

Biomedical Protocols for Free-ranging Brown Bears, Wolves, Wolverines and Lynx

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PREFACE

Compilation of this document was initiated by the Norwegian Directorate for Nature Management in order to establish recommended protocols for capture, chemical immobilization, anesthesia and radiotagging of free-ranging brown bears (*Ursus arctos*), gray wolves (*Canis lupus*), wolverines (*Gulo gulo*) and Eurasian lynx (*Lynx lynx*). In addition, procedures to ensure proper sampling of biological materials for management, research and banking purposes have been included.

The current protocols are based on nearly 3,000 captures of free-ranging brown bears, wolves, wolverines and lynx carried out from 1984 through 2012 in Scandinavia. Some of the results have been published as peer reviewed papers, conference presentations, theses, and reports. However, a large amount of data are still on file and will be published in the future. In addition, comprehensive reviews of the global literature on brown bears, wolves, wolverines and lynx have been carried out in order to include pertinent information from other sources.

These protocols have been approved by all ongoing research projects on brown bears, wolves, wolverines and lynx in Scandinavia.

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This document will be updated on a regular basis and will be available in pdf format at: <http://www.rovviltportalen.no/content.ap?thisId=500039688>

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Cover Photos: Staffan Widstrand[©] (bear) and Jon M. Arnemo[©]

INTRODUCTION

Chemical immobilization of wild animals is a form of veterinary anesthesia conducted under the most difficult of circumstances. Anesthetic drugs are never completely devoid of toxicity and anesthesia invariably carries a risk to the life of even healthy patients. The risk of severe side effects, injuries or death can never be completely eliminated. In addition, all immobilizing drugs are toxic and some are potentially lethal to humans.

Chemical immobilization of free-ranging wildlife should only be considered if it is necessary to accomplish research or management goals, and should be carried out by a team of professionals with proper training, experience and expertise in wildlife capture, veterinary anesthesia, animal handling and basic first aid and CPR techniques. If captures are carried out by darting from a helicopter, the skill of the pilot and the crew members is of paramount importance for a safe and successful outcome.

All captures must be properly planned. Chemical immobilization of brown bears, wolves, wolverines and lynx should preferably be carried out in winter or spring, on snow-covered ground. High ambient temperatures, open water, and bare ground make captures more difficult and increase the risk of accidents and mortality. The choice of capture method is also important and needs to be adapted to the species in question and the specific landscape. Most bears, wolverines and wolves are captured from helicopters in Scandinavia, although lynx are also captured using snares and box-traps.

DRUGS AND DOSES FOR CHEMICAL IMMOBILIZATION AND ANESTHESIA

Brown bears

Brown bears are usually captured in early spring, shortly after emergence from their winter dens. Although brown bears are sometimes chemically immobilized during summer or shortly before denning, such captures are more difficult due to lack of snow cover, open water, high ambient temperatures and increased dose requirements due to seasonal changes in physiology and body fat. Bears are sometimes immobilized during wintertime in their dens for hibernation related research.

Except for hibernating animals, brown bears are darted from a helicopter using a remote drug delivery system (Dan-Inject[®]). Currently, the following standard doses of medetomidine (M) (Domitor[®] 1 mg/ml, Zalopine[®] 10 mg/ml) and tiletamine-zolazepam (TZ) (Zoletil[®]) are used for immobilization of free-ranging bears in April-May: Yearlings (15-45 kg) 1.25 mg M + 62.5 mg TZ; small bears (2-3 years, 45-70 kg) 2.5 mg M + 125 mg TZ; adult females and small males (70-120 kg) 5 mg M + 250 mg TZ; medium-sized adult males (120-200 kg) 10 mg M + 500 mg TZ; large males (> 200 kg) 15 mg M + 750 mg TZ. A fixed M:TZ ratio is used so that doses can be split or combined. The doses and darts are made up as follows:

- 1.25 mg M + 62.5 mg TZ (yearlings): 1 ml of Zalopine[®] and 1.8 ml of sterile water are used to dissolve 500 mg of Zoletil[®]; split into 8 doses; use 1.5 ml darts with 1.5 x 25 mm barbed needles
- 2.5 mg M + 125 mg TZ (2-3 year old subadults): 5 ml of Domitor[®], 0.5 ml of Zalopine[®] and 0.8 ml of sterile water are used to dissolve 500 mg of Zoletil[®]; split into 4 doses; use 1.5 ml darts with 2.0 x 30 mm barbed needles

