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Abstract One-hundred and fifty-five free-ranging Northern chamois *(Rupicapra rupicapra)* were anaesthetised in the course of a restocking programme using xylazine plus ketamine. Mean±SD dosages for xylazine and ketamine were 1.9±0.5 and 2.2±0.7 mg/kg, respectively. In 57 chamois, sedation was reversed using 0.3 ±0.1 mg/kg atipamezole. Although all the anaesthetic dosages tested immobilised free-ranging Northern chamois, shorter induction times (4.8±2.6 min), deeper sedation with no reaction to handling in >90% of the animals and quick reversal (4.0±2.7 min) were obtained using 2.5 mg/kg xylazine plus 3.0 mg/kg ketamine reversed with 0.25 mg/ kg atipamezole. Under the conditions of this study, suggested standard doses are 63 mg/animal xylazine plus 76 mg/animal ketamine reversed by 6.3 mg/animal atipamezole. This anaesthetic protocol improves the results from the previous study of Dematteis et al. (Vet Rec 163:184-189, 2008) using xylazine alone.

Keywords: Xylazine, Ketamine, Atipamezole, Chamois, Anaesthesia.

Introduction

Several anaesthetic protocols have been used to immobilise chamois *(Rupicapra* spp.): ketamine plus medetomidine antagonised with atipamezole (Jalanka and Roeken 1990; Walzer et al. 1996, 1998); etorphine plus acepromazine antagonised with diprenorphine (Wiesner et al. 1982); xylazine alone (Bauditz 1972; Gauthier 1993a; Peracino and Bassano 1993; Dematteis et al. 2008); xylazine plus carfentanyl antagonised with naloxone plus yohimbine (Duchamps 1985); xylazine plus fentanyl antagonised with naloxone plus yohimbine (Jensen 1982); xylazine plus ketamine (Wiesner and von Hegel 1985; Fico 1988; Moran et al. 1994; von Hardenberg et al. 2000) and tiletamine plus zolazepam (Chaduc et al. 1993). However, most of these studies did not evaluate physiological responses to treatment, and the suitability of tested drugs was mainly assessed based on observation, experience and anecdotal evidence.

The aim of this retrospective study was to analyse dosages of xylazine-ketamine combination reversed by atipamezole in free-ranging Northern chamois in order to identify a specific and safe anaesthetic protocol. The drugs were analysed for their ability to achieve rapid and deep immobilisation and effective recovery safely. Furthermore, the possibility of administering a standard dosage irrespective of sex and body mass is discussed, since environmental operating conditions in the Alps hinder prompt adaptation of dosage to the characteristics of the individual target (Dematteis et al. 2008).

Xylazine is an alfa-2-adrenergic tranquilizer used to immobilise wildlife both as a sole immobilising agent (Jorgenson et al. 1990, 1991; Haviernick et al. 1998) and in combination with other anaesthetics (Delvaux et al. 1999; Kilpatrick and Spohr 1999; Janovsky et al. 2000). It has a large safety margin since its lethal level is more than ten times the normal dosage (Delvaux et al. 1999). Among its potential side effects, respiratory depression, rumen stasis and bradycardia may appear at high dosages (Jacobsen 1983; Hsu and Shulaw 1984; Jessup et al. 1985; Wallingford et al. 1996). Its half-life is approximately 50 min, but effects may last for 1.5-2 h (Plumb 1999).

Ketamine affects the central nervous system, inducing dissociative anaesthesia (Lin 1996). Desirable characteristics include retention of normal reflex actions such as coughing and swallowing. Muscle rigidity and violent recoveries occur when ketamine is used alone (Lin 1996); therefore, it is frequently used in combination with xylazine (Kilpatrick and Spohr 1999; Telesco and Sovada 2002; Grassman et al. 2004). Ketamine half-life, reported in cattle and other domestic species, is approximately 60 min (Adams 2001).

Atipamezole is a potent and selective alpha-2-adrenoceptor agonist with a competitive nature (Lin 1996; Virtanen 1989) which can antagonise xylazine (Jalanka and Roeken 1990). Currently, there are no effective antagonists for ketamine (Kreeger et al. 2002).

