Immobilization of confined European bison with a medetomidine – zolazepam/tiletamine – butorphanol combination using readily available veterinary medicinal products

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Abstract: Various protocols have been used to immobilize European bison in recent years. Nevertheless, availability, human toxicity, animal side effects, costs and legal obstacles continue to cause problems for breeders of the European bison. We have therefore applied an immobilization protocol that is essentially based on medetomidine, zolazepam/tiletamine and butorphanol using products, which are readily available on the market. The basic prerequisite for the application is the ability to separate individual animals and apply larger volumes using a jab stick. This paper shows the results of 21 immobilizations of animals that were intended for transport to other breeding enclosures or for reintroduction projects between 2019 and 2021. The combination is suitable for most immobilization reasons and meets all requirements for safe and animal-friendly immobilization.

Keywords: European bison, immobilization, medetomidine, zolazepam/tiletamine, butorphanol, jab stick

Introduction

Breeding and keeping European bison (*Bison bonasus*) requires repeated veterinary interventions for which reliable immobilization is a basic prerequisite. The monitoring of animal health as well as veterinary regulations for the exchange, transnational transport or reintroduction require blood samples to be taken, TB tests to be carried out, markings to be made, transmitters to be attached, etc. (Olech & Perzanowski 2022). A low-risk and animal-friendly implementation of immobilization with medicines available at all times is therefore essential for the successful conservation breeding of European bison. There have been reliable protocols for the immobilization of the European, as well as the American bison, for quite some time. The protocols are mainly based on opioids, α 2-adreneergic agonists or dissociative anaesthetics or combinations thereof. Commonly used agents are etorphine, xylazine, ket-amine, medetomidine or zolazepam/tiletamine (Krasiński *et al.* 1982; Wiesner *et al.* 1982; Kania *et al.* 1985; Caulkett *et al.* 2000; Bielecki *et al.* 2005; Krzysiak & Larska 2014; Wolfe *et al.* 2017; Harms *et al.* 2018; Didkowska *et al.* 2022). Unfortunately, the drugs required are not always reliably available on the market in the needed concentration. Besides the difficult sourcing, they are often difficult to dose, very expensive, dangerous for the user and – at least some of them – associated with numerous side effects on the animals. Especially the ultrapotent opioid etorphine, which is one of these disadvantages (Milnes *et al.* 2022).

We have therefore developed an immobilization protocol that is based on ready-to-use products that are regularly applied to various other species and are therefore easily available. Each of these drugs has been used in a variety of anaesthesia protocols of wild and domestic species as well as immobilization of European or American bison. Nevertheless, the protocol described here requires the application of larger volumes and only partially takes into account the requirements of immobilisation of free roaming animals (Larska & Krzysiak 2019). Our study was realized in captive condition with easy access to animals.

For the initiation of immobilization, we use the following agents:

Medetomidine is a potent α 2-adreneergic agonist, generally regarded as sedative-hypnotic and is most commonly administered to induce sedation (Short 1987). Its analgesic and sedative properties supports a smooth induction and muscle relaxation during immobilization (Wolfe *et al.* 2017). α 2-adrenergic receptor agonists are commonly used in horses in combination with butorphanol, which has a.o. synergistic analgesic properties (Clutton 2010). The combination enhances and prolongs analgesia and has been used for some years to make horses easier to handle during veterinary procedures. Further on sedation is more reliable and animals are less responsive to external stimuli when an α 2-adrenergic agonist is given with butorphanol or another opioid (Tranquilli *et al.* 1983; Bush *et al.* 2012). Medetomidine can be antagonized with atipamezole (Wolfe *et al.* 2017).

Butorphanol is a mixed agonist-antagonist opioid. In veterinary medicine, it is widely used as a sedative and analgesic in dogs, cats and horses (Bush *et al.*

2012). It is less potent than other opioids used for (wildlife) immobilization, but has reduced respiratory and cardiovascular side effects (Harms *et al.* 2018). The drug is commonly used in horses with α 2-agonists (Clarke & Paton 1988; Kim *et al.* 2021) and with a variety of sedatives and tranquilizers in other domestic or wildlife species for sedation, anaesthesia or neuroleptanalgesia. Butorphanol combined with α 2-adrenergic agonists may produce safer anaesthesia procedures by minimizing many adverse effects. These combinations use lower doses of each agent and use the synergistic effects of the various drugs in the combination (Bush *et al.* 2012). Butorphanol can be antagonized with naltrexone (Mich *et al.* 2008).