- 5 mg M + 250 mg TZ (adult females and small adult males): 5 ml of Domitor[®] and 0.5 ml of Zalopine[®] are used to dissolve 500 mg of Zoletil[®]; split into 2 doses; use 3 ml darts and 2.0 x 40 mm barbed needles
- 10 mg M + 500 mg TZ (medium-sized adult males): 1 ml of Zalopine[®] and 1.5 ml of sterile water are used to dissolve 500 mg of Zoletil[®]; one dose; use 3 ml dart and 2.0 x 40 mm barbed needle
- 15 mg M + 750 mg TZ (large adult males): 1 ml of Zalopine[®] and 0.5 ml of sterile water are used to dissolve 500 mg of TZ; make up three vials that are split into two doses; use 3 ml darts and 2.0 x 40 mm barbed needles
- For capture of bears late in the fall: Consider increasing the spring dose by 25-50% and using longer needles, but the injection site is probably the most important factor to avoid delayed absorption from subcutaneous fat
- For hibernating bears in late winter, spring dose is reduced by 50% and ketamine is added. For 45-65 kg subadult bears, the combination of 1.25 mg M + 62.5 mg TZ + 75 mg ketamine provided sufficient anesthesia; for small subadults, 20-30 kg, half of this dose is used.

Hypoxemia (low levels of oxygen in the blood) is a common side effect documented by arterial blood gases in both captive and free-ranging brown bears immobilized with different doses of MTZ. Intranasal oxygen supplementation markedly improves the arterial oxygenation and is routinely given throughout anesthesia, as part of the standard field procedure for the Scandinavian Brown Bear Research Project. In relation to the body mass, the following flow rates from oxygen cylinders are adequate during immobilization with MTZ doses used for free-ranging bears in April-May: 0.5 L/min to bears up to 25 kg, 1 L/min to bears up to 50 kg, 2 L/min to bears up to 200 kg, and 3 L/min to bears up to 250 kg. Preliminary studies indicate that in wintertime 0.5 L/min is probably sufficient for bears up to 55 kg. In addition to oxygen cylinders, a portable battery driven oxygen concentrator (EverGo[™] Portable Oxygen Concentrator) is being used to efficiently provide supplemental oxygen to immobilized bears in Scandinavia.

Tiletamine-zolazepam was formerly the drug combination of choice for immobilization of bear species. Tiletamine-zolazepam has a wide margin of safety and has no major cardiopulmonary or thermoregulatory side effects in bears. The main disadvantage of this combination is extended recovery times. However, in combination with medetomidine, the effective dose of tiletamine-zolazepam can be reduced by as much as 75%, and atipamezole (Antisedan[®]), the antagonist for medetomidine, is used to shorten the recovery times.

Wolves

Wolves are immobilized by darting from a helicopter in winter on snow-covered ground. Animals \geq 6 months of age, regardless of sex and body mass, are darted with 250 mg tiletamine-zolazepam (Zoletil[®]) per animal using a remote drug delivery system (Dan-Inject[®]). A 3 ml dart syringe with a 1.5 x 25 mm barbed needle is used. Once recumbent, administration of 0.5-1.0 mg medetomidine or 0.3 mg/kg midazolam i.m. may be required to induce complete immobilization. Mean (range) body weights in wolves > 18 months old captured in Scandinavia were 48 (36-57) kg for males and 38 (31-46) kg for females. Juveniles 7-10 months old weighed 34 (24-44) kg.

Wolverines

Adult wolverines (females 9-11 kg, males 14-16 kg) and juveniles (> 8 months, 8-14 kg) are usually immobilized from a helicopter or in den sites (only secondary dens, never primary

natal dens) under boulders or snow. Juveniles of approx. 3 months age (3-8 kg) are captured at den sites with a snare pole followed by drug-injection by hand. Adult wolverines are darted with a dose of 4 mg medetomidine (Zalopine[®]) + 100 mg ketamine (Narketan 10[®]) per animal using a remote drug delivery system (Dan-Inject[®]). A 1.5 ml dart syringe with a 1.5 x 25 mm barbed needle is used. Juveniles (up to 8 kg) are manually restrained, weighed and immobilized with 0.1 mg/kg medetomidine (Domitor[®]) + 5 mg/mg ketamine (Ketalar[®]) i.m. (induces 30-40 min of immobilization).

