

Capture and Anaesthesia of Wild Mongolian Equids – the Przewalski's Horse (*Equus ferus przewalskii*) and Khulan (*E. hemionus*)

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Abstract

Science-based conservation efforts in general, and wide-ranging equid conservation specifically, often require capture and subsequent handling of the subject animal. Safe and animal-welfare appropriate wild equid capture and anaesthesia is a complex operation necessitating a multitude of skills that require appropriate veterinary training. The agent of choice for wild equid capture and anaesthesia is the potent opiate ethorphine in combination with specific opiate antagonists that allow for the complete reversal of the anaesthetic effects. The recommended dosage for a healthy, wild adult Przewalski's horse is 2.5-3.0 mg ethorphine, 10 mg of the alpha2-agonist detomidine and 10 mg of the opioid agonist-antagonist butorphanol. In Przewalski's horses ethorphine is reversed with the opioid antagonist naltrexone (200 mg). In khulan procedures anaesthesia was induced with a combination of 4.4 mg Ethorphine, 10 mg Detomidine and 10 mg Buthorphanol. Anaesthesia was reversed with the opioid antagonist-agonist diprenorphine or a combination of 200 mg naltrexone and the alpha2-antagonist 20 mg atipamezole. All equids were standing and alert approximately two minutes following administration of the antagonists.

Key words: Anaesthesia, *Equus ferus przewalskii*, *Equus hemionus*, Khulan, Mongolia

Introduction

Science-based conservation efforts in general, and wide-ranging equid conservation specifically, often require capture and subsequent handling of the subject animal. Probably, the two most common research reasons that require the capture of animals are the placement of radio-telemetry devices and the collection of biomedical materials (Osofsky & Hirsch 2000). In order to develop management plans for Mongolian equid species, which range over vast areas, the use of radio-telemetry is an essential tool. Radio-telemetry allows the researchers to better understand habitat requirements and to delineate the areas of potential wildlife-human conflicts. Mongolia has two wild equids that need to be considered: the Przewalski's horse (*Equus ferus przewalskii*) and the Mongolian wild ass (*E. hemionus*). Wild equid capture and anaesthesia is a complex operation requiring a multitude of skills.

Prior to capture, the purpose and circumstances of the procedure must be considered to be both practical and essential (Osofsky & Hirsch, 2000). Every anaesthetic event bears the inherent risk of significant injury and potential death. Though this risk is for the most part very small it must be ascertained that the procedure is necessary and that the potential gains outweigh the risks (Kreeger et al., 2002). Necessary permits must be procured well in advance of the planned event.

The purpose of this paper is to provide information and a minimum set of guidelines towards planning and performing safe capture and anaesthesia events in wild Mongolian equids in-situ.

The environmental and logistical constraints in Mongolia are taken into consideration. Additionally, special reference is given to the transport of the Przewalski's horse, as this activity has been essential in re-establishing this species in Mongolia and will be once again in the near future when horses are exchanged between the various reintroduction sites.

Methodology and Discussion

Przewalski's horse (*Equus ferus przewalskii*). The Przewalski's horse (takhi in Mongolian) became extinct in the wild by the mid 1960's. The last recorded sightings of Przewalski's horses occurred in the Dzungarian Gobi desert in SW Mongolia. The species has only survived due to captive breeding based on 13 founder animals. A private fund and the Mongolian Society for the Conservation of Rare Animals of the Ministry of Environment initiated the Takhiin Tal Project with the support of various international sponsors. In 1999 the International Takhi Group (ITG) was established to continue and extend this project in accordance with the IUCN reintroduction guidelines. In 1992 the first group of captive born Przewalski's horses were airlifted to the Takhiin Tal site (45.53.80 N, 93.65.22 E) at the edge of the 9000 km² Greater Gobi-B Strictly Protected Area (SPA) and International Biosphere Reserve. Subsequent transports were carried out in the following years and to date a total of 89 horses have been transported. In 1997 the first harem group was released into the wild from the adaptation enclosures and in 1999 the first foals were successfully raised in the wild. At present 96 Przewalski's horses live at the Takhiin Tal site, with 87 horses belonging to 6 harems and one bachelor group ranging freely in the Gobi-B National Park. Due to its important symbolic value in Mongolian culture, the Przewalski's horse has become an important vehicle for national park development. The Gobi-B is a cultural landscape and management aims to conserve it as a biosphere reserve in the sense of the IUCN. The vision is the integral protection of the Gobi habitat and the life style of the semi-nomadic herders. Przewalski's horses have also been reintroduced into the Hustai Nuruu National Park and recently 12 Przewalski's horses were brought to Khomiin Tal where they are being held in adaptation enclosures in view of a possible future reintroduction.