The phencyclidine derivative tiletamine induces a dissociative cataleptoid state, somatic analgesia, and altered consciousness. The patient is immobilized but not relaxed or fully unconscious, and analgesia is incomplete. Pharmacodynamics of tiletamine are similar to those of ketamine, but tiletamine is more potent and acts longer (Lin *et al.* 1993). It is available for use only in combination with the benzodiazepine zolazepam in a 1:1 ratio. Zolazepam is a benzodiazepine agonist producing sedative, anxiolytic, muscle relaxant, and anticonvulsant effects in most animals. Thus, the agents have complementary effects. This combination provides slight cardiovascular stimulation, causing the heart rate to increase (Riebold 2007).

Combination of Tiletamin-Zoletil with the α 2-agonist medetomidine will enhance analgesia, and decrease recovery times following antagonism. These combinations will produce a better quality of immobilization and recovery, particularly in ungulates (Caulkett & Arnemo 2007). Generally, the addition of medetomidine will greatly decrease Tiletamin-Zoletil requirements (Bush *et al.* 2012).

Material and methods

21 animals were immobilized in this study. The animals were intended for transport to other breeding enclosures or for reintroduction projects. All of them were in good physical condition and, as far as could be assessed, clinically healthy.

The animals to be immobilized were separated from the herd via a system of gates integrated into the enclosure (Berneisch 2021). Each animal was isolated in a stall (floor area: 7,3m x 8,4m) for at least 2 days until it was ensured that it had calmed down and got used to the new environment. The animal was not fed for 6-12 hours before the injection. Before treatment, two or three experienced animal attendants estimated the weight of the animal visually. To improve the estimation, the exact weight of some animals was determined after loading on a calibrated truck scale. For the injection, each animal was crowded into a small treatment box (floor area: 3,2m x 1,1m; height 2,7m) with sliding doors (Berneisch 2021).

With the exception of two animals, all animals were immediately released from the treatment box back into the stall after application to ensure undisturbed laying down.

The substances were applied from an upper position into the neck or shoulder muscles with an automatically discharging 2m long jab stick, (DAN-INJECT Smith GmbH, Walsrode / Germany) with the corresponding equipment (10 ml Nylon syringe, specially produced injection needles 3.0 x 40mm). The antagonist drugs were applied into the jugular vein.

The following animal drugs were used:

Cepetor® (CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany) or Sedin® (Alvetra GmbH, Neumünster, Germany) containing 1.0 mg/ml medetomidine hydrochloride. Zoletil® (Virbac Tierarzneimittel GmbH, Bad Oldesloe, Germany) containing 50 mg/ml tiletamine hydrochloride and 50 mg/ml zolazepam hydrochloride after reconstition with 5.0 ml diluent. Alvegesic® (Alvetra GmbH, Neumünster, Germany) or Butorgesic® (CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany) containing 10.0 mg/ml Butorphanol. Revertor® (CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany) or Nosedorm® (alfavet Tierarzneimittel GmbH, Neumünster, Germany) containing 5.0 mg/ml Atipamezole hydrochloride.

Apart from the first four immobilizations (Tabl. 1), the following dosages were used per 10 kg estimated live weight: 0.6 ml Cepetor® or Sedin®, 0.12 ml Zoletil®, 0.20 ml Alvegesic® or Butorgesic® and for antagonisation 0.36 ml Revertor® or Nosedorm®.

After recumbency, sufficient immobilization and vital functions were checked and the necessary measures (attaching GPS collars, TB testing, several sampling etc.) were carried out rapidly by a trained team. The animals were not moved further or relocated and were always treated at the place where they went down. Time was measured between the last immobilizing injection and sternal recumbency, as well as between the application of the antagonist and the spontaneous standing up of the animal. Peculiarities and reactions of the animal during the whole procedure were recorded.

After the procedure, they remained in the individual box under observation for about 24 hours before being moved to a separate enclosure where they remained until loading. They were loaded directly from the treatment box without further immobilization.

Results

A total of 21 immobilizations were performed using the described procedure. By fixing the animal to be treated in a box and using a jab stick, the required volumes could be applied without any problems even if several injections were necessary. In all cases, the animals reacted to the injection with varying degrees of defensive movements (kicking, rearing, horn thrust). Due to a rapid evasive movement of the animal, in one case only an unknown part of the required amount of medetomidine could be administered, so that an estimated subsequent re-dosing was necessary. After the first four animals showed a very good depth of anaesthesia but repeated muscle twitching after laying down, the medetomidine dose administered was slightly reduced and the butorphanol dose slightly increased. The selected dosage (Tabl. 1) was well effective in all cases, so that all manipulations could be performed without any problems. No anaesthetic incidents occurred. At no time, (un-) coordinated defensive movements of the recumbent animals endangered the treating staff.

