CHEMICAL IMMOBILISATION OF LETEA FERAL HORSES (*EQUUS CABALLUS*) USING KETAMINE AND MEDETOMIDINE

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Summary

Thirty-five free-range feral horses were successfully remotely anaesthetised for immunocontraception using different combinations of ketamine/medetomidine with or without hyaluronidase. Only horses where fully discharged darts induced anaesthesia (without any top-up darts and/or previous deflected darts) were included in our study. A mean ($\bar{x}$) induction time of 8.85 minutes (SD = 4.76) was recorded for a group of 28/35 horses that were immobilised with ketamine (1.45 - 3.8 mg/kg, $\bar{x}$ = 2.32 mg/kg) and medetomidine (0.05 - 0.15 mg/kg, $\bar{x}$= 0.09 mg/kg) and a mean induction time of 9 minutes (SD = 4.83) for another group (7/35) that received ketamine (1.25 - 2.2 mg/kg, $\bar{x}$ = 1.74 mg/kg), medetomidine (0.08 - 0.17 mg/kg, $\bar{x}$ = 0.119) and hyaluronidase (2.85 - 4.4 IU/kg, $\bar{x}$ = 3.64 IU/kg). Approx. 25 minutes after induction five horses in the first study group (n = 28) and two from the second (n = 7) required additional 1.4 mg/kg ketamine I.V. to achieve a deeper anaesthesia level. The mean duration of anaesthesia was 69.56 min (SD = 12.87) for the first study group and 73.8 min (SD = 20.54) for the second study group. Heart rate, respiratory rate, temperature and SpO_2 were measured and recorded during recumbency. No specific antidote (atipamezole) was given, except for one individual due to critical clinical conditions. During reversal the horses were manually assisted to sternal position with the front limbs extended, which facilitated their raising. Once standing most of the horses preferred to remain stationary if not disturbed. Some tachypnoea and one case of a stormy awakening was reported, however, there were no post-anaesthestic complications or injuries.

Introduction

Free-ranging feral horses are commonly captured in round-ups, using horse riders, motorcycles or helicopters with which they are herded into corral traps. This stressful process may result in capture of horses of mixed sexes and ages from different herds, with a high risk of injuries for the captured individuals and the people involved in the process.

An immuno-contraception programme of overabundant Letea feral horses, requiring ear-tag identification, health check and PZP vaccination of mares, would have required the round-up of hundreds of horses to selectively contracept the targeted individuals.

The risks associated with round-ups were the reasons for the present study evaluating the feasibility of selective capture using on ground remote tranquilisation. The drug of choice for wild equids immobilisation in general, and for feral horses in particular, is considered to be the highly potent opiate ethorphine (WALZER, 2007; SEAL et al., 1985). Due to the restricted availability of ethorphine and previous positive experience with the combination of dissociatives and alpha-2 agonists in feral horses (ROSU et al., 2012) and other equids (MATTHEWS et al., 1995), different combinations of ketamine and medetomidine with or without hyaluronidase were taken into consideration.

The purpose of the study was to find a suitable ketamine/medetomidine combination, fitting into a single dart that would offer a suitable anaesthesia for free-ranging feral horses (*Equus caballus*) in the field.