Hypoxemia is a common side effect in wolverines immobilized with medetomidine-ketamine, as documented in an arterial blood gas study. Drug-induced physiological changes and high altitude (500-1,300 m above sea level) contributes to the low levels of oxygen in the blood. Supplemental oxygen is recommended to prevent hypoxemia and improve safety for immobilized wolverines.

Lynx

Adult lynx and juveniles (> 8 months) are either immobilized from a helicopter or captured using box traps or snares set around fresh roe deer kills. Hunting dogs have also been used to chase the lynx into a tree. Adults (males 18-28 kg, females 14-19 kg) are darted with an initial dose of 4 mg medetomidine (Zalopine[®]) + 100 mg ketamine (Narketan 10[®]) per animal using a remote drug delivery system (Dan-Inject[®]). In adults captured in box traps (calm animals) and in juveniles (6-12 months 9-16 kg, yearlings 12-21 kg), the doses can be reduced to 2 mg medetomidine + 50 mg ketamine. A 1.5 ml dart syringe with a 1.5 x 25 mm barbed needle (Dan-Inject[®]) is used. Kittens (4-5 weeks of age; mean body mass 1.5 kg) are captured by hand in their natal lairs, weighed and immobilized with 0.1 mg/kg medetomidine (Domitor[®]) + 5 mg/kg ketamine (Ketalar[®]) i.m.

Supplemental dose

Supplemental dosing depends on the situation, species and whether surgical anesthesia is required or not. Animals that are not recumbent 15 minutes after the initial dose, are redarted with a full dose (all species). If the animal is recumbent but incompletely immobilized, administration of additional drugs is usually necessary:

Brown bears: In large bears (adult females and adult and subadult males), redarting with either a full dose or half the initial dose is recommended for safety reasons. In yearling and subadult bears 1 mg medetomidine (Domitor[®]) can be given i.m. by hand syringe injection.

Wolves: Wolves are usually easy to handle, even if they are not completely immobilized (often the case after darting with tiletamine-zolazepam). To reduce stress and to facilitate sampling, 1 mg medetomidine (Domitor[®]) i.m. is recommended to induce complete immobilization.

Wolverines and lynx: If the animal is down but incompletely immobilized, 25-50% of the initial dose can be given i.m. by hand syringe injection.

In case of a prolonged procedure or signs of spontaneous recovery, 0.5-1.0 mg medetomidine (Domitor[®]) i.m. can be given to keep juvenile and adult wolves, wolverines and lynx and yearling bears immobilized for another 15-30 minutes. For personnel safety 2 mg medetomidine (Domitor[®]) should be combined with 1-2 mg/kg ketamine (Narketan 10[®]) in adult bears. If extra time is needed to finish surgery or other painful procedures, medetomidine-ketamine should always be administered. Due to the long elimination time, additional tiletamine-zolazepam should not be used, unless for human safety reasons in large bears.

CHASING, TRAPPING AND STRESS

Animals that have not been previously captured from or chased by a helicopter are usually naïve when approached and darting can be performed within a few minutes of initial observation if the snow conditions and the landscape are optimal (proximity to ice-covered lakes, clear-cuts, open terrain etc.). Animals that have been captured previously, especially wolves, will usually run for cover when they hear the helicopter and are more difficult to approach. To avoid stress and physiological side effects (hyperthermia, lactic acidemia) during immobilization, intensive chasing should be kept to a minimum (less than 3 min), and the total time of pursuit (the time from initial observation, including alternating periods of intensive and extensive pursuit) should never exceed 30 minutes.

Lynx are sometimes immobilized after being captured in walk through wooden box-traps, spring-loaded foot-snares or after being chased into a tree by hunting dogs. Spring-loaded foot-snares placed at lynx-killed prey should be continually monitored using radio-alarms, and the reaction time upon capture should never exceed 20 minutes. Reaction time upon capture in box-traps should never exceed 12 hours. Intensive chasing with dogs should be kept to a minimum, and should never exceed 30 minutes. Special care should be given to avoid accidents or physiological side effects due to prolongation of the capture attempt.