Materials and methods

One-hundred and fifty-five Northern chamois *(Rupicapra rupicapra;* Shackleton and the IUCN/SSC Caprinae Specialist Group 1997) were immobilised in the Maritime Alps Natural Park (44° 14' N, 7°23' E) in the Southwestern Italian Alps, between 1991 and 2003, for restocking purposes. Two or three capture teams operated during the spring (April and May) at 1,000- to 1,300-m elevation. Stone shelters were built within 20-50 m of salt licks to facilitate close approach of the chamois. All chamois were observed for at least 5 min prior to darting; only animals that appeared to be healthy were darted. Following recovery, animals were housed in a dedicated small stable and observed for 4 h to 3 days before the release.

Dart guns (11-mm barrel diameter; G.U.T. 50®, Telin-ject, Sturzelbronn, France) fitted with 3-ml syringes and 1.5x38-mm plain needles were used to inject the anaesthetic mixture, preferably in the upper thigh or in the shoulder blade. Darting distances were determined using a laser distance measurer (Rangemaster LRF 800®, Leica, Solms, Germany).

Free-ranging Northern chamois (7V=191) were immobilised using various dosages of xylazine combined with ketamine; however, only chamois darted with a single dose and for which all pertinent anaesthetic, physiological and biometric parameters were recorded (see below) have been included in this study (7V=155). The effects of xylazine were reversed with atipamezole in 57 of these animals. The combination of xylazine-ketamine was obtained dissolving 500 mg xylazine (Rompun® dry substance, Bayer, Leverkusen, Germany) in 5 ml of sterile water (solution 100 mg/ml) and then mixing 0.2-0.7 ml of this solution to, respectively, 0.8-0.3 ml ketamine (Ketavet® 100, Parke-Davis GmbH, Berlin, Germany, 100 mg/ml). For ballistic matters, a standard volume of 1 ml was administered to each animal, obtaining the dosages of Table 1.

Once captured, animals were placed in right lateral recumbency, hobbled, blindfolded and marked with plastic ear tags. Body mass, ascertained with spring scales to the nearest 500 g, and body measurements were recorded for each animal. Age was estimated by counting horn rings (Schroder and von Elsner-Schack 1985), and animals were classified as juveniles (11 to 24 months) or adults. Females were tested for pregnancy by abdomen palpation. After processing, atipamezole (5 mg/ml; Antisedan®, Orion Corporation Farmos, Turku, Finland) was injected intramuscularly (2.5-13 mg/animal) in 57 chamois. Length of spontaneous recovery from anaesthesia was determined in 82 of the remaining 98 chamois.

Physiological parameters were recorded at two intervals from darting in order to monitor for variations related to capture: (1) immediately after restraint and (2) 30 min after restraint. Respiratory rate (RR), heart rate (HR) and rectal temperature (RT) were measured using, respectively, a clinical phonendoscope and a mercury thermometer. Depth and regularity of breathing, colour of the oral and ocular mucous membranes, capillary backfill time, rumen activity and muscle tone were monitored at various intervals throughout the study. In the event that physiological abnormalities were detected, intravenous atipamezole was on hand to reverse sedation. Postmortem examinations were not performed.

For the purposes of this study, "induction" was defined as the interval from sedative injection to complete immobilisation (head down); "handling" as the interval between induction and the injection of atipamezole; "reversal" as the interval from antagonist administration until the animal was standing and "natural recovery" as the time from the anaesthetic injection until the animal was standing (it applied to animals which were not injected with atipamezole).

The depth of sedation was estimated and animals were categorised into three classes based on reaction to approach of the operators after induction, standard manipulations (tying limbs and blindfolding) and sensitivity to pain (ear tagging). The three classes were: deep sedation (the animal did not react when approached, hobbled, blindfolded and tagged); weak sedation (the animal did not react when approached, but reacted during containment operations and tagging) and light sedation (the animal tried to escape when approached and had to be contained during all manipulations).