Materials and methods

The studies were conducted in Grindul Letea (45°20'43"N, 29°30'45"E), Danube-Delta, Romania, between October 2013 and January 2014. Ambient temperatures ranged from +5 to +23 ºC.
A total of 52 horses were successfully immobilised in this period, (49 mares and three subadult males) from which 35 anaeasthesias were included in the present study. Due to the lack of sexual dimorphism the three young males were confused to be females and mistakenly darted.
Animals appeared healthy based on body weight and physical examination. The age was estimated to range from 2 to 20 years ($\bar{x}$ = 5.8 yr) and the body weight from 200 to 400 kg ($\bar{x}$ = 310 kg).
The working protocol included health check, ear tagging, vaccination (Porcine Zona Pellucida, The Science and Conservation Center, Billings, USA) blood and faecal sampling, age estimation from dental surface and taking pictures for future identification. This protocol took up to 40 min to finish from the time of induction.
After being located, the mares were slowly approached and darted from the car. Distances between 15 and 45 meters allowed targeting the rump while the individuals remained relaxed and not moving.
Different combinations of ketamine 100 mg/ml (Vetased®, Romvac, Filipesti de Padure, Romania), ketamine HCL dry powder 1 g/20ml (Ketamine 1 g®, Kyron Laboratories, Benrose, South Africa) and medetomidine 40 mg/ml (Medetomidine 40 mg/ml®, Kyron Laboratories, Johannesburg, South Africa) were used. In seven immobilisations hyaluronidase 5000 IU (Hyaluronidase 5000 IU®, Kyron, Johannesburg, South Africa) was added to the dart combination.
Drugs were delivered remotely using 5.0, 6.0 or 7.0 ml, Ø13 mm aluminum disposable darts (Pneu-dart Type 'P'®, Pneudart, Inc., Wiliamsport, USA), with either 3.81 cm or 5.08 cm barbed needles, delivered by Ø13 mm compressed air rifle (X-Caliber® Gauged Projector, Pneudart, Inc., Wiliamsport, USA).
After an animal was successfully darted it was left alone and observed from afar (100 - 200 m) with binoculars. Slowing or ataxia were considered as onset of induction. Induction time (minutes from darting until the animal went down and stayed down in sternal or lateral recumbency) and total time of recumbency (duration of anaesthesia)were recorded. The duration of anaesthesia was defined from the moment of recumbency to the moment the horse was standing again without assistance. Also the time of standing was considered to be the end of the procedure.
Once recumbent the horses were blindfolded and their body positioned laterally with their necks and lower front limbs extended.
In most of the cases the vital parameters were assed (temperature, pulse, respiration, pulse-oximetry, capillary refill time) and noted every 10 min. The temperature was taken with an oral digital laser thermometer. In some cases two different pulse-oximeters were used. During three procedures an Oxi-100 VET® was used, and during 15 procedures a Nonin® 2500 A. The pulse oximeter probes were placed on the tongues. Respiratory rate was counted by visual assessment of thoracic and abdominal distension and air movement at the nares.

Different combinations of ketamine and medetomidine were used. In seven cases (20 %), hyaluronidase was added to the anaesthesia mix. The rest of the inductions (28/35, 80 %) were performed with ketamine/medetomidine combinations only.
Only the 35 cases of fully discharged darts (without any top-up darts and/or previous deflected darts) were included in the calculations and table. They are represented by Group 1 (ketamine + medetomi-

dine) including 28 horses and Group 2 (ketamine + medetomidine + hyaluronidase) including seven horses.
Statistics consisted of mean values and standard deviations (table 1).

Results

A total of 52 feral horse immobilisations were successfully performed in 16 days of fieldwork, from which 35 were induced with only one delivered dart.
The rest of the inductions (17/52) either required a top-up dart of 500 mg ketamine (6/17) for recumbency, and/or repetition(s) of darting with full dosages (one repetition in five individuals, two repetitions in two individuals) due to deflection of the darts.
The darts were mainly projected from the car. While the animals remained relatively calm with approaching vehicles, the horses tended to become nervous and move on as soon as the car stopped for darting, allowing only about 5 seconds for taking aim.
In the first study group (28/35; Group 1) induction was achieved in 8.85 minutes (SD = 4.76) using a mean of 2.32 mg/kg ketamine (1.42 - 3.8 mg/kg) in combination with 0.09 mg/kg medetomidine (0.05 - 0.15 mg/kg) (see table 1 and figure 1). In this group, in the case of five horses (approx. 25 minutes after induction) a supplementary 1.4 mg/kg ketamine I.V. was considered necessary to acquire a deeper plane of anaesthesia. The mean overall duration of anaesthesia in Group 1 for the horses (23/28) which did not require supplementary I.V. ketamine was of 69.56 minutes (SD = 12.87).
In Group 2 (7/35, $\bar{x}$ = 20 %), a mean of 1.74 mg/kg ketamine (1.25 - 2.2 mg/kg) in combination with 0.119 mg/kg medetomidine (0.08 - 0.17 mg/kg) and 3.64 IU hyaluronidase (2.85 - 4.4 IU/kg), resulted in induction within 9 minutes (SD = 4.83) from darting. In this group 2/7 horses received supplementary 1.4 mg/kg ketamine I.V. approx. 25 minutes after induction. The mean overall duration of anaesthesia for the horses (5/7) without supplementary I.V. ketamine was of 73.8 (SD = 20.54) minutes (table 1 and figure 1).