In the time between application and laying down, no negative effects were observed in any animal. The animals became calmer and moved more slowly. Laying down was coordinated in all cases. No excitations, strong restlessness, increased movements or evident ataxic events occurred during this phase. The recorded times are summarised in table 1. The time between last injection and laying ranged from 2 to 15 minutes (mean 5.8 ± 3.4). Younger and male animals tended to lay down faster. Depending on the measures performed, the manipulation time ranged from 9 to 31 minutes (mean 19.1 ± 6.0). The animals stood up voluntarily, i.e. without human intervention, 2 to 43 minutes (mean 9.4 ± 12.5) after application of the α 2-antagonist (Tabl. 1).

No side effects were observed in any animal after immobilization. No further treatments were required and all animals reached their destination safe and sound.

Discussion

Although various protocols for immobilizing European bison have been in use for many years (Krasiński *et al.* 1982; Wiesner *et al.* 1982; Bielecki *et al.* 2005; Krzysiak & Larska 2014; Didkowska *et al.* 2022) availability, human toxicity, animal side effects, costs and legal obstacles continue to cause significant problems. An immobilization procedure for the most common routine procedures (e.g. blood sampling, TB-testing, marking), based on readily available veterinary medicinal products which is not dangerous and can be used Table 1. Details of the immobilized animals, injected volumes of readily available veterinary medicinal products containing medetomidine (Cepetor® or Sedin®), tiletamine/zolazepam (Zoletil®), butorphanol (Alvegesic® or Butorgesic®), atipamezole (Revertor® or Nosedorm®) and time to onset of drug effect, duration of treatment and antagonisation

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and
Handling | 17 | 15 | 10 | 6 | 23 | 24 | 27

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| Atipamezole
(5.0 mg/ml) | 20.0 | 20.0 | 20.0 | 23.0 | 6.1 | 7.2 | 7.9

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 | 8.0 | 15.1 | 10.8 | 12.6 | 8.0 |
| Butorphanol
(10 mg/ml) | 5.0 | 8.0 | 5.0 | 5.0 | 3.5 | 4.1 | 4.5

 | 8.2 | 5.6 | 8.2
 | 3.1 | 7.2 | 4.4
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 | 4.5 | 8.6 | 6.1 | 7.2 | 4.5 |
| Tiletamine/
Zolazepam
(50/50mg/ml) | 4.8 | 5.0 | 4.8 | 4.8 | 2.0 | 2.4 | 2.6

 | 4.8 | 3.4 | 4.8
 | 1.8 | 4.8 | 2.0
 | 1.8 | 3.0 | 3.8
 | 2.6 | 5.0 | 3.6 | 4.2 | 2.6 |
| Medetomidine
(1.0 mg/ml) | 30.0 | 30.0 | 30.0 | 30.0 | 10.2 | 12.0 | 13.2

 | 24.0 | 16.8 | 24.0
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 | 13.2 | 25.2 | 18.0 | 21.0 | 13.2 |
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| est.
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(kg) | 400 | 450 | 450 | 450 | 170 | 200 | 220

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 | 150 | 350 | 170
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 | 220 | 420 | 300 | 350 | 220 |
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(yrs) | 3.7 | 8.7 | 11.1 | 16.9 | 1.9 | 3.0 | 2.8

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 | 1.9 | 2.7 | 3.2
 | 2.1 | 4.6 | 3.4 | 4.2 | 2.2 |
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reliably, has been lacking. This might be one reason why no or too few essential health parameters are examined when European bison are exchanged among the individual breeding enclosures. This contradicts the current recommendations (Olech & Perzanowski 2022) and is hardly an acceptable risk from a veterinary or biosecurity point of view, given the continuing threat to the species. In addition, in many breeding enclosures there is still a lack of possibilities to separate animals to be immobilized, to keep them isolated and then to immobilize them in a non-agitated state (Poettinger 2011; Berneisch 2021). In many cases, immobilization in the enclosure does not differ from capturing and immobilizing wild animals. The same applies to transport for the exchange of breeding animals.

An essential prerequisite of the immobilization procedure described here is the possibility to separate individual animals from the herd and to keep them separated for at least few days. This method allows access to the animal, makes the use of a blowpipe and/or an injection rifle obsolete, and thus considers aspects of animal welfare (Berneisch 2021). Finally, it is possible to immobilize non-excited, calm and unfed animals that are accustomed to the environment. Additional stress or risks such as capture myopathy (Breed *et al.* 2019) or aspiration pneumonia can be minimised (Arnemo *et al.* 2014). The latter is also reduced by the fact that the animals do not have to be moved for further manipulation after they have been placed in the stall. Overall, the standardised procedures in a defined environment reduce the risk of incidents during immobilization.

