND METHODS

The study was conducted in October and November 2005 at the Waswanipi Cree Model Forest, municipality of James Bay, Québec, Canada (49°70′N, 76°50′W). This project was carried out according to animal utilization protocols approved by the animal care committees of the institutions involved in this project, both of which operate under the auspices of the Canadian Council on Animal Care.

Martens were captured in live traps (model 202, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) covered with branches of spruce or fir, and baited mostly with beaver or hare flesh and trapping lures. Each trap was checked daily. Trapped martens were transferred into an open-ended restraining cone made of fabric with a 25-mm-diameter opening that allowed safe restraint of the animal with direct open access to its snout (Fig. 1). Anesthesia was induced with a facial mask (Guinea pig mask, 11/16" diameter, J. A. Webster, Inc., Sterling, Massachusetts, USA) placed over the snout with isoflurane (AErrane®, Baxter, Toronto, Ontario, Canada) set at 3% and delivered in 1 l/min oxygen using a custom-made portable anesthesia machine. This machine was built using a semiopen, nonrebreathing Mapleson D modified Bain circuit connected to an isoflurane vaporizer (Tec-3 Model, Dispomed, Joliette, Québec, Canada) and an oxygen flow meter (Dispomed). The system was firmly fixed inside a solid plastic case (57 × 46 × 33 cm, Stormcase model IM2750, Hardigg, Saint-Jean-sur-le-Richelieu, Québec, Canada) to provide adequate resistance to rough conditions during transport. The total weight of this assembly was 10.2 kg. A portable type E medical oxygen cylinder (67 cm long ×

10 cm in diameter and weighing 7.7 kg) was used as a source of oxygen.

The procedures were performed on the back seat of a crewcab at ambient temperatures varying from 1.5 to 25.8 C (10.4±3.9 C, mean±SD) in the truck. When sufficient levels of anesthesia were obtained, the isoflurane setting was decreased to an appropriate level, defined as maintaining inhibition of animal movement. Martens were weighed and morphometric measurements were made. Heart rate and hemoglobin oxygen saturation (SpO_2) were monitored with a pulse oximeter (OxiMax NPB-75® Handheld Capnograph/Pulse Oximeter, Nellcor Puritan Bennett, Plesanton, California, USA) with the probe placed on a forelimb digit. Respiratory rates were determined by counting complete thoracic cycles for 30 sec every 5 min. End-tidal carbon dioxide partial pressures ($ETCO_2$) were measured in some individuals with a microstream capnograph (OxiMax NPB-75®) via a pediatric nasal cannula (diameter 2.5 mm, Smart Capnoline® Oral/Nasal circuit, Nellcor Puritan Bennett, Plesanton, California, USA) which was inserted about 5–7 mm inside the nares. Rectal temperatures were monitored throughout the procedure and recorded once on average 12 min after the beginning of anesthesia. Ambient temperatures were measured at the beginning and the end of the procedure in the truck, near the vaporizer. When ambient temperatures were low, hypothermia was prevented by turning the engine on with the door closed but window partially opened.

After a complete physical examination, blood was sampled via a jugular vein, feces were collected from the rectum or in the trap, and a first premolar tooth was extracted using a small root elevator (Super Slim Feline Elevator, J. A. Webster Inc., Sterling, Massachusetts, USA) and extraction forceps. An ear swab was taken for parasitologic examination and an ear tag was installed. Radio transmitters were also attached in selected females (18 of 27 females) using a collar. Isoflurane administration was discontinued when the procedure was completed. Oxygen was delivered via face mask until the marten started to move.

The animals were left in the trap to recover and released as soon as the level of wakefulness was believed to be sufficient. Length of induction was defined as the interval between the beginning of mask induction and a lack of response to tactile stimuli. Length of recovery was defined as the interval between the end of anesthesia (isoflurane=0%) and the moment when the marten was ready for release (normal gait and normal behavior such as vocalization and signs of aggression).

Statistical analyses were performed using SAS 9.1 (Cary, North Carolina, USA). *t*-tests for unequal variances were used to examine the effects of period of the day (AM versus PM, which could represent the time spent by the marten inside the trap) on the induction, recovery, and procedure times. Linear regression models were used to test the relationship between weight, body index (weight divided by the total length), ambient temperature, minimum and maximum settings of isoflurane, and on the lengths of induction, recovery, and procedure. Alpha was set to 0.05.