HANDLING AND MONITORING OF IMMOBILIZED ANIMALS

Immobilized animals should be monitored and clinically examined by professionals with experience in wildlife medicine and anesthesia. Possible side effects include hypoxemia (inadequate amount of oxygen in the blood), respiratory depression (hypoventilation; increased carbon dioxide levels in the blood) and thermoregulatory dysfunction (hyperthermia or hypothermia). Drug overdose in individuals with poor body condition, aspiration of vomitus/saliva, pneumothorax due to misplaced dart, and vomiting (in wolves) are other possible complications. If several animals are being captured at the same time, e.g.: members of a pack, family group, they should be brought together for monitoring and processing.

To prevent aspiration of saliva or vomitus, immobilized animals should be kept in lateral recumbency with the mouth and head low relative to the body. An eye gel (Viscotears[®]) should be applied to the cornea to prevent drying. Animals should be protected from direct sunlight into the eyes. A blind-fold and ear plugs should be used.

Thermoregulation should be monitored by frequent measurements of the rectal temperature (RT). “Normal” body temperature in brown bears is 37-37.5 C based on preliminary studies with abdominal temperature logging devices. Typical and acceptable RT during immobilization of bears, wolves, wolverines and lynx is thought to be 38.0-39.0 C. Hyperthermic animals (RT > 40.0 C) should be cooled by applying snow (or water in summertime) to the axilla, groin, and/or tongue. If the hyperthermia is not resolving within 15 minutes, or the animal has an RT > 41.0 C i.v. fluid therapy should be initiated (10-15 ml/kg of Ringer[®]-acetat). Oxygen supplementation is recommended for hyperthermic animals because the oxygen demand increases 10% for each degree C increase in body temperature. Hypothermic animals (RT < 36.0 C in bears and < 37.0 C in the other species) should be protected from wind and cold surfaces to avoid further cooling using a Wolverine Bag[®]. Hot water bottles can be placed in the groin and axilla as an external heat source in the field. In case of prolonged immobilization and recovery, hypothermic animals should be warmed, and prewarmed fluid (38 C) (Ringer[®]-acetat) should be administered intravenously.

The color of the mucous membranes in the mouth (gums) can be used to assess blood oxygenation. A pink or red color is normal; bluish membranes indicate hypoxemia. The capillary refill time (CRT) can be used to assess peripheral circulation by pressing a finger on the gums, release and counting the seconds it takes for color to return. Normal CRT is 2 sec or less. Cardiorespiratory function should be monitored using a pulse oximeter (Nellcor[®]) with the sensor (VetSat[®]) applied to the tongue. Hemoglobin oxygen saturation (SpO₂) should be above 95%. A decreasing trend indicates hypoxemia and supplementation with intranasal oxygen should be given to improve oxygenation and the safety for the immobilized animal. A portable oxygen cylinder should be part of the standard field equipment, as well as a laryngoscope, endotracheal tubes, and a ventilation bag. In case of apnea (absence of breathing), intubation and ventilation with a bag valve mask (e.g. AmbuBag[®]) should be carried. Doxapram (Dopram[®]) at 5-10 mg/kg i.v. can be given to stimulate respiration. The effect of doxapram is short-acting and, as a general central nervous system stimulant, it may lead to the animal waking up from anesthesia. Field personnel should be able to intubate (place an endotracheal tube in the wind pipe) and provide assisted ventilation to an animal with apnea.

A small surgical kit for treating wounds and an electrical clipper should be part of the standard equipment.

TAGGING, SAMPLING AND DOCUMENTATION

Most animals are captured for tagging or sampling purposes and should be processed according to the aim of the project. Capture data should be recorded according to an established animal capture form and photos should be taken.

Radiocollars should be fitted according to the size, age and sex of the animal. The weight of the radiocollar should not exceed 2% of the animal's body mass. When changing the collar the neck should be examined for hair loss and possible skin irritation.

Brown bears: The collar should be fitted so that it can be pulled on and off over the head. Drop-off collars or a break-away zone (double webbing in males, single in females) should be used on all growing bears and on bears of unknown age. For adult males, which may have a greater circumference of the neck than the head, consider clipping hair on the neck to avoid losing the collar. Ensure that it is possible to pass a flat hand between the collar and the neck.

Wolves: Minimum collar circumference should be 44.5 cm for females and 48.0 cm for males. Ensure that there is enough space for two fingers (4 cm) between the collar and the neck.