For Mongolia three distinct aspects in the anaesthetic management of the Przewalski's horse in Mongolia have to be considered: a.) the capture and transport of animals to Mongolia from abroad and the subsequent translocation within Mongolia; b.) the capture of animals for medical management reasons following injury or disease within a controlled environment at the respective project sites; and c.) capture of horses in the wild for the placement of radio-telemetry devices or the collection of biomedical materials.

Due to the distance and limited accessibility of the respective project sites (especially Takhiin Tal and Khomiin Tal) the transport of wild horses represents a general logistic challenge. Figure 1 gives an overview of the essential steps in planning and implementing a transport to the Takhiin Tal site. The process is evaluated at each step and allows for adaptations if they become necessary. Essential "plan B" scenarios have to be developed for various phases. This is especially important for the final phase as bad weather could hinder landings on the desert airstrips at Takhiin Tal and Khomiin Tal. During the pre-shipment phase various procedures need to be completed: veterinary examinations, sample collection, freeze branding and therapeutic actions. These need to be carried out to fulfil the various authorities' requests and also to fulfil IUCN (IUCN 1998) and project guidelines (Walzer et al., 2000). The various procedures that need to be taken into consideration at this phase have been published previously (Walzer et al., 2004). Over the years specific techniques and methods have been developed in order to facilitate loading of the horses. For the first time in 2002 non-chemical capture and crating was possible for the majority of the horses. The system was based on training each individual horse to feed in large crates. These crates could subsequently be closed using a remote system and the horses moved into the smaller transport crates. In 2005 this system was used successfully in Takhiin Tal in order to move eight horses into the west of the Gobi B strictly protected area.

Chemical restraint and capture. In addition to this non-chemical capture method, chemical restraint and anaesthesia of horses has been markedly refined over the years (Walzer, 2003). The agent of choice for wild equid immobilization and anaesthesia is the potent opiate ethorphine. The opiates interact in the central nervous system (CNS) with stereo-specific and saturable receptors (Kreeger