Data normality was tested according to the Shapiro-Wilk *W* test (Altman 1991). Student's *t* test or the Mann-Whitney test or the Wilcox signed rank test were used to assess differences between (1) intramuscular vs. thoracic-abdomen xylazine-ketamine administration; (2) drug dosage according to age class; (3) induction time according to age class; (4) induction time based on the three classes of reaction; (5) physiological measures between intervals 1 and 2 and (6) reversal time according to sex and age class. Correlations between xylazine-ketamine dosages and induction time, antidote dosage and reversal time, xylazine dosage and reversal time and between atipamezole/xylazine ratio and reversal time were evaluated determining Pearson's or Spearman's correlation coefficients.

Data are reported as means±SD; statistical significance was set at P<0.05. Statistical analyses were performed using R-2.6.0 software (Ihaka and Gentleman 1996).

Results

One-hundred and fifty-five free-ranging Northern chamois, aged 1-12 years, were immobilised with a xylazine-ketamine mixture, including 66 males (5.1 ±3.1 years) and 89 females (5.3±2.5 years). Mean body mass was 25.2± 4.2 kg: juvenile males *{N=\l)* 19.2±3.2 kg (11-23.5 kg), adult males (N=49) 26.8±3.6 kg (18.6-33 kg), juvenile females *{N=9)* 19.3±3.5 kg (15-26 kg) and adult females (Af=80) 26±3.2 kg (20-33 kg). Forty-eight females were pregnant by abdomen palpation (60% of females older than 2 years); no abortion was recorded during the observation period after recovery or during transport to release sites (10-18 h).

Most chamois (Af=119; 76.8%) were immobilised by intramuscular injection into the rump, hip or thigh; the remaining 36 chamois were darted in the thoracic (12.9%) or abdomen areas (10.3%). Since no differences were observed in induction time, degree of sedation and mortality, between intramuscular and thoracic-abdominal administrations, these data were analysed as a single group.

The chamois were immobilised with xylazine dosage ranging from 0.8 to 3.6 mg/kg (1.9±0.5 mg/kg) plus ketamine dosage ranging from 1.1 to 5.4 mg/kg (2.2± 0.7 mg/kg). Adults (xylazine 1.8±0.47 mg/kg plus ketamine 2.06±0.53 mg/kg) received significantly lower (P<0.001) doses than juveniles (xylazine 2.46±0.58 mg/ kg plus ketamine 2.91 ±0.85 mg/kg).

Mean induction time was 7.2±5.1 min (range, 1-37 min). Induction time in adults was longer than in juveniles (7.5 vs. 5.3; *t=2.9,* P<0.01). The mean dose of xylazine received by the chamois who were immobilised within 10 min (7V=132; 85.2%) was 1.9±0.5 mg/kg, whereas the mean dose of ketamine was 2.2±0.7 mg/kg.

Mean handling time was 71.7±13.4 min.

Table 2 shows the anaesthetic data for Northern chamois according to the depth of sedation.

Small but significant differences were detected for RT and RR measured at interval 1 vs. interval 2: RT1=39.4± 1.1°C and RT2=39.1±1.2°C (7=1320, P=0.02), RR1 = 88.4±28.8 breaths/min and RR2=84.8±26.9 breaths/min (F=1242.5, P=0.03).

Hyperthermia (RT>41°C) was observed in seven chamois (4.5%). Eight animals (5.2%) showed signs of sudden respiratory, cardio-circulatory depression (shallow and quick respirations, hypoxic aspect of mucous membranes, increased capillary backfill time) during handling. Two of these chamois presented respiratory depression after hyperthermia appearance. Minor bloat was observed in other five chamois (3.2%), but rumenal punction was not deemed necessary. No sign of muscle stiffness or rigidity was recorded. Animals showing one or more of these abnormalities were immediately treated with atipamezole. Overall, six animals (3.9%) died (one juvenile and five adults). Four of these animals had shown signs of respiratory or cardio-circulatory depression associated in two cases with hyperthermia. The other two chamois died during induction time due to injuries suffered after a fall from rocks. Chamois with unfavourable outcomes were darted with 2.4±0.7 mg/kg of xylazine (1.6-3.3 mg/kg) plus 2.8±1.2 mg/kg of ketamine (1.6-5.0 mg/kg). No unusually stressful pre-darting conditions of these animals were recorded.