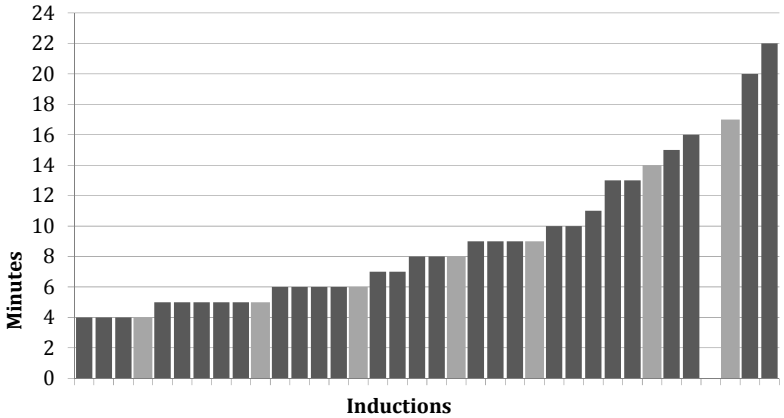

Fig 1: Distribution of induction times for 28 horses immobilised using ketamine and medetomidine (dark grey), seven horses immobilised using ketamine, medetomidine and hyaluronidase (light grey).

Tab. 1: Descriptive statistics (mean, SD, median, min, max and n) for feral horses under two different immobilisation protocols.

	keta-mine (mg/kg)	medetomi dine (mg/kg)	hyalu-ronidase (IU/kg)	Induc-tion (min)	Time of re-cumbency (min)	Tem-perature (ºC)	Respiratory rate (bpm)	Heart rate (bpm)
Group 1								
Mean	2.32	0.09	-	8.85	69.56	37.6	25.4	41.44
Standard deviation	0.56	0.02	-	4.76	12.87	1.34	9.29	8.84
Median	2.24	0.1	-	7.5	68	37.2	24	40
Minimum	1.42	0.05	-	4	60	34.1	14	26
Maximum	3.8	0.15	-	22	110	40.4	68	88
n	28	28	-	28	23	86	93	65
Group 2								
Mean	1.74	0.119	3.64	9	73.8	38.5	32.57	45.16
Standard Deviation	0.32	0.02	0.54	4.83	20.54	1.22	12.10	6.30
Median	1.85	0.11	3.57	8	65	38.2	31	44
Minimum	1.25	0.08	2.85	4	48	36.3	11	36
Maximum	2.2	0.17	4.4	17	92	40.8	56	65
n	7	7	7	7	5	44	40	36

All of the above combinations offered good muscle relaxation and adequate anaesthetic depth.

Despite several moments of transitory tachypnoea, all the vital parameters, except SpO_2 were within the normal physiological range without any significant differences between the two groups. Nevertheless the mean heart rate was at the upper limit, both in Group 1 ($\bar{x}$ =41.11 bpm, SD = 8.84) and Group 2 ($\bar{x}$ = 45.16 bpm, SD = 6.3) (table 1). The pulse-oximeters registered values under 90 % in all of the cases.

After 65 to 70 minutes of anaesthesia, the horses (25/28 in Group 1, and 7/7 in Group 2), were manually assisted into sternal position with their front limbs extended. The rest of the horses were able to position themselves sternally or woke up to standing position instantly. After approx. 9 minutes ($\bar{x}$ = 8.9 min) in sternal position, the horses were given a manual stimulus on the head or back to stand up.

The majority of the horses from the study groups (91.4 %) remained on site upon standing. Several horses remained in the same area for up to three hours, however, if approached they would move away at pace or gallop. One horse (2.8 %) went back to sternal position shortly after standing to raise again after a couple of minutes.