In the 21 cases described here, application by means of a jab stick was feasible without any problems. The system injects up to 10 ml in one second or less on contact, so that even large volumes can be administered. This allows the use of commercially available medicines that are readily available and pose little danger to the user compared to the previously used highly concentrated substances.

The individual active ingredients have already been used in various protocols for American bison and have proven to be effective (Caulkett *et al.* 2000; Wolfe *et al.* 2017; Harms *et al.* 2018). The combination presented here has various synergistic effects, so that the dose of individual active substances as well as negative effects can be reduced. In particular, it is known that the combination butorphanol with α 2-adrenergic agonists decreases many adverse effects and lowers doses of each agent. Furthermore combinations of α 2-adrenergic agonists with tiletamine/zoletil will produce a better quality of immobilization and recovery and will greatly decrease Tiletamin-Zoletil requirements (Caulkett & Arnemo 2007; Riebold 2007; Bush *et al.* 2012). Accordingly, no adverse effects were observed in the animals until they went down. In particular, the side effects known after the use of etorphine, such as evident ataxic events and high stepping gait was not observable. The effect of immobilization proved to be extremely reliable and allowed the necessary measures to be carried out without any problems in all cases. All European bison recovered smoothly after reversal with i.v administration of atipamazole.

The duration from application to recumbancy, averaging 5.8 ± 3.4 minutes, was comparable to other studies (Caulkett *et al.* 2000; Harms *et al.* 2018). We could not determine the reasons for the considerable differences from 2 min to 15 min. Even though the same body region was always applied, it can be assumed that differences in local absorption contributed to this observation.

After i.v. application of atipamazole, 16 of the 21 animals stood without further action in less than 10 min, another two animals in less than 15 min. Three animals took up to 43 minutes to get up. However, a good effect of the antagonist was also observed in these animals. Only the standing up of the animals was delayed. The criterion "standing up" for reliable antagonism is a disadvantage here. It nevertheless remains superior to all other criteria in field trials due to its distinctiveness.

In few cases physiologic parameters associated with immobilization, including heart and respiration rate as well as oxygen saturation via pulse oximetry were recorded additionally. Although the results will be presented later, some effects such as hypoventilation and hypoxemia were evident. With the relatively short handling time of 9 to 30 minutes described here, we do not consider the effect to be of particular clinical concern. In the case of prolonged immobilization, immobilization of agitated or severely ill animals, further monitoring and the administration of oxygen may be necessary.

As the animals got up quickly after the administration of atipamezole, there was no need to antagonise butorphanol with naltrexone. However, in some cases (weak or ill animals, clinically evidenced respiratory depression, etc.) it could bring further benefits.

Even though studies are still needed to determine further anaesthetic parameters, we assume based on our observations that the combination of medetomidine – zolazepam/tiletamine – butorphanol – atipamezole is suitable for most immobilization reasons and meets all requirements for safe and animal-friendly immobilization.

Acknowledgements

We thank Paula Fletcher, Annette Reindl, Gisela Schütz, Michael Strobel & Alfred Wiedmann for patience, assistance, suggestions, support and dedicated care of the animals.

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Immobilizacja żubrów w zagrodzie przy użyciu połączonych, łatwo dostępnych środków: medetomidiny – zolazepam/tiletaminy – butorfanolu

Streszczenie: W ostatnich latach stosowano różne metodyki służące unieruchamianiu żubrów. Rozwiązania najlepsze jednak są problematyczne dla hodowców z powodu dostępności, toksyczności dla ludzi, skutków ubocznych dla zwierząt, kosztów i przeszkód prawnych. Dlatego w niniejszej pracy zastosowano protokół immobilizacji, który zasadniczo opiera się na medetomidynie, zolazepamie/tyletaminie i butorfanolu – produktów, które są łatwo dostępne na rynku. Podstawowym warunkiem aplikacji większych objętości przy użyciu jab-stick jest możliwość oddzielania poszczególnych zwierząt. W pracy przedstawiono wyniki unieruchomień 21 zwierząt, które były przeznaczone do transportu do innych zagród lub do projektów reintrodukcji w latach 2019–2021. Kombinacja leków jest odpowiednia i spełnia wszystkie wymogi bezpiecznego dla zwierząt unieruchomienia.