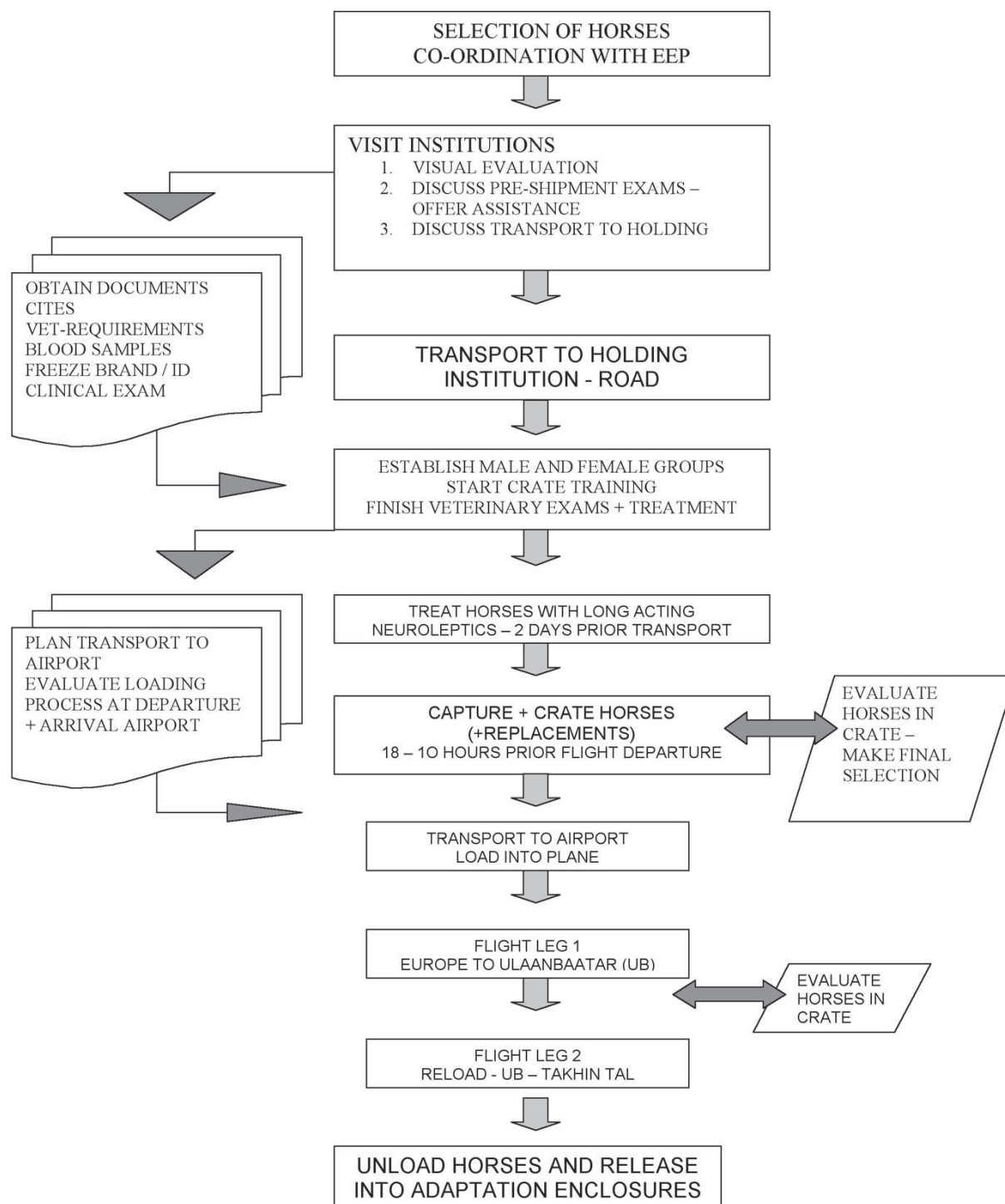


Figure 1. Flow chart showing the most important steps in the transportation process.

et al., 2002). Various receptors have been identified. These are classified as kappa, delta, sigma and mu receptors. A major advantage in the use of the opiates is the specific opiate antagonists that allow for the complete reversal of the anaesthetic effects. Whereas some agents can be classed as sole antagonists (e.g. naltrexone) others have agonist-antagonist properties (e.g. diphrenorphine).

The opiate ethorphine is an analogue of thebaine and is in humans 500 times more potent than morphine (Jasinski et al., 1975; Kreeger et al., 2002). Ethorphine at 2.45 mg / ml is available in Europe and many other parts of the world in combination with acepromazine 10 mg / ml (Large Animal Immobilon, C-Vet Veterinary Products, Leyland, UK). Furthermore ethorphine is available as a

mono substance at 4.9 mg / ml and 9.8 mg / ml (M99, Vericore Ltd., Dundee, Scotland). All products are supplied in a container together with the antidote diprenorphine or M5050 in the respective adequate dosages. In North America, due to the unavailability of ethorphine, a similar even more potent opiate, carfentanil (Wildlife Pharmaceuticals, USA) has been used extensively in equids. However, the effects of carfentanil cannot be equated with those of ethorphine as the procedure is markedly rougher with significant muscle contractions (Morris, 1992). In the past years several additional non-narcotic immobilization protocols have been developed and used more or less successfully in wild equids (Matthews, 1995; Morris, 1992; Vitaud, 1993). For prolonged procedures intubation and inhalation anaesthesia with isoflurane or halothane is recommended.

