

Biomedical Protocol for Free-ranging Gray Wolves (*Canis lupus*) in Scandinavia

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Photo: Håkan Sand

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Biomedical protocol for free-ranging gray wolves (*Canis lupus*): Capture, anaesthesia, surgery, tagging, sampling and necropsy procedures

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General

Capture and chemical immobilization of free-ranging wolves should be carried out by a team of professionals with proper training, experience and expertise in wildlife capture, veterinary anaesthesia, and animal handling. Capture data should be collected according to the animal capture form (“Immobiliserings- og märkprotokoll”).

Chemical immobilization

Immobilization of wolves from helicopter and immobilization of trapped wolves: All animals ≥ 6 months of age, regardless of sex and body mass, are darted with 500 mg tiletamine-zolazepam (Zoletil®¹) per animal using a remote drug delivery system (Dan-Inject®²). The drugs are transferred to a 3 ml dart syringe (Dan-Inject®). A 1.5 x 25 mm barbed needle (Dan-Inject®) is used. To prevent the drug mixture from freezing, the needle can be filled with 70% ethanol before assembling of the dart.

Supplemental dose

Animals that are not down 15 minutes after the initial dose, are redarted with a full dose of 500 mg tiletamine-zolazepam (Zoletil®). If the animal is down but incompletely immobilized, 1 mg medetomidine (1 ml Domitor®³) can be given i.m. by hand syringe injection. If required, medetomidine (1 mg) i.v. or i.m. can be used to keep the animal immobilized without causing prolonged recovery.

Helicopter chasing and stress

Wolves which have not been captured from (or chased by) a helicopter, are usually naive when approached and darting can be performed within a few minutes of observation if the snow condition and the area are optimal (ice-covered lakes, clear-cuts, open terrain etc). Wolves that have been captured before will usually run for cover when they hear the helicopter and are much more difficult to approach. To avoid stress and physiologic side

¹ Zoletil® 500 mg/vial, Virbac, Carros, France

² Dan-Inject®, Børkop Denmark

³ Domitor® 1 mg/ml, Orion Pharma Animal Health, Turku, Finland

effects (hyperthermia) during immobilization in such animals, intensive chasing should be kept to a minimum and the total time of pursuit should not exceed 30 minutes.

Handling of immobilized animals

Immobilized animals should be monitored and clinically examined by a wildlife veterinarian. Possible side effects include respiratory depression (drug overdose in individuals with poor body condition, aspiration of vomitus/saliva, pneumothorax due to misplaced dart), vomiting, and thermoregulatory dysfunction. If several animals are being captured at the same time, they should be brought together for monitoring and processing.

To prevent aspiration of saliva or vomitus, immobilized animals should be kept in sternal or lateral recumbency with the mouth and head low relative to the body. An eye gel (Viscotears®⁴) should be applied to the cornea to prevent drying. Animals should be protected from direct sunlight into the eyes. Preferably, a blind-fold should be used.

Thermoregulation should be monitored by frequent measurements of the rectal temperature (RT). “Normal” RT in wolves 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. In case of persistent hyperthermia or RT > 41.0 °C i.v. fluid therapy should be given (10-15 ml/kg/hr of Ringer®⁵ or 500 ml/hr in an adult wolf). Hypothermic (RT < 36.0 °C) animals should be protected from wind and cold surfaces to avoid further cooling using a Wolverine Bag®⁶. In case of prolonged immobilization and recovery, hypothermic animals should be brought into a heated room and prewarmed Ringer® (38 °C) should be administered

Cardiorespiratory function should be monitored using a pulse oximeter (Nellcor®⁷) with the sensor (VetSat®, Nellcor) applied to the tongue. Tiletamine-zolazepam (combined with medetomidine) have no major respiratory or cardiovascular side effects in gray wolves. A relative arterial oxygen saturation (SpO₂) > 80 % is considered to be clinically acceptable in a field situation. A decreasing trend or SpO₂ < 70% indicate inadequate ventilation and treatment with 5-10 mg/kg of doxapram i.v. (10-20 ml of Dopram®⁸) should be considered. A laryngoscope, endotracheal tubes and a ventilation bag should be available, and access to supplemental oxygen is recommended.

Tagging and sampling

Most wolves are captured for tagging or sampling purposes and should be processed according to the aim of the project.

Radiocollars (VHF, GPS or satellite) should be fitted according to the size, age and sex of the animal. Minimum collar circumference should be 44.5 cm for females and 48.0 cm for males. The transmitter (VHF) should be activated by removing the magnet and should be tested with the receiver before the animal is released.

A microchip should be implanted s.c. at the base of the right ear in all animals. The microchip should be tested with the scanner after implantation. In wolves which are not collared, plastic ear tags with numbers should be applied to both ears.

Body measurements should be recorded according to the animal capture form. Photos, both whole body pictures and close-ups of fur, teeth, and eyes, should be taken of all animals.

⁴ Viscotears®, CIBA Vision AG, Hetlingen, Switzerland

⁵ Ringer®-acetat, Pharmacia & Upjohn, Oslo, Norway

⁶ Jerven AS, Odda, Norway

⁷ Nellcor Inc., Pleasanton, CA, USA

⁸ Dopram®, Wyeth Lederle, Wyeth-Ayerst International Inc., Philadelphia, PA, USA

Blood is sampled from the cephalic or the femoral vein (Venoject® II⁹): 4 x 5 ml tubes with EDTA as anticoagulant, and 5 x 10 ml tubes with gel and clot activating factor should be collected. Blood for genetic studies (4 x 5 ml EDTA) should be stored at –20 °C. Tubes without anticoagulant 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®¹⁰). Serum for banking (serology and back-up) is stored at –20 °C.