In the attempt to define an optimal anaesthetic protocol for the xylazine/ketamine combination, we considered separately the chamois immobilised with dosages higher than the mean values of both drugs, obtaining a subsample of 25 animals immobilised with a mean dosage of 2.5 mg/kg xylazine plus 3 mg/kg ketamine. The recorded induction time was 4.8± 2.6 min; all animals were immobilised within 10 min and 92% of chamois presented a deep sedation.

Atipamezole (0.3±0.1 mg/kg) was randomly administered to 57 chamois (36.3%; 25 males and 32 females) 81.7± 11.0 min after the xylazine-ketamine injection, except for animals that received a "therapeutic" treatment because of their abnormal vital signs (Af=18). The mean atipamezole/xylazine dose ratio was 1:8.9±3.2. Reversal times varied from 15 s to 19 min (4.0±2.7 min). The remaining 82 chamois (52.9%) recovered naturally in 139.7±10.2min.

Discussion

Behavioural and physiological responses to different anaesthetic regimes may vary widely between species. Consequently, well-designed experimental protocols can provide valuable objective data to inform wildlife operators avoiding unpleasant incidents. In the case of wildlife, however, small sample sizes and casual observations are often the only sources of information; this is the case with chamois. Jalanka and Roeken (1990) published a review on the use of medetomidine-ketamine combination in nondomestic mammals, with recommended dosages for captive Northern

chamois *(N*— 49), and provided a range of induction periods (0.7-11.7 min). The same combination, but with lower dosages, was used by Walzer et al. (1996, 1998), obtaining an effective plan of anaesthesia in 11 free-ranging chamois that showed lateral recumbency on average after 6.25 min. Etorphine-acepromazine, fen-tanyl-xylazine, carfentanyl-xylazine and tiletamine-zola-zepam combinations were successfully used to immobilise captive chamois (Wiesner et al. 1982; Jensen 1982; Duchamps 1985; Chaduc et al. 1993). Detailed information about anaesthetic parameters cannot be derived from the original papers. In addition, even at low doses, opioids (etorphine, fentanyl and carfentanyl) are potent toxic substances in both animals and humans, and extra care and concentration are required by operators when working with these drugs (Kreeger et al. 2002). Finally, xylazine alone and combinations of xylazine plus ketamine have been frequently used in Northern and in Southern chamois, and some data about anaesthetic times and physiological responses were reported (Bauditz 1972; Appolinaire et al. 1984; Wiesner and von Hegel 1985; Fico 1988; Gauthier 1993a; Peracino and Bassano 1993; Moran et al. 1994; von Hardenberg et al. 2000; Bassano et al. 2004; Dematteis et al. 2008).

Induction times have been reported for Southern chamois anaesthetised with xylazine plus ketamine, namely 10 and 6.1 min (Appolinaire et al. 1984; Fico 1988). Induction time in this study falls in the above range and is similar (7.2±5.1 vs. 7.6±4.4 min) with the average one reported in Northern chamois captured in the same study area using xylazine alone (Dematteis et al. 2008).

In this study, RT and RR slightly decreased between the first and the second interval (during chamois handling) with similar ranges as Dematteis et al. (2008), indicating normal physiological reactions of chamois to these drugs. Similar RT (39.2±0.5°C) but lower RR (70±19.2 breaths/min) and HR (53±11.3 beats/min) were reported in Southern chamois (Fico 1988), whereas lower RT (37.7±1.3°C), RR (55.6±19.2 breaths/min) and HR (46.7±24.3) were recorded in Northern chamois immobilised with xylazine alone (Peracino and Bassano 1993). These differences can be explained by sample size, methodological or climatic influences, anaesthetic dosages, timing of measurements, variations in instrumentation, ambient temperature or humidity (Jorgenson et al. 1991). Comparing our values with the ones recorded in wakeful Northern chamois (Couturier 1938), RT was similar (39.0-39.3°C) and RR was lower (25 breaths/min); actually, the elevated RR of our study could reflect a sub-excitement state throughout the handling time, as suggested for other chemically captured wild ruminants (Walter et al. 2005; Dematteis et al. 2006).