RESULTS

Forty-one male (weighing 953±131 g) and 27 female (weighing 680±107 g) martens were anesthetized during the project. Each captured marten was anesthetized once. Although some animals appeared subjectively thin, no significant anomalies were observed during the physical examination. All procedures were uneventful and no anesthesia-related mortality occurred. Minimum and maximum maintenance isoflurane settings ranged from 1.0% to 2.5% (1.4±0.3%) and from 2.0% to 4.0% (3.0±0.2%), respectively. Inductions and recoveries were usually rapid and smooth, with animals exhibiting minimal agitation. Mean length of induction was 1.85±1.20 min (range: 0.5–6 min) and the whole procedure lasted 16.4±7.1 min (range: 9–35 min). Recovery took place in 6.3±2.8 min (range: 2–17 min). Respiratory parameters and heart rates were recorded in 18 and 8 martens, respectively, on average 10 min after the beginning of anesthesia. Mean respiratory rate was 31±11.5 movements per minute (mpm) (range: 12–64). Mean heart rate was 216±17 beats per minute (bpm) (range: 200–240). Mean $ETCO_2$ was 40.4±5.8 mm Hg (range: 32–53 mm Hg) and all values of SpO_2 remained above 95% during the procedure. Mean rectal temperature, recorded on average 12 min after induction, was 37.4±1.5 C (range: 34.0–40.1 C). No relationship was ob-

TABLE 1.   Comparison of induction and lengths of recovery with different anesthesia techniques described in American martens.

Anesthetic agent[a]	Route of administration	n	Induction (min) Mean±SD (Range)	Recovery (min) Mean±SD (Range)	Reference
Isoflurane	Semi-open, non-rebreathing	68	1.8±1.2 (0.5–6.0)	6.3±2.8 (2.0–17.0)	Current report
Isoflurane	Open drop	62	1.6±0.8 (0.5–4.8)	3.6±2.4 (0.5–9.7)	Potvin et al., 2004
Halothane	Open drop	264	2.6±0.7 (1.5–8.3)	3.1±1.5 (1.0–10.0)	Herman et al., 1982
TZX	Injectable	19	2.5±1.8 (0.8–6.1)	70.8±31.9 (30–122)	Belant, 2005
KX	Injectable	5	1.8±0.5 (1.2–2.5)	100.4±43.2 (62–175)	Belant, 1992

[a] TZX: tiletamine-zolazepam + xylazine; KX: ketamine + xylazine.

served between the other variables tested and the lengths of induction, recovery, and procedure.

We compared the lengths of induction and lengths of recovery obtained in our study with results from previously published anesthetic protocols in martens (Table 1). Twenty-seven of the 68 martens (40%) were recaptured at least once between one and 33 days after anesthesia. All recaptured animals were very active and appeared to behave normally. Less than 75 ml of isoflurane and three type E medical oxygen cylinders were necessary to anesthetize the 68 martens used in this study.

DISCUSSION

Anesthesia of free-ranging animals in a field setting is often challenging. Age and health status are often uncertain, and the body condition of wild animals is regularly suboptimal. In addition, capture-related stress can lead to increased plasma levels of glucocorticoids and catecholamines (Spraker, 1982). As a consequence, any adverse effects of anesthetic agents are likely to be greater than those observed in domestic animals. Capture and prolonged containment in live traps, followed by handling and anesthesia, certainly carries some physiological implications, as well as negative effects on the animal's well-being (Mathews et al., 2002). As animal users, scientists are ethically responsible for

refining handling techniques used in order to minimize any negative effects of their interventions on individuals and the population as a whole. Field biologists benefit considerably from refining interventions to reduce sampling variances.

The anesthesia protocol used in our study enabled us to perform all procedures rapidly and safely for both staff and animals; no mortality or anesthesia problems occurred in the 68 martens. Induction and recovery were rapid and smooth, and cardiorespiratory parameters remained within the acceptable range for anesthetized animals at all times.

Kreeger et al. (1998) noted that a great deal of maintenance for the vaporizer was required when using isoflurane with an anesthesia machine. The vaporizer we used was calibrated only once, at the beginning of the project. The possibility of inaccuracy due to decalibration or variation in ambient temperature was overcome by adjusting the vaporizer setting according to animal response. Nevertheless, because we used this vaporizer outside its recommended range of temperatures (15 C to 35 C), the reported settings of isoflurane are not necessary equal to the actual concentration delivered. Even though isoflurane has a slightly pungent and ethereal odor and can irritate the upper airways (Calvey and Williams, 2001), martens tolerated induction via face mask well; this is consistent with observations of Siberian polecats (Gaynor

et al., 1997), sea lion pups (Heath et al., 1997), beavers (Breck and Gaynor, 2003), and ferrets (Lawson et al., 2006). Isoflurane is a volatile agent with a low blood:gas solubility coefficient (1.4 versus 2.4 for halothane) and low tissue solubility, resulting in extremely rapid induction and recovery (Calvey and Williams, 2001).