Only in one case (2.8 %) with more than 90 min of recumbency, medetomidine antagonisation was considered necessary (atipamezole 0.1 mg/kg i.v., Alzane®, Pfizer, León, Spain).

No post-anaesthetic morbidity or mortality was observed. The oldest mare in the study (estimated age 20 yrs) died six weeks after the anaesthesia procedure, however, this event was considered unrelated to the anaesthesia due to the lapsed time, poor body condition and the harsh weather from that period.

Discussion

The goal of this study was to find the best combination of easily available drugs with an overall quick induction, suitable anaesthetic depth for the PZP vaccination protocol and a smooth reversal in Letea feral horses.

A combination of approx. 2.32 mg/kg ketamine and 0.09 mg/kg medetomidine resulted in a satisfactory induction in 8.89 minutes and 69.56 minutes of anaesthesia. This combination is very similar with previously reported immobilisations in Przewalski's horses (2.1 mg/kg ketamine and 0.09 mg/kg medetomidine) (MATTHEWS et al., 1995).

The use of hyaluronidase (3.64 UI/kg) as an agent that increases the absorptions of other drugs offered similar results compared to the simple ketamine/medetomidine combinations: induction in 9 minutes and a duration of anaesthesia of 73.8 minutes. However, those results were achieved with lower doses of ketamine (1.74 mg/ml) and higher doses of medetomidine (0.119 mg/kg).

Nevertheless, adding hyaluronidase to the darts increased the total volume, which required the use of bigger darts (e.g. 7.0 ml) and, consequently, shorter target ranges and decreased accuracy. Associated with the fact that both combinations' results were similar, and that for ketamine/medetomide, smaller 6.0 ml darts were used, the latter combination was preferred.

In five horses from Group 1 and two from Group 2, approx. 25 minutes after induction, a top-up of intravenous ketamine (≈ 1.4 mg/kg) was considered necessary to acquire a suitable anaesthesia depth to continue with the working protocol. Reasons for those top-ups were that slight eye and/or ear movements were seen and thus considered a light plane of anaesthesia that had to be addressed. Nevertheless, once several anaesthesias were performed it was seen that even in the presence of some cases of eye and/or ear movements our working protocol could be performed safely, without the risk of the horse to wake up, consequently no more top-ups were considered to be necessary.

Due to the fact that in the Letea region there are no direct predators and administration of reversal agents would have been beyond the financial possibilities, the authors considered recovery without medical reversal acceptable. No deaths or injuries were reported in the days following the immobilisations.

Most of the horses were able to stand up at first attempt. Once standing they seemed to prefer remaining stationary and only moved if approached, either in pace or gallop. If their herd was in vicinity the horses slowly followed the other group members. This prolonged state of lethargy is considered to be the sedative effect of the medetomidine.

The authors found out that positioning the horses sternally with their front limbs extended quickened the reversal and helped them acquire the standing position without stumbling and falling.

From the total amount of immobilisations done, in 9/52 cases the darts hit other parts of the body (five times the abdominal area, one time at the base of the tail, one time in the tibia, one time in the neck and one time in the masseter muscle) which, depending on the site injected, delayed the time of induction. From all those injections the longest period of inductions was seen to be those of the darted that hit the abdominal area (more than 20 minutes). This long period of induction might be explained either by a slower absorption of the abdominal muscles or because the anaesthetic could have been delivered and absorbed intraperitoneally. Poor dart placement and deflected (bounced) darts contributed to poor induction.

Peripheral oxygenation (SpO_2) in most of the immobilisations has been seen to have values under the normal range (< 90 %). Those low readings could be explained by the decreased peripheral oxygenation effect of medetomidine (MATTHEWS et al., 1995, SARAZAN et al., 1989) and insufficient oxygenation due to the perfusion/ventilation mismatch acquired during the horses' lateral recumbency (GREEN and KEEGAN, 2002). Although no pathology has been reported the following days, supplemental oxygenation should have been provided.

The authors conclude that using ketamine/medetomidine combinations (with or without hyaluronidase) is a reliable alternative to ethorphine for field feral horse immobilisations, offering good muscle relaxation and adequate anaesthetic depth, with few complications and smooth reversals, even without antagonisation.

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