Hyperthermia appeared with a lower frequency than reported by Dematteis et al. (2008), 4.5% vs. 7.9%. This is clinically relevant since hyperthermia represents a negative risk factor for respiratory/circulatory depression (odds ratio=9.5). The corresponding odds ratio value in Dematteis et al. (2008) was 21.1.

Reported mortality rates for chemically captured free-ranging *Caprinae,* under a variety of scenarios, varied between 0% and 26%, most of which being around 5% (Bergerud et al. 1964; Houston 1969; Kock et al. 1987; Jolicouer and Beaumont 1986; Gauthier 1993b; Jorgenson et al. 1990; Peracino and Bassano 1993; Perez et al. 1997; Kilpatrick and Spohr 1999; Bassano et al. 2004; Dematteis et al. 2008). In free-ranging Northern chamois captured using medetomidine-ketamine (Walzer et al. 1998) and xylazine alone (Peracino and Bassano 1993; Dematteis et al. 2008), mortality rates were 9.1%, 8% and 4.6%, respectively. In this study, except for the two traumatic events which occurred during induction time, death was always preceded by cardiorespiratory depression. Accurate postmortem analyses could not be performed due to the lack of adequate facilities; therefore, definitive causes of death remain unknown, but as suggested by Jorgenson et al. (1990), greater though unrecognised individual stress before and during immobilisation leading to cardiopulmonary problems was a likely explanation. Respiratory/ circulatory depression is a recognised side effect of xylazine (Kreeger et al. 2002). In our study, it was observed with a lower frequency (5.2%) than following the use of xylazine alone (8.8%; Dematteis et al. 2008), suggesting a reduction of drawbacks when xylazine is used in association with a dissociative drug like ketamine. This is consistent with reports by other authors (Knight 1980; Lin 1996; Nielsen 1999; Plumb 1999).

Abortion is another well-known side effect of xylazine in advanced pregnant ruminants (Richter and Gotze 1986; Knight 1980), whereas no risk is reported for ketamine (Fowler and Mikota 2006) and when these drugs are combined together (Festa-Bianchet and Jorgenson 1985). In small ruminants, the trans-abdominal palpation of the foetus from the fourth month of pregnancy is considered to be an accurate diagnostic method with a reliability of 92% (Richter and Gotze 1986). No signs of abortion were detected during the observation period. This is consistent with data on Northern chamois immobilised with xylazine alone (Peracino and Bassano 1993; Dematteis et al. 2008) and with similar experiences on other wild ungulates (Del Giudice et al. 1986; Larsen and Gauthier 1989; Jorgenson et

al. 1990; Gauthier 1993b).

A frequent disadvantage with immobilising drugs is the lack of an antidote and the resulting long duration of immobilisation and slow recovery. Currently, alfa-2-adrenergic tranquilizers can be reversed by a specific antidote, whereas no effective antagonist exists for ketamine (Kreeger et al. 2002). Notwithstanding, in this study, the use of atipamezole significantly reduced the length of the recovery phase proving to be efficient in neutralising xylazine in chamois; since atipamezole was administrated 81.7±11.0 min after anaesthetic injection, it is probable that ketamine action was vanished and only xylazine effects needed to be reversed (Plumb 1999; Adams 2001). Atipamezole can be particularly useful in emergencies (as happened in this study) or when a rapid release of captured wildlife is desirable, but Kreeger et al. (2002) reported that the reversal agent should not be injected until at least 30 min after the initial anaesthetic administration, that is, when ketamine serum concentration starts to decrease. Atipamezole was administered to a sample of 57 animals at the same mean dose of 0.3±0.1 mg/kg previously used by Dematteis et al. (2008) and a similar atipamezole/ xylazine mean ratio (1:8.9±3.2 vs. 1:9.4±4.3). Reversal time in this study was lower (4.0±2.7 vs. 5.0±2.7 min). In both studies, there was not a significant correlation between atipamezole/xylazine ratio and reversal time, suggesting that atipamezole might be effective within both ranges and that the recommended atipamezole/xylazine dose ratio of 1:10 (Jalanka and Roeken 1990; Arnemo et al. 1993) represents a reference ratio also in this study. Chamois which were not injected a reversal agent naturally recovered in 147.2± 11.3 min, similar to 139.7± 10.2 min recorded by Dematteis et al. (2008) using xylazine alone and 126.4±26.8 min recorded by Fico (1988) using xylazine-ketamine combination in Southern chamois.