Specifically in the Przewalski's horse these authors presently recommend a combination of the opiate ethorphine (M99, C-Vet Veterinary Products, Lancs, UK), the sedative alpha2 agonist detomidine-HCl (Domosedan, Orion Corp. Farnos Finland) and the mixed antagonist-agonist opioid butorphanol (Torbugesic, Fort Dodge Animal Health, Iowa, USA). Detomidine acts on the alpha2-adrenergic receptors where it inhibits the release of norepinephrine. Butorphanol is a mu-opioid receptor antagonist that alleviates the marked respiratory depression induced by the ethorphine at the mu-receptor and potentiates the sedative effect at the kappa and sigma receptors. Furthermore this combination has significantly limited the ethorphine specific pacing which greatly reduces the distance a horse travels after darting. This is particularly important in the open steppe habitat where horses darted without the addition of butorphanol can cover several kilometres before becoming recumbent. However the combination still allows for "walk-in" crate loading. The recommended dosage for a healthy, wild adult Przewalski's horse is 2.5-3.0 mg ethorphine, 10 mg detomidine and 10 mg butorphanol. Ethorphine is reversed with the opioid antagonist naltrexone (Trexonil, Wildlife Laboratories Inc., Fort Collins, Colorado, USA) that has a longer half-life than the standard antagonist-agonist diprenorphine (Revivon, C-Vet Veterinary Products, Lancs, UK) and eliminates in- and post-transport renarcotization. Renarcotization is an effect that occurs when using opioids. Several hours after antagonist application the animals, once again

comes under the influence of the opioid agonist (Kreeger et al., 2002). Especially in horses captured in the wild this effect could be fatal as it potentially makes an individual more prone to predation and injury. However, it is important to note that due to the long half-life a subsequent anaesthesia induction with ethorphine (or any other opioid), in case of emergency, would not be possible and an alternative method (e.g. the alpha2-agonist medetomidine and ketamine) needs to be considered.

In crated animals the drugs are easily applied with a jab-stick or standard syringe with an attached butterfly-type peripheral venous catheter that allows for evasive animal movement. However, when the drugs need to be applied over a greater distance specific remote delivery systems need to be used. For an excellent review of the various available systems the reader is referred to Kreeger et al. (2002). These authors recommend the use of a CO2 propelled dart gun such as the Daninject JM model (Danimject JM™, Wildlife Pharmaceuticals, Fort Collins, CO 80524, USA). This type of gun is considered more versatile when compared to models that use gas generated from .22 cal blank cartridges. When working in the wild these authors prefer to use new 3 ml darts discharged by expanding compressed air (Danimject, Wildlife Pharmaceuticals, Fort Collins, CO 80524, USA). Old darts are not used, as these are never as accurate. By shortening the dart stabilizers to 3 cm the effective range is 80 meters under ideal conditions (Lengger et al., 2002). However, this distance is significantly reduced in the windy conditions commonly encountered in the Gobi region. A sufficiently long dart needle of 55 mm is required to safely dart a wild horse during the summer and fall seasons due to significant layers of fat in the rump region. The use of wire barbs or collars on the needle to securely retain the dart in the animal is recommended in order to enable complete drug expulsion. Once an animal is successfully darted one should attempt to keep it in sight. However, it is very important at this stage to not disturb the animal any further by chasing it or approaching before the drugs have taken full effect. Once the animal has become recumbent, an approach on foot from behind and immediate fixation of the head is recommended (see Figure 2). Be aware that in the first few minutes of recumbency the animal may become aroused by voices or loud noises and attempt to rise and flee further.

Anaesthesia monitoring should be implemented as soon as the animal is fixed. Sequential rectal temperature measurements, thorax excursion to determine breathes per minute and auscultation for heart rate is the absolute bare minimum in anaesthesia monitoring. Relative percent oxyhemoglobin saturation measured with a battery-powered pulse oximeter (e.g. Nellcor NP-20, Nellcor Incorporated, Pleasanton, California, USA) is an

extremely useful tool to determine anaesthesia depth and progression.