Wolverines: The circumference of the animal's head and neck should be measured before fitting the collar. The circumference of the collar should be adjusted so it is slightly less than the circumference of the head, but larger than the circumference of the neck. Ensure that the collar is not too tight (make room for one finger between the neck and the collar) or that it can be pulled over the head of the animal (backwards). In some cases the difference in circumference of the head and neck is very small (especially in males) and fitting the collar can be difficult.

Lynx: The minimum collar circumference should be 26 cm for females and 30 cm for males. Ensure that at least one finger can be passed between the collar and the neck. In Norway all collars have to have a break-away zone. Collars for juvenile males should have a break-away zone or an implant should be used if 30 cm is too great to be retained by the animal.

All species: The transmitter (VHF) should be activated by removing the magnet and should be tested with the receiver before the animal is released. Be sure that the GPS unit is working properly before any capture is initiated.

A microchip should be implanted s.c. at the base of the nose of brown bears and the insertion hole sealed with a drop of tissue or super glue. In all the other species the microchip is implanted s.c. at the base of the right ear. The microchip should be tested with the scanner after implantation. Application of ear tags and tattooing depend on the species, age of the animal, and aim of the project. The area around previously applied ear tags should be inspected for signs of infection or irritation (redness, swelling or discharge) and if found, the ear tag should be removed and a new one placed on the other ear (if an eartag is required).

All species: Body measurements should be recorded according to the animal capture form.

Blood can be sampled from the jugular vein (all species), cephalic vein (wolves, lynx), or the femoral vein or artery (all species) using evacuated plastic tubes and multisample needles. Blood for genetic studies (5 ml EDTA) should be stored at -20°C until shipment to the laboratory. Tubes without anticoagulant for serum biochemistry and serology should be kept at room temperature for 1-2 hours to ensure complete coagulation. Serum should then be separated by centrifugation (1500 g for at least 15 minutes) and transferred to 2 ml cryogenic vials (Nalgene[®]). Serum for banking (serology and back-up) is stored at -20°C until shipment to the laboratory. Blood samples are taken from all captured carnivores for ongoing and future studies regarding health, disease and stress, and are available for investigations of marked animals submitted for necropsy results.

In brown bears and wolverines, the rudimentary first maxillary premolar is extracted for age determination. At least five minutes before tooth extraction, local anesthesia (Lidokel-Adrenalin[®]) is injected under the mucus membrane above the second premolar (over the maxillary foramen) and carprofen (Rimadyl[®]) is administered i.m. The tooth is preserved in 96% alcohol in a 2 ml cryogenic vial (Nalgene[®]).

Hair should be collected with pliers and transferred to 15 ml sterile plastic tubes (Sarstedt[®]) (brown bears and wolves) or 5 ml sterile cryogenic vials (Nalgene[®]) (wolverines and lynx). Hair samples can be preserved by drying in paper envelopes or by freezing at -20°C in 96% ethanol. Skin biopsies are taken from the inside of the ear, after clipping the hair and cleaning the skin with Klorhexidin sprit[®], by the use of a 4 mm sterile dermal biopsy punch (e.g. Miltex[®]). The skin biopsy is transferred to a 2 ml cryogenic vial (Nalgene[®]) and preserved by adding 96% ethanol. To stop bleeding, pressure is applied to the area where the biopsy was taken, using a piece of gauze held in place with a clothespin.

In brown bears and wolves, feces is collected with the index finger from the rectum using examination gloves. The feces is transferred to 50 ml sterile plastic tubes (Sarstedt[®]). In wolverines and lynx, feces is sampled by inserting a sterile cotton swab (Transwab[®]) into the rectum.

Urine is collected when relevant for research purposes using a 0,6 X 40 mm needle with a 20 ml syringe. The animal is placed in dorsal recumbency and the location of the bladder is determined by palpation or ultrasound. The skin can be wiped with alcohol for disinfection and to improve contact of the ultrasound probe with the skin. The needle is inserted into the bladder through the midline of the abdomen and urine is withdrawn using the syringe.

If muscle biopsies are required, they are taken from the biceps femoris. Routine surgical preparation of the skin overlying the biopsy site is done prior to the biopsy. A short incision is made through the skin and fat overlying the biopsy site and the required amount of muscle tissue is excised. Muscle tissue is washed with sterile saline before placement in cryovials. Fat samples are taken from the same surgery site before closing in two layers

(muscle fascia and skin) using 2-0 PDS or Vicryl[®] sutures. If there is significant subcutaneous fat, it can also be opposed using 2-0 PDS or Vicryl[®] sutures.