Hair (4 x 15 ml sterile Sarstedt®¹¹ plastic tubes), skin biopsies (four pieces of skin are taken from the inside of the ear using a sterile 4 mm biopsy punch¹² and transferred to 4 x 2 ml cryogenic vials), feces (1 x 50 ml sterile Sarstedt® plastic tube), and other biological materials should be sampled according to the study protocol. Skin biopsies are preserved using 96% ethanol while hair and feces are frozen.

If applicable, sperm should be collected from adult males either by digital manipulation or by electroejaculation during anesthesia. Standard methods in veterinary medicine should be applied and sperm samples should be processed according to instructions from the laboratory.

Analgesia and anaesthesia for surgery

For implantation of intraperitoneal radiotransmitters (Telonics®¹³) in animals darted with 500 mg of tiletamine-zolazepam, medetomidine at 0.05 mg/kg i.m. is required to achieve surgical anaesthesia. Pups (4-6 weeks old) are anaesthetized with 0.04 mg/kg of medetomidine (Domitor®) combined with 5 mg/kg of ketamine (Ketalar®¹⁴) i.m. The drugs can be given in the same syringe. [A separate protocol for gas anaesthesia in pups is available.] For post operative analgesia, 4 mg/kg of carprofen (1 ml Rimadyl®¹⁵ per 12.5 mg body mass is administered s.c.

Surgical procedure for implantation of intraperitoneal radiotransmitters

The animal is kept in dorsal recumbency. A 6-8 x 4 cm area caudal to the umbilicus is clipped and swabbed with chlorhexidine in 60% ethyl alcohol (Klorhexidin®¹⁶). 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 (BM) of the animal, i.e. a Telonics® IMP/400/L (90 grams, 9.7 x 3.3 cm) should only be used in animals with a BM > 4.5 kg. In pups with BM < 4.5 kg, smaller Telonics® implant models should be applied. The radiotransmitter should be tested with the receiver before implantation into the animal. Implants should be gas sterilized (ethylene oxide) or disinfected by soaking in 10 mg/ml benzalkonium chloride (non proprietary) for at least 24 hours. 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, using a simple interrupted pattern for the *Linea alba* (US 0 for adults and US 2-0 for pups < 4.5 kg with round needle) and a interrupted horizontal mattress pattern for the skin (US 2-0 with cutting needle in all animals). The skin wound is covered with a

⁹ Venoject® II, Terumo Europe N.V., Leuven, Belgium

¹⁰ NALGENE®, Nalge Company, Rochester, NY, USA

¹¹ Sarstedt AS, Ski, Norway

¹² Produkte für die Medizin AG, Köln, Germany

¹³ Telonics Inc., Meza, AZ, USA

¹⁴ Ketalar® 50 mg/ml, Warner Lambert, Morris Plains, New Jersey, USA

¹⁵ Rimadyl® 50 mg/ml, Orion Pharma Animal Health, Turku, Finland

¹⁶ Klorhexidin 5 mg/ml, Galderma Svenska AB, Bromma, Sweden

spraydressing (OpSite®¹⁷). Before surgery the animal is injected with a “long-acting” combination of procaine penicillin and benzathine penicillin at 100.000 IU/kg (100 mg/kg) i.m. (3 ml PENI-kél L.A. 15+15®¹⁸ per 10 kg) in order to minimize the risk of postoperative wound infections.

Reversal of immobilization

For reversal of immobilization in wolves that have been given medetomidine, animals should receive 5 mg of atipamezole (Antisedan®¹⁹) i.m. or s.c. per mg of the total dose of medetomidine administered. Due to the long elimination time of tiletamine-zolazepam, atipamezole should not be given until 40-50 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 5 mg midazolam (1 ml Midazolam®²⁰) i.v. or i.m.

All animals should be observed by trained personnel until full recovery is evident. This may take 4-6 hours in animals immobilized with 500 mg of tiletamine-zolazepam. Possible side effects and dangers during and immediately after recovery include vomiting, hypothermia, attack from other wolves, open water, lack of fear, traffic, and poaching.

Other treatment

Captured animals with health threatening diseases should be treated according to accepted standards in veterinary medicine. In animals with terminal illness, euthanasia should be considered. Recommended treatment for severe sarcoptic mange is 0.3-0.4 mg/kg of ivermectin s.c. (0.3-0.4 ml of Ivomec®²¹ per 10 kg body mass). Vaccination of free-ranging wolves in Scandinavia is currently not recommended.

Necropsy procedures

In case of a capture related mortality, the carcass should be sent to a diagnostic laboratory for complete necropsy (Sweden: Statens Veterinærmedicinska Anstalt, Uppsala; Phone: + 46 18674000. Norway: Veterinærinstituttet, Trondheim; Phone: + 47 73580727). To ensure rapid cooling, the carcass should be skinned and eviscerated. 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 consultations with the laboratory.

¹⁷ OpSite®, Smith & Nephew Medical Limited, Hull, England

¹⁸ PENI-kél L.A. 15+15, Kela Laboratoria NV, Hoogstraten, Belgium

¹⁹ Antisedan® 5 mg/ml, Orion Pharma Animal Health, Turku, Finland

²⁰ Midazolam® 5 mg/ml, Alparma AS, Oslo, Norway

²¹ Ivomec® 10 mg/ml, Merial SAS, Lyon, France

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Wolf immobilized with tiletamine-zolazepam



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