Due to the general lack of cover in the Gobi area Przewalski's horses are extremely difficult to approach in the field. During the past years we have employed various methods to get within shooting range such as approaching on a motorcycle and horseback or waiting at water points. Using the protocol described above we have been



Figure 2. Once an equid has become recumbent it is essential to fix the head to prevent attempts at rising. This is best accomplished by placing weight on the maxilla.

able to successfully anaesthetize 12 horses in the wild and additionally have carried out approximately 35 procedures in the very large adaptation enclosures at Takhin Tal. Initial effects were noticed after 3-5 minutes when the animal exhibited a stiff, high stepping gait and became ataxic. With the exception of 3 cases, where drug application was partial, induction to lateral recumbency occurred within 5-10 minutes. Procedures lasted on average 35 minutes. Following intravenous (IV) antagonist application anaesthesia was smoothly reversed without any signs of excitement and the animals were back on their feet within 2 minutes. It is important to note that the head of all equids should be fixed to the ground as long as possible following antagonist application to prevent premature uncoordinated attempts at getting up, as these could result in injury (see figure 2).

Long-Acting Neuroleptics. When long-term sedation is required, as during a transport process, this is best achieved using one of the long acting neuroleptics (LAN). This group of drugs can be used to reduce anxiety and stress during long-distance translocation, and for reintroduction into novel enclosures and habitats. As a result of the delayed absorption of the neuroleptics it is prudent to combine a long acting tranquillizer with a short acting analogue (Ebedes, 1992). Haloperidol belongs to the butyrophenone group of neuroleptics with a longer duration of activity. It is available in Europe as a 5 mg/ml injectable solution (Haldol, Janssen-Cilag, Vienna, Austria) that can be applied intramuscularly (IM) and IV. Furthermore 1 mg and 10 mg tablet forms (Haldol, Janssen-Cilag, Vienna, Austria) for oral application are also available. It is important to note that in Europe,

haloperidol is also available as a decanoate ester (Haloperidol decanoate, Janssen-Cilag, Vienna, Austria) an oily form that results in a long-term deposit. Haloperidol decanoate results in a prolonged sedation of up to 25 days and its effects are generally unsatisfactory due to adverse side effects such as inappetence and central nervous system symptoms (Swan, 1993). Zuclopenthixol acetate is a thioxanthene similar to the phenothiazine group of tranquilizers. Through esterification with the acetate and dissolution in a vegetable oil, absorption and duration has been extended. It is available as 50 mg / ml injectable solution (Ciatyl-Z-Accuphase, Bayer, Leverkusen, Germany). Similar to haloperidol, zuclopenthixol is also available in some countries as a decanoate ester, again the duration is extended, but once again the effect seems inadequate (Swan, 1993). Perphenazine is a phenothiazine derivative with a piperazine side chain. It is available as a 100 mg / ml injectable solution in the enanthate ester form, dissolved in sesame oil (Decentan Depot, Merck KGaA, Darmstadt, Germany). When compared to haloperidol and zuclopenthixol, perphenazine has a markedly prolonged duration of action that can subsist for 10 days. These neuroleptics have been used successfully in various zebra species (Swan, 1993) and in the Przewalski's horse (Walzer et al., 2000). Extrapyramidal symptoms (EPS) a neurological side effect causes a variety of symptoms, e.g. involuntary movements, tremors, changes in breathing and heart rate and inappetence have been recorded as the most important side effects of LAN. The EPS can be treated with biperidine (Akineton, Knoll, South Africa) and Diazepam (Valium, Roche, Switzerland). The use of long acting neuroleptics has greatly facilitated the in-crate phase during flight and re-loading of Przewalski's horses. These authors presently recommend treatment with a combination of 0.2 - 0.3 mg/kg haloperidol (Haldol, Janssen-Cilag, Vienna, Austria) and 150-200 mg/adult horse perphenazine (Decentan-Depot, Merck KGaA, Darmstadt, Germany). It is important to carry out this treatment at least 12-24 hours prior to transport or anticipated stressor influence.