Depending on the situation and the study protocol, other biological materials should be sampled according to current standards in veterinary medicine or specific instructions from the laboratory.

ANALGESIA AND ANESTHESIA FOR SURGERY

In brown bears, wolverines and lynx, surgical anesthesia is induced by the recommended immobilizing drugs and doses. For post operative analgesia, 4 mg/kg carprofen (Rimadyl[®]) or 0.2 mg/kg meloxicam (Metacam[®]) is administered s.c. as soon as possible after immobilization is induced and before surgery is initiated (all species). The carprofen dose is reduced to 1 mg/kg in hibernating bears (due to decreased metabolic rate and drug excretion).

SURGICAL PROCEDURES FOR IMPLANTATION

Intraperitoneal transmitters

For surgery, the animal is kept in dorsal recumbency. An appropriate area caudal to the umbilicus is clipped and swabbed with chlorhexidine in 60% ethyl alcohol (Klorhexidin[®]). To avoid frostbite and excessive heat loss at low ambient temperatures, clipping should not be done in the fall or winter. Instead an antiseptic cream (Brulidine[®]) is rubbed into the fur along the midline and the hair is parted to expose the skin. For access to the peritoneal cavity, a ventral midline incision is made using standard surgical procedures. The weight of the implant should not exceed 2% of the body mass of the animal. The radiotransmitter should be tested with the receiver before implantation. Implants should be gas sterilized or disinfected by soaking in 10 mg/ml benzalkonium chloride (non-proprietary). They should be prewarmed and, in the case of chemically disinfected implants, thoroughly rinsed with sterile saline before being placed aseptically into the peritoneal cavity. The incision is closed in two layers with absorbable sutures (PDS or Vicryl[®]), using a simple interrupted pattern for the *Linea alba* (US 1 in all bears except yearlings, US 0 in juvenile and adult wolves, wolverines, lynx and yearling bears and US 2-0 in wolf pups, wolverine cubs and lynx kittens; use a round needle) and an intradermal or interrupted horizontal mattress pattern for the skin (US 0 in all bears except yearlings and US 2-0 in all other animals; use a cutting needle). The skin wound is covered with a spraydressing (OpSite[®]).

Temperature loggers, ECG monitors and physiological sensors

Surgery should be carried out according to accepted standards in veterinary medicine. For temperature loggers and physiological sensors, surgery is carried out as described for intraperitoneal transmitters with the incision length appropriate for the size of the logger or sensor. Temperature loggers, ECG monitors and physiological sensors are sterilized using ethylene oxide gas for at least three hours. Sensors are placed into the peritoneal cavity (free-floating), whereas temperature loggers are placed in the peritoneal cavity but sutured to the body wall under the umbilicus using a non-absorbable suture. ECG monitors are placed subcutaneously on the left thorax at the level of the heart. All devices must be programmed and tested before implantation.

REVERSAL OF IMMOBILIZATION

For reversal of immobilization in animals that have received medetomidine-combinations, 5 mg of atipamezole (Antisedan[®]) per mg of medetomidine is administered i.m. Due to the long elimination time of tiletamine-zolazepam, atipamezole should not be given until earliest 50-60 min after darting. In an emergency, atipamezole can be given at any time, but recovery may then be rough with possible incoordination, excitation and convulsions. Such an animal can be calmed by administration of midazolam (Midazolam[®]) i.m. (suggested dose 0.1-0.2 mg/kg).

Immobilized animals can usually be left to recover undisturbed at the site of capture. Possible side effects and dangers during and immediately after recovery include vomiting (wolves), hypothermia (especially in animals with a small body mass relative to body surface or in case of extended procedures), hyperthermia (due to extensive chasing prior to capture, sun and/or high ambient temperatures), intraspecific strife (attack by pack members, males attacking other males, males trying to mount immobilized females in estrus, males attacking dependent young), open water, lack of fear, traffic, and poaching. Wolves should be observed by trained personnel until full recovery is evident. This may take several hours in wolves immobilized with tiletamine-zolazepam. All GPS and radio-instrumented animals are checked the day after capture.

OTHER TREATMENT

Captured animals with health-threatening diseases should be treated according to accepted standards in veterinary medicine. In animals with severe or terminal illness, euthanasia should be considered.

