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RAPID REVERSIBLE IMMOBILIZATION OF FERAL STALLIONS USING ETORPHINE HYDROCHLORIDE, XYLAZINE HYDROCHLORIDE AND ATROPINE SULFATE

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ABSTRACT: Forty-eight newly captured free-ranging feral stallions (*Equus caballus*) from two different locations and six captive stallions were immobilized using combinations of etorphine hydrochloride, xylazine hydrochloride and atropine sulfate with or without acepromazine. Six animals were immobilized twice, 1 mo apart. The drugs were administered either intramuscularly ($n = 13$) or intravenously ($n = 44$). Mean immobilization time (\pm SE) after intravenous (i.v.) injection of etorphine, xylazine and atropine was 55 ± 4 sec (range 20 to 185 sec) compared to 708 ± 131 sec (range 390 to 1,140 sec) for intramuscular (i.m.) injection. Immobilization was reversed with i.v. administration of 3 to 11 mg diprenorphine hydrochloride and 16 to 24 mg yohimbine hydrochloride. Average time from administration to standing and walking was 86 ± 7 sec ($n = 55$). Reversal of etorphine-induced immobilization with an amount of diprenorphine equal to the etorphine and administered i.v. was as effective as a 2:1 ratio of diprenorphine to etorphine. Acepromazine had no effect on induction time, but decreased relaxation after immobilization and prolonged ataxia after reversal of the etorphine and xylazine. Eight free-ranging horses were immobilized in 708 ± 132 sec by darting with 5.5 mg etorphine, 1,300 mg xylazine and 15 mg atropine from a helicopter. Three animals died during the study: one immediately after reversal of an i.v. administration, one from a broken neck during induction from darting, and one was found a week later at the site of darting. Comparisons of hematological values before and 15 min after drug immobilization demonstrated a small but significant decline in hemoglobin, red cells, and hematocrit with no significant effects on calculated red cell parameters. The horses captured in the Flanigan area had significantly lower values of hemoglobin, hematocrit and red cells ($P < 0.001$, 0.01, and 0.06, respectively) than stallions from Beaty's Butte. This correlated with the poorer condition of the horses in the Flanigan area. The effectiveness of yohimbine as an antagonist for xylazine facilitated capture and immobilization of free-ranging feral horses and allowed their immediate release after handling with a minimum of postreversal depression.

Key words: Feral horses, etorphine, diprenorphine, xylazine, yohimbine, immobilization, anesthesia, hematology, blood, *Equus caballus*.

INTRODUCTION

The chemical immobilization of free-ranging feral horses has been laborious and complicated by mortality of 10-37% (Borchard, 1980; Berger et al., 1983). The most commonly reported immobilizing agent has been succinylcholine chloride. Borchard (1980) reported capture of 23 stallions and one mare with nine deaths and Berger et al. (1983) reported three deaths after capture of 23 feral horses with succinylcholine chloride.

There are few reports of immobilization of feral equids in North America using etorphine hydrochloride (etorphine). Blake et al. (1981) reported capturing feral donkeys (*Equus asinus*) in Death Valley, California, but details of immobilization procedures were not presented. In a report to the Bureau of Land Management (BLM), Moore (1979) described immobilizing 34 horses with etorphine and tranquilizers. Eight mortalities were incurred. However, details of drug doses or procedures were not provided. More recently, Seal et al.

(1985b) reported the immobilization of 87 free-ranging feral mares with etorphine and xylazine hydrochloride (xylazine). Five mares died at the time of capture and the remains of three other mares were found near the site of capture 4 mo later.

The addition of xylazine to immobilization mixtures has resulted in several problems including long down-times, unstable body temperatures and various physiological and environmental hazards associated with such conditions (Jessup et al., 1983). In feral horses, prolonged recoveries and animal losses could result in disruption of band integrity and social structure (Berger et al., 1983).

The effectiveness of yohimbine hydrochloride (yohimbine) as an antagonist to xylazine (Jessup et al., 1983; Hsu and Shulaw, 1984, 1985; Mech et al., 1985) for white-tailed deer (*Odocoileus virginianus*) prompted this study to determine the effectiveness of etorphine and xylazine for immobilization of feral horses with rapid reversal using diprenorphine, a commonly accepted etorphine antagonist, and yohimbine. Physical data and samples of blood were collected before and after immobilization for evaluation of drug effects and animal condition.

MATERIALS AND METHODS

Six captive feral stallions were immobilized at the BLM wild horse holding facility in Lovelock, Nevada during December 1985. Twenty-six feral stallions were captured from the Flanigan wild horse management unit in northwestern Nevada during December 1985 and twenty-two feral stallions were captured from the Beaty's Butte unit in southern Oregon during February 1986. The free-ranging animals were captured in corrals utilizing a Bell B1 helicopter and professional roundup service. One stallion in the Flanigan area would not go into the trap and was darted from the helicopter with a 7 ml dart containing a mixture of etorphine and xylazine. Barbed needles (NC-2, "Cap-Chur®" Equipment, Great Western Serum Co., Albuquerque, New Mexico 87106, USA) were used to enhance retention of the dart and maximize delivery of the drugs. One animal in the

corrals at Lovelock was darted also. The remainder of the animals were run into a squeeze chute and hand injected either intravenously or intramuscularly. One mo later, one new stallion and six previously captured animals from the Flanigan area were immobilized by darting from the helicopter.

The drugs used were etorphine, 1 mg/ml (M99®, Lemmon Co., Sellersville, Pennsylvania 18960, USA), xylazine, 100 mg/ml (Rompun®, Haver-Lockhart, Bayvet Division, Cutter Laboratories, Inc., Shawnee, Kansas 66024, USA), AcePromazine® (Aveco Co., Inc., Ft. Dodge, Iowa 50501, USA), and atropine, 15 mg/ml (Med-Tech, Inc., Elwood, Kansas 66024, USA). Drugs were mixed in a single syringe. Atropine was included in the mixture to prevent the secondary atrioventricular block induced by xylazine (Kerr et al., 1972a, b; Muir et al., 1979). Animals anesthetized in the corrals were given 20 mg of diazepam intravenously (Valium®, Hoffmann-LaRoche Inc., Nutley, New Jersey 07110, USA) to reduce the muscular rigidity and tremors associated with the immobilization (Daniel and Ling, 1972; Butera et al., 1978). All animals were given 10.5 million units of benzathine penicillin, 300,000 units/ml (Flo-cillin®, Bristol Laboratories, Syracuse, New York 13201, USA) intramuscularly as an antibiotic. Reversal was accomplished with the i.v. administration of a mixture of diprenorphine, 2 mg/ml (M50/50®, Lemmon Co., Sellersville, Pennsylvania 18960, USA) and yohimbine, 4 mg/ml in 5% dextrose prepared from the powder (Sigma Chemical Co., P.O. Box 14508, St. Louis, Missouri 63178, USA) given in the same syringe. Various doses of yohimbine were used to determine a minimal effective dose. A special mixture of etorphine and xylazine plus atropine was prepared for injecting the animals from the helicopter. Five grams of xylazine was lyophilized and dissolved in 20 ml of 45% propylene glycol containing 20 mg of etorphine. This resulted in a final concentration of 0.77 mg etorphine and 192 mg xylazine per ml solution, thus allowing a large amount of the xylazine to be injected using a 7 ml dart.

The drug dosages were grouped as follows: Group I contained 30 stallions, 20 stallions that were anesthetized by i.v. injection of a mixture of 5.5 mg etorphine, 800 mg xylazine and 15 mg atropine and 10 other stallions that received the same drugs along with 30 mg acepromazine in the same syringe. Immobilization was reversed by i.v. injection of a mixture of 4–11 mg diprenorphine and 16–24 mg of yohimbine in the same syringe. Group II contained 11 stallions that were anesthetized by i.v. injection of 3.5

mg etorphine, 600 mg xylazine and 15 mg atropine sulfate in the same syringe. They were reversed by i.v. injection of 3–6 mg diprenorphine and 20 mg yohimbine in the same syringe. Group III contained 12 stallions that received 5.5 mg etorphine and 7.5 mg atropine along with either 550 ($n = 2$), 800 ($n = 2$) or 1,300 ($n = 8$) mg xylazine in the same syringe. Immobilization was reversed by i.v. injection of a mixture of 11 mg diprenorphine and 16–26 yohimbine in the same syringe.

The free-ranging feral horses were captured using the Bell helicopter on a band by band basis. A band was located, brought up to and run into the traps. As many as seven bands could be held at one time without mixing. Often one or two bands would be held overnight for processing the next morning. This occurred in both the Flanigan and Beaty's Butte areas. All animals were graded according to condition and bled for hematology (Seal et al., 1977). Body lengths and heart girths were measured and respiratory rates and rectal temperatures were recorded. Heart rate was counted with the aid of a stethoscope while the animal was anesthetized (Beaty's Butte only). Ages of all animals were determined according to tooth eruption and wear (Ensminger, 1969). Records of the immobilizations included induction time (sec from drug administration until the animal went down and stayed down), duration of immobilization (time from when the animal first stayed recumbent to administration of reversal drugs), and time for recovery (time to standing) after antidote administration. Residual effects or status of the animal after recovery were estimated subjectively.

Statistics in the text are means (\pm SE). Comparisons were made using ANOVA and Student's t -test for paired samples utilizing the Number Cruncher Statistical System version 4.21 for the IBM computer (Dr. Jerry L. Hintze, Kaysville, Utah 84037, USA). This program follows the procedures outlined by Snedecor and Cochran (1967) and Ostle (1969).

RESULTS

Mean induction time for the 10 Group I stallions receiving acepromazine was 46 ± 7 sec and 60 ± 7 sec for the 20 animals which did not receive acepromazine (NS, Table 1). Two stallions were not immobilized with the initial presumed i.v. injection and required a dart injection of additional drug. Data from these two animals were not included in the immobili-

TABLE 1. Effect of drug dosages on induction time in feral stallions.

Dosage (mg)				Time (sec)			
				Induction		Recovery	
M 99*	Xyl	Ace	n	\bar{x}	SE	\bar{x}	SE
5.5	800	0	20	60	7	84	10
3.5	600	0	11	60	9	90	8
5.5	800	30	10	46	7	96	10

* M 99 = etorphine, Xyl = xylazine, and Ace = acepromazine. All animals received atropine.

zation times because their values were greater than three standard deviations from the mean of the rest of the animals. All animals exhibited some degree of muscular rigidity and tremors. Relaxation was achieved by the intravenous administration of 20 mg diazepam. Mean duration of immobilization was $2,448 \pm 168$ sec.

Mean recovery time for the 10 animals receiving acepromazine was 96 ± 10 sec, whereas the mean recovery time for animals which did not receive acepromazine was 84 ± 10 sec (NS, Table 1). A mild ataxia was present for about 20 min after arousal only in animals receiving acepromazine. Subjectively, there was less muscular rigidity in animals not receiving acepromazine.

The induction time for the 11 Group II stallions was 60 ± 9 sec (Table 1). Duration of immobilization for these stallions was $2,094 \pm 216$ sec and mean recovery time was 90 ± 8 sec.

The dose ratio of diprenorphine to etorphine was varied from 1:1 to 2:1 with no significant difference in time to standing and no apparent difference in residual effects. None of the animals remained in the vicinity of the corrals or experienced a relapse into profound immobilization.

Mean induction time in the 12 Group III stallions receiving the drug i.m. was 708 ± 132 sec. None of these horses received acepromazine, but six received 20 mg diazepam after being immobilized. Mean immobilization time for these ani-

TABLE 2. Biological data on immobilized free-ranging stallions in two different areas.

	Beaty's Butte, Oregon			Flanigan, Nevada			<i>P</i>
	\bar{x}	SE	<i>n</i>	\bar{x}	SE	<i>n</i>	
Induction time							
Intravenous (sec)	60	7.6	19	55	6.0	21	NS
Intramuscular (sec)	1,500	60.0	2	660	18.0 ^a	9	—
Recovery (sec)	84	10.0 ^b	18	103 ^c	17.0	29	NS
Temperature (C)	40.4	0.2	22	39.7	0.1	26	0.01
Heart rate (beats/min)	120	10.0	19	Not taken			
Respiratory rate (breaths/min)	10.6	1.0	22	7.4	0.8	24	0.01
Age (yr)	11	2.0	22	11	1.0	26	NS
Body length (cm)	195	2.4	21	194	3.7	25	NS
Girth (cm)	177	1.8	21	181	1.7	25	NS

^a Does not include two animals that required additional drug i.m. to be immobilized.

^b Does not include one animal that took 23.4 min to get up.

^c Does not include one animal that took 23.0 min to get up.

mals was $2,274 \pm 312$ sec. Immobilization was reversed by i.v. injection of 11 mg diprenorphine and 16–26 mg yohimbine. Mean time for reversal (83 ± 10 sec) was the same as in animals immobilized with i.v. injections and no different residual effects were noted. Varying the yohimbine had no apparent effect.

Death of three stallions could be attributed to the immobilization procedure: One 17-yr-old stallion in Group I died from apparent cardiac arrest after administration of the diprenorphine-yohimbine mixture, a 7-yr-old stallion was found dead 1 wk later at the site where he was darted and one stallion died from falling and breaking his neck after being darted.

Body temperature for the Beaty's Butte horses averaged 40.4 ± 0.2 C ($n = 22$) as compared with 39.7 ± 0.1 C ($n = 24$) for the Flanigan horses ($P < 0.01$) (Table 2). Heart rates were measured in the Beaty's Butte horses only and averaged 120 ± 10 beats/min during immobilization. Respiratory rate after immobilization was significantly lower in Flanigan horses than Beaty's Butte horses (7.4 ± 0.8 versus 10.6 ± 1.0 , $P < 0.01$, Table 2).

Blood was collected from 29 stallions handled in the trap before and 15 min after administration of the anesthetics. No

significant differences were noted in white blood cell counts, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) or mean corpuscular volume (MCV) between Flanigan horses and Beaty's Butte horses (Table 3).

There was a decline in hemoglobin ($P < 0.04$), hematocrit ($P < 0.01$) and red blood cells ($P < 0.01$) at 15 min postinjection of the immobilizing drugs (Table 3) in the samples collected from both areas. The differences were relatively small, but consistent, at this short interval. There was no change in WBC, MCV, MCHC or MCH in drugged horses versus undrugged horses. Hemoglobin ($P < 0.001$), hematocrit ($P < 0.01$) and red cell concentrations ($P < 0.06$) were significantly lower in unanesthetized Flanigan horses than in unanesthetized Beaty's Butte horses (Table 3).

DISCUSSION

Immobilization of wild animals has been hampered by the lack of ability to reverse some drugs. Xylazine has been used quite effectively for immobilization and anesthesia of domestic animals. However, these immobilizations are usually performed in a controlled environment. Since wild animals are usually immobilized under less

TABLE 3. Location and drugging effects on hematologic values of feral stallions.

Assay	Drug status, location and sample size			
	Undrugged		Drugged	
	Beaty's Butte, Oregon (n = 22)	Flanigan, Nevada (n = 26)	Beaty's Butte, Oregon (n = 22)	Flanigan, Nevada (n = 7)
Hgb (g/dl)	17.4 ± 0.4*	15.9 ± 0.3	17.1 ± 0.5	15.5 ± 0.5
Hct (vol %)	50.1 ± 1.0	46.2 ± 1.0	48.4 ± 1.1	48.2 ± 1.5
RBC (10 ⁶ /μl)	9.9 ± 0.3	9.1 ± 0.3	9.4 ± 0.2	9.0 ± 0.4
WBC (10 ³ /μl)	7.0 ± 1.6	6.6 ± 0.3	7.0 ± 0.4	6.7 ± 0.9
MCV (fl)	50.9 ± 0.8	51.0 ± 0.7	51.7 ± 0.9	53.4 ± 0.7
MCHC (g/dl)	34.9 ± 0.3	34.5 ± 0.4	35.2 ± 0.5	32.1 ± 0.2
MCH (pg)	17.7 ± 0.3	17.6 ± 0.3	18.2 ± 0.3	17.2 ± 0.2

* Mean ± SE.

than optimal conditions, problems associated with the use of xylazine have limited its usefulness. These problems include long down times, loss of thermoregulation and death of animals. The ability of the α_2 adrenoreceptor antagonist yohimbine to rapidly reverse the effects of xylazine has allowed the controlled use of xylazine with and without other drugs for immobilization and anesthesia of wild animals (Goldberg and Robertson, 1983; Jessup et al., 1983, 1985; Jacobson et al., 1985; Mech et al., 1985). Sedation times from 180 to 300 min have been reported in deer from xylazine dosages of 0.30 to 0.34 mg/kg. Immobilization of the same animals was reduced to 13 min or less after reversal of the xylazine with yohimbine and 4-aminopyridine (Renecker and Olsen, 1985). Mech et al. (1985) reported a median walk time of 9.5 ± 2.9 min after administration of yohimbine alone for xylazine-induced immobilization in white-tailed deer.

Our experience with xylazine in conjunction with etorphine demonstrates that this combination is effective for rapidly immobilizing feral stallions. Seal et al. (1985b) demonstrated the effective use of xylazine in combination with etorphine in free-ranging feral mares under field conditions. They demonstrated that this combination could be used in mares with mortality of <10%. This drug combination appears less hazardous to the horses than

succinylcholine (Zinn et al., 1970; Benson et al., 1979; Moore, 1979; Borchard, 1980; Berger et al., 1983) and provides full analgesia and anesthesia. Although xylazine and etorphine have been used in combination in domestic horses with mortality of <0.1% (Hillidge and Lees, 1974; Fuentes, 1978), animals in their studies were not under the excitement stress experienced by animals in this study. In addition, the time of induction of captive animals is not as critical as under field conditions when the cost of people and helicopter time become factors.

Seal et al. (1985b) pointed out the difficulty of getting enough xylazine with the required etorphine into a dart for effective field capture of feral mares. Since stallions were expected to require a higher dose of drugs than mares, concentrating xylazine appeared more desirable than increasing the size of the syringe dart. Larger darts would be difficult to deliver accurately and safely from a helicopter. Haigh (1978) reported the use of lyophilized xylazine in conjunction with ketamine for immobilizing exotic species. Lyophilizing and reconstituting xylazine in the etorphine-propylene glycol has two advantages: increasing the dose of xylazine and yielding a solution that will not freeze in the dart or needle, a problem frequently encountered during the winter.

Schlarmann et al. (1973) have reported

that the predominant side effects of i.v. mixtures of acepromazine and etorphine given to domestic horses include muscular tremor and rigidity, mydriasis, sweating, and pronounced hypertension and tachycardia. They hypothesized that these effects were due to sympathetic stimulation by etorphine resulting in massive release of catecholamines from postganglionic neurons. Similar effects were observed in this study. Although the addition of 800 mg or more xylazine to the immobilization mixture reduced these side effects, in several instances diazepam was necessary to allow enough relaxation for surgery.

The dosages of xylazine used in this study were five- to six-fold greater than we used for immobilization of feral mares (Seal et al., 1985b). These dosages achieved similar induction times for the stallions at the same dose of etorphine. In contrast to previous experience with feral mares (Seal et al., 1985b) and with moose (*Alces alces*) immobilized with carfentanil and xylazine and reversed with naloxone and diprenorphine (Seal et al., 1985a), reversing the animals with diprenorphine and yohimbine minimized postimmobilization depression of activity. The stallions dispersed from the handling chute shortly after reversal and were not in the vicinity the following day. We feel yohimbine is an important antagonist for xylazine-induced immobilization and allows use of larger doses of xylazine without the risk of prolonged residual effects. We recommend immobilization of captive feral stallions with i.v. injection of 3.5 mg etorphine, 800 mg of xylazine, and 7.5 mg atropine sulfate. This may be reversed with 3.5–7.0 mg of diprenorphine and 16 mg of yohimbine. For free-ranging stallions, we recommend 5.5 mg etorphine with 1,300 mg of xylazine and 7.5 mg atropine sulfate. Although the lower drug scheme was effective with restrained stallions, it was not reliable when the animal was not restrained (unpubl. obs.). Our loss of three

animals or slightly less than 6% was lower than reported by other investigators for wild equids and supports the effectiveness of this procedure