Mongolian wild ass (*E. hemionus*). The Gobi area of Mongolia is home to an estimated 20,000 Asiatic wild asses, (khulan or dziggetai in Mongolian); (Reading et al., 2001; Feh et al., 2001; Ministry of Nature and Environment of Mongolia, 2003; Kaczensky & Ganbaatar unpublished

data). In the IUCN Equid Action Plan the status of *Equus hemionus* is qualified as "insufficiently known" and the species is listed as Vulnerable (Feh et al., 2002). Most probably no more than 5,000 individuals remain outside of Mongolia and China and therefore Mongolia is a globally important stronghold of the Asiatic wild ass (Feh et al., 2002). In Mongolia the khulan has been fully protected since 1953. It is listed in Appendix I of the Convention on International Trade of Endangered Species (CITES) and in 2002 was included in Appendix II of the Convention of Migratory Species (Bonn Convention, CMS, 2002). However, due to human population growth in conjunction with severe winters in the past years (United Nations Disaster Management Team, 2000), the occurrences of herder - khulan conflicts appear on the increase. Competition for pastures and water and poaching for meat seem to be increasingly becoming a problem in Mongolia. For some parts of the local population, wild ass meat seems to provide a substitute or even a cheap alternative protein source to meat from domestic animals (Kaczensky & Ganbaatar unpublished data). In 2005, a national survey based on questionnaires suggested that up to 2,000 wild asses might be poached each year throughout their distribution range in Mongolia (Wingard & Zahler, 2006). Moreover, political changes in the early 1990's forced urban populations to return to nomadic land use, resulting in a sharp increase in human- and livestock numbers in many rural areas (Fernandez-Gimenez, 1999; Bedunah & Schmidt, 2004; Mearns, 2004).

In June 2002 we initiated a khulan project. In order to monitor movement patterns and habitat use we captured 16 and equipped 14 free-ranging Asiatic wild asses with ARGOS and GPS-ARGOS satellite collars in the Great Gobi B Strictly Protected Area (7 animals) and the south Gobi in the Umngovi and Dorngovi aimags (9 animals) (additional project information at www.wildvet.at and <http://www.waza.org/conservation/projects/>).

Field capture procedures. The Mongolian khulan is extremely skittish – most probably due to poaching activities – and in some areas flees human presence at several kilometers distance (e.g. in Great Gobi B SPA). We have employed three distinct techniques to capture this species in the wild. In the summers of 2002 and 2005 we used a modified high pressure CO₂ dart gun (Daninject JM™, Wildlife Pharmaceuticals, Fort Collins, CO 80524, USA) from a pre-placed hide, 60-80 me-

ters distant from water points. This method was especially useful in the south Gobi as the khulan are readily approached in the area. Some water points additionally offer good cover which allows for a shooting distance of 40-55 meters. If possible, it is a distinct advantage to take a position high above the water point, as the animals appear to never look up. As open water is lacking in large parts of the distribution range in the south Gobi the khulan must dig to a depth of approximately 45 cm to access ground water. At this time it is very difficult for animal to see movements in its vicinity. Furthermore the use of ground water increases the amount of time the animals have to remain stationary which additionally greatly facilitates darting.

In 2003 and 2005 we also employed a chase method where the khulan was darted from a moving jeep. This method has been used to collar a wild Bactrian camel (*Camelus bactrianus ferus*) and is traditionally employed by khulan poachers with 12 gauge shotguns (Blumer et al., 2002). When using the local UAZ jeeps it is important to

remove the window from the passenger side and to provide seatbelts for the driver and shooter. If using the Daninject JM CO₂ dart gun, a short 4 cm barrel can be used instead of the standard barrel as this greatly facilitates movement in the jeep.

Once an animal is identified, it is chased till the jeep is able to approach within approximately 10-15 meters on a parallel track (see Figure 3). It is then easily darted in the rump musculature using standard pressure settings. It is essential to define a chase cut off time before the procedure is started. Our experience has shown that a cut off time of 15 minutes is adequate. To date we have captured 5 animals with this very time-efficient method. The shortest chase time was 2 minutes and the longest 13 minutes. In all cases induction was extremely rapid and smooth (4-8 minutes) and body temperature was below 40°C. A severe limitation to this method is that one is only able to capture males or juveniles without foals. A chase of a female with a foal would result in (permanent) separation of the young from the mare and is therefore unacceptable.