Wolves: For immigrant wolves, several additional samples and tests are performed. Treatment with praziquantel is given (5mg/kg s.c.) for internal parasites including echinococcus. Selamektin (Stronghold[®]) is applied topically between the shoulder blades at doses of 1 tube (120 mg) for animals under 20 kg, 2 tubes for 20-40 kg and 3 tubes for 40-60 kg. After release, at least 3 more feces samples are collected from the ground during the first few weeks at 1-3 day intervals. Rabies vaccines are given to wolves which have been in Sweden less than 6 months.

TRANSLOCATIONS

In some cases, genetically valuable wolves in problem areas are moved to places where they are less likely to cause conflict and have a higher likelihood of survival. In these cases, wolves are darted with the standard dose of tiletamine-zolazepam and 0.3 mg/kg midazolam is given after the wolf is captured, and repeated immediately before the wolf is loaded into the transport crate in order to prolong the effect of the midazolam. All wolves to be translocated over 1 hour are given 500 ml IV fluids (1L if hyperthermic due to ambient temperature or extended chasing). The wolf must be capable of sitting with its head elevated and not showing any signs of vomiting before transport begins, this is usually at least one hour from darting.

NECROPSY PROCEDURES

In case of a capture-related mortality, the carcass should be sent to a diagnostic laboratory for necropsy (Sweden: Statens Veterinärmedicinska Anstalt, Uppsala; Norway: Veterinærinstituttet, Tromsø/Trondheim/Oslo). To ensure rapid cooling, skinning and evisceration should be considered. If transportation to the laboratory is not possible within 24-48 hours, the carcass should be frozen. As an alternative, a field necropsy can be carried out by a veterinarian after consultation with the laboratory.

LEGAL ASPECTS

All captures must be approved by the appropriate animal ethical committee (Norway: Utvalg for forsøk med dyr; Sweden: Djurförsöksetiska nämnden) and the wildlife management authority (Norway: Direktoratet for naturforvaltningen; Sweden: Naturvårdsverket). The use of motor vehicles may require special permits from local, regional and/or national authorities as well as landowners. Prior to starting capture activities, the police, animal welfare and local wildlife authorities should be informed according to the permit. The use of radiotelemetry equipment requires a permit (Norway: Post- og teletilsynet; Sweden: Post- och telestyrelsen). Note that additional permits may be needed from county authorities when working in protected areas.

Immobilizing agents are prescription drugs and must be used by or on the order of a licensed veterinarian (Norway: Statens legemiddelverk; Sweden: Läkemiddelsverket). Some of these drugs are also controlled substances, i.e. drugs that can be abused, for which specific regulations apply. In Norway, non-veterinarians can legally use immobilizing agents if a valid veterinarian/client/patient relationship is established; i.e. the veterinarian should ensure that the animal in question is under his/her care. In Sweden a special permit is required for non-veterinarians (Jordbruksverket).

New regulations in Sweden (July 2010) require that the person doing the darting has a hunting license (Class one license, either Swedish or valid in Sweden), that a veterinarian experienced with wildlife be readily available, that all missed darts be retrieved, that darts are clearly labeled "WARNING! Do not touch the needle. May contain poison. Call (own phone. No) or police (police tel. No) ", that a tracking dog is available and that any mortalities must be necropsied by the National Veterinary Institute (Viltsektionen vid Statens veterinärmedicinska anstalt, SVA).

Withdrawal times (general considerations): According to the current legislation in force in the European Union (EU), any substance to be used in food producing animals must be assessed by the European Medicines Evaluation Agency (EMA) in order to establish Maximum Residue Limits (MRLs) (applies in Norway as well). After assessment, substances may be listed in one of four Annexes of Council Regulation (EEC) No 2377/90 of 26 June 1990: Annex I – substances for which a full MRL has been fixed; Annex II – substances for which an MRL is not required; Annex III – substances for which a provisional MRL has been fixed; Annex IV – substances for which no MRL can be fixed. If the animal is a "food producing animal" (i.e. an animal, domestic or wild, captive or free-living, whose flesh or products are intended for human consumption), the veterinarian or the person acting under his/her direction may *only* administer a substance listed in Annex I, II, or III. Substances in Annex IV or substances that do not have an Annex entry (I, II, or III) may *not* be used in food producing animals. As of January 2010, very few of the drugs currently used for wildlife immobilization have been authorized for use in food producing animals in the EU. In the EU, a withdrawal period is set within the procedure of granting a marketing authorization, i.e.

either by the national authority concerned (Norway: Mattilsynet; Sweden: Läkemedelsverket) or, in case of a centrally authorized product, by the EMEA. However, for substances that do not have an Annex entry, no marketing authorization can be granted for use in food producing animals.

Withdrawal times (brown bears in Sweden): Permission to use medetomidine-tiletamine-zolazepam and atipamezole has been granted by Läkemedelsverket, but the drugs should not be used less than 3 months before the opening of the hunting season.

RECOMMENDED DRUGS AND EQUIPMENT

Disclaimer: The list does not indicate approval by any authorities or manufacturer for use on wildlife. Drugs and equipment mentioned in the text can be purchased from other manufacturers than those listed.

Anexate[®], 0.1 mg/ml, F. Hoffmann-La Roche, Basel, Switzerland
Antisedan[®], 5 mg/ml, Orion Pharma Animal Health, Turku, Finland
Brulidine[®], Aventis Pharma, Oslo, Norway
Dan-Inject[®], Børkop, Denmark
Domitor[®], 1 mg/ml, Orion Pharma Animal Health, Turku, Finland
Dopram[®], Wyeth Lederle, Wyeth-Ayerst International Inc., Philadelphia, PA, USA
Ketalar[®], 50 mg/ml, Warner Lambert, Morris Plains, New Jersey, USA
Klorhexidin, 5 mg/ml, Galderma Svenska AB, Bromma, Sweden
Lidokel-Adrenalin[®] Kela Laboratoria NV, Hoogstraten, Belgium
Metacam[®], 5mg/ml, Boehringer Ingelheim, Reihn, Germany
Midazolam[®], 5 mg/ml, Alpharma AS, Oslo, Norway
Miltex[®], Miltex GmbH, Tuttlingen, Germany
Nalgene[®], Nalge Company, Rochester, NY, USA
Narketan 10[®], 100 mg/ml, Chassot, Dublin, Ireland
Nellcor[®] NP-20, Nellcor Inc., Pleasanton, CA, USA
OpSite[®], Smith & Nephew Medical Limited, Hull, England
PENI-kél L.A. 15+15[®], Kela Laboratoria NV, Hoogstraten, Belgium
Rimadyl[®], 50 mg/ml, Orion Pharma Animal Health, Turku, Finland
Ringer[®]-acetat, Pharmacia & Upjohn, Oslo, Norway
Sarstedt[®], Sarstedt AS, Ski, Norway
Telonics[®], Telonics Inc., Meza, AZ, USA
Transwab[®], Medical Wire & Equipment Co. Ltd., Corsham, Wiltshire, UK
VetSat[®], Nellcor Inc., Pleasanton, CA, USA
Vicryl[®], Ethicon, Norderstadt, Germany
Viscotears[®], CIBA Vision AG, Hetlingen, Switzerland
Wolverine Bag[®], Jerven AS, Odda, Norway
Zoletil[®], 500 mg/vial, Virbac, Carros, France
Zalopine[®], 10 mg/ml, Orion Pharma Animal Health, Turku, Finland

AUTHORITIES

Direktoratet for naturforvaltning, Norge: <http://www.dirnat.no/>

Djurförsöksetiska nämnder, Sverige:

<http://www.jordbruksverket.se/amnesomraden/djur/forsoksdjur.4.7850716f11cd786b52d80001724.html>

EMEA: <http://www.emea.eu.int/>

Forsøksdyrutvalget, Norge: <http://www.mattilsynet.no/fdu/>

Jordbruksverket, Sverige: <http://www.jordbruksverket.se/>

Livsmedelsverket, Sverige: <http://www.slv.se/>

Läkemedelsverket, Sverige: <http://www.mpa.se/index.shtml>

Mattilsynet, Norge: <http://www.mattilsynet.no/>

Naturvårdsverket, Sverige: <http://www.naturvardsverket.se/>

Post- og teletilsynet, Norway: <http://www.npt.no/>

Statens legemiddelverk, Norge: <http://www.legemiddelverket.no/>

Statens Veterinärmedicinska Anstalt, Sverige: <http://www.sva.se/>

Utvalg for forsøk med dyr, Norge - <http://www.fdu.no/fdu/om/>

Veterinærinstituttet, Norge: <http://www.vetinst.no/>