Anaesthetic parameters correlated with the aforementioned optimal xylazine-ketamine dosage compare favourably with the ones reported by Dematteis et al. (2008) using their "optimal xylazine dosage" (2.6-3.6 mg/kg). In particular, we recorded a lower induction time (4.8±2.6 vs. 6.9±5.2 min), a greater number of chamois immobilised within 10 min (100% vs. 86%) and a greater rate of animals in the deep sedation class (92% vs. 80.7%). Furthermore, the association of the two drugs permits to reduce the xylazine dosage (up to 30.5%) and, in perspective, to decrease the related risk of side effects (Plumb 1999). The optimal xylazine-ketamine dosage will also imply a lower atipamezole dosage (and therefore a cheaper reversal) than using xylazine alone (Dematteis et al. 2008).

Based on the reported results, we defined a xylazine-ketamine standard dosage irrespective of sex and body mass. Chemical captures of free-ranging chamois are frequently performed by operators waiting in emplacement, and it is necessary to prepare an effective dose regardless of age and sex of the animal. Moreover, a standard weight of the dart syringe (^standard volume of inoculation) is required for ballistic precision. Under field conditions, the optimal dose of drugs can be calculated considering the mean weight of the target population during the selected capture period (Dematteis et al. 2008). For example, in this study, the mean weight of captured chamois was 25.2 kg and the standard dose, based on the optimal combined dosage previously defined (xylazine 2.5 mg/kg plus ketamine 3 mg/kg), corresponded to xylazine 63 mg plus ketamine 76 mg. A standard volume of 1.4 ml per inoculation can be obtained mixing 0.6 ml of a 10% xylazine solution plus 0.8 ml of a 10% ketamine solution. To reverse the action of xylazine (in case of alfa-2-adrenergic side effects or to obtain a pharmaco-induced recovery starting at least minutes after the administration of the anaesthetic solution), it is possible to administer 6.0 mg of atipamezole per animal, corresponding to the suggested and generally accepted atipamezole/xylazine ratio of 1:10 (Jalanka and Roeken 1990; Arnemo et al. 1993).

In conclusion, we suggest using xylazine associated with ketamine for the immobilisation of free-ranging Northern chamois, and we recommend atipamezole as a prompt pharmacological agent to reverse the alfa-2-adrenergic action. Though the "Hellabrunn mixture" with a xylazine/ ketamine ratio of 1:0.8 (Wiesner and von Hegel 1985) has been widely used to immobilise the two species of chamois (Gauthier 1993a; Wiesner and von Hegel 1985; Fico 1988), we suggest a ratio of 1:1.2 to induce better anaesthetic performance.

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Table 1 Different dosages of xylazine—ketamine administered to chamois *(R. rupicapra)* in this study

N	Xylazine–ketamine (mg)
2	20–80
19	30–70
47	40–60
51	50–50
32	60–40
4	70–30

Table 2 Mean anaesthetic dosages administered to Northern chamois according to the depth of sedation

Sedation class	*N*	Percentage	Dose (mean±SD)		Induction time (mean±SD; min)
			Xylazine (mg/kg)	Ketamine (mg/kg)	
Deep	114	73.6	1.9±0.5	2.3±0.7	6.1±4.4[a]
Weak	21	13.5	1.8±0.4	1.9±0.6	7.8±4.8[a]
Light	20	12.9	2.0±0.7	2.1±0.5	11.6±5.0[b]

Values with different superscript are significantly ($P<0.05$) different from each other

N number of chamois in each category, *SD* standard deviation