Figure 3. A khulan is chased until the jeep is able to approach within approximately 10-15 meters on a parallel track. It is then easily darted in the rump musculature using standard pressure settings.

Finally we have used a video-enabled remote controlled CO₂ gun (Walzer & Boegel, 2003) at several water points in attempts to capture khulan in 2003 and 2005. To date this method has not been successful for khulans mainly due to the abundance of water in the areas it was employed. In the authors view this method has great potential in areas with small waterholes that the animals have to visit (e.g. Great Gobi A SPA).

Chemical capture. In all khulan procedures anaesthesia was induced with a single 3 ml dart containing a combination of 4.4 mg Etorphine (M99, C-Vet Veterinary Products, Lancs, UK), 10 mg Detomidine-HCl (Domosedan, Orion Corp. Farnos Finland) and 10 mg Buthorphanol (Torbugesic, Fort Dodge Animal Health, Iowa, USA). Anaesthesia was reversed in the first seven cases with an IV combination of 200 mg Naltrexone (Trexonil Wildlife Laboratories Inc., Fort Collins, Colorado, USA) and the alpha₂-antagonist 20 mg Atipamezole (Antisedan, Orion Corp. Farnos Finland). Reversal was rapid and generally smooth but some signs of excitation related to the collar – head shaking - were noted. In the south Gobi the opioid antagonist-agonist diprenorphine (Reviron, C-Vet Veterinary Products, Lancs, UK) was

used. This eliminated head shaking and provided a smoother reversal. All animals were standing and alert approximately two minutes following administration of the antagonists (see Figure 4). Presently we have captured 16 and out-fitted 14 khulans in Mongolia. The jeep-chase method proved the most efficient in our primary study area. However, this method is not necessarily applicable to all areas.

The use of LANs in asses has not been reported to date. Though the dosage rate is reported to vary between the various ungulate species, it appears that the dosages within the equids are similar and therefore use of the zebra and Przewalski's horse dosages adapted for body weight seem acceptable for potential use in asses.

Human exposure to capture drugs. Using potent capture drugs bears the inherent risk of human injury. Though prevention is the mainstay in avoiding capture drug related accidents it is important to establish a protocol to deal with eventual problems. Accidental capture drug injection is always to be considered an emergency that will require calm, prompt and organized action (Walzer & Fahlman, 2006). Be aware that the legal implications of administrating medical treatment to



Figure 4. GPS-Argos collared khulan, standing and alert approximately two minutes after the IV administration of the opioid antagonist-agonist diprenorphine (M5050).

accident victims by persons that are not qualified vary from country to country (Morkel, 1993). As a bare minimum in the field the following precautions should be adhered to: use capture drugs only with a second, trained person present; respect the potency of the drugs and do not take chances and underestimate a dangerous situation; never work with opioid drugs without having the human antidote in the emergency kit (see table 1); limit personnel present when working with the drugs. See Morkel (1993) for an excellent review on dealing with drug related accidents in the field.

Conclusions

The capture and anaesthesia of Przewalski's horses and khulan in Mongolia, with the subsequent placement of radio-telemetry equipment and the collection of biomedical samples can make a substantial contribution to the knowledge and conservation of these two endangered species. In order for procedures to be safe for both the equids and humans involved, a significant amount of veterinary knowledge and training is required. Capture and general anaesthesia can only be as safe as the acquired skills and knowledge of the person performing the procedure allow. These in combination with an adequate anaesthetic protocol determine the outcome. The described protocols using species-specific combinations of the potent opiate ethorphine in combination with the alpha2-agonist detomidine and the opioid agonist-antagonist butorphanol provide rapid and safe anaesthesia for the Przewalski's horse and the Asiatic wild ass. The use of the specific opioid antagonists provides a smooth and rapid reversal of the anaesthetic effects.

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