The two reported protocols using injectable agents in martens were associated with prolonged and variable length of recovery when compared to inhaled agents (Table 1). These lengthy recoveries could be detrimental for the animals by increasing the level of stress and the potential for detrimental hypothermia, especially at relatively cold ambient temperatures. Enduring residual anesthetic effects could also prevent the animal from resuming normal activities such as hunting and caring for young. In addition, long recoveries increase post-procedural monitoring time by handlers, which diminishes the productivity of the field team and therefore adds to the cost of the project. Because dosages of injectable agents are approximate, premature recoveries can occur, potentially exposing the handler to bites and zoonotic disease transfer. Inhalant anesthesia allows for rapid modifications of anesthesia depth and therefore prevents such undesirable outcomes. The definition of the length of recovery varies between studies limiting useful comparisons of the different protocols. Belant (1992, 2005) defines recovery length as the time between immobilization and the animal's ability to maintain an upright posture and respond to external stimuli. Potvin et al. (2004) described recovery time as the time between recumbency and the moment when the animal stood up once released. In the Herman et al. (1982) study, the length of recovery was not clearly defined.

Among the volatile anesthetics commonly used in veterinary anesthesia, isoflurane is considered to have the fewest effects on the heart but its effects on respiration are more pronounced than with halothane (McKelvey and Hollingshead, 2003). Only 0.2% of the isoflurane dose is metabolized (Calvey and Williams, 2001), which allows for its utilization in neonatal and geriatric animals, as well as in animals with hepatic or renal damage (McKelvey and Hollingshead, 2003). Cardiovascular depression is a common side effect of most injectable agents. Mean heart rates measured during tiletamine-zolazepam-xylazine and ketamine-xylazine anesthesia were, respectively, 163 ± 34 bpm (Belant, 2005) and 125 ± 7.5 (SE) bpm (Belant, 1992), compared to 216 ± 17.2 bpm in the present study. Mean respiratory rates were superior with injectable agents (67 ± 30 mpm and 65 ± 15.1 [SE] mpm, respectively) compared to inhalation agents (31 ± 11.5 mpm, similar to normal respiratory rates of a similarly-sized mustelid, the domestic ferret (33–36 mpm; Fox, 1998)). Moreover, isoflurane is delivered in 100% oxygen, and oxygen arterial partial pressure is likely to be greater using this technique. In our study, ventilation was assessed in 18 martens by measuring $ETCO_2$. All values obtained were in the expected range for a small mammal and indicated that ventilation and pulmonary gas exchanges were adequate throughout the procedure. An added safety feature of precision vaporizer delivered inhalant anesthesia is that endotracheal intubation and assisted manual ventilation are possible if apnea occurs. General anesthesia can induce hypothermia, because muscular activity and metabolism are depressed. In the present study, mean rectal temperature 12 minutes after induction with isoflurane (37.4 ± 1.5 C) were similar to those reported with the combination of tiletamine-zolazepam-xylazine (37.0 ± 2.2 C) (Belant, 2005).

Inhaled agents have been used in free-ranging martens with open drop exposure by injecting the liquid agent into a closed chamber (Herman et al., 1982; Potvin et al., 2004). Halothane and isoflurane are compounds with a high vapor pressure

and evaporate so easily that they can reach a concentration of more than 30% at a barometric pressure of 760 mm Hg, depending on ambient temperatures (Mathew et al., 2002). That level could cause a fatal anesthetic overdose (McKelvey and Holligshead, 2003). A precision vaporizer limits the evaporation of these agents and allows for their safe use in anesthesia. Use of an anesthesia machine also permits maintenance of the animal under an appropriate level of anesthesia for as long as needed.

Even if toxicity to isoflurane has not been clearly demonstrated, exposure of staff to the anesthetic gas is always an issue. Induction via a face mask reduces this exposure when compared to induction in a closed chamber.

The major disadvantages of the use of isoflurane anesthesia in the field are the weight and bulk of the equipment for remote use, and that mask induction without injectables requires adequate control and restraint of the animal. Our method for induction worked very well for this purpose. Oxygen cylinders can be a safety issue for air transport. Kreeger (1999) reported that a great deal of training was necessary to work with an anesthesia machine. For our study, wildlife biologists and technicians were trained by a veterinarian at the beginning of the study until an adequate number of animals were handled ($n=10–15$) and they did not encounter any difficulties using the machine on their own. Because no mortalities occurred, training appeared sufficient. Anesthesia is never risk-free and whatever the technique used, adequate training of the staff is recommended to improve anesthesia safety as much as possible.

In our study, isoflurane anesthesia proved safe, easy to use, and provided smooth and rapid inductions and recoveries. Additional costs of this technique were considered to be balanced by the improvement of animal welfare and overall anesthesia security, and by the gain in productivity of the field team. When equipment transport is possible, isoflurane inhalant anesthesia with a precision vaporizer provided excellent results in the American marten under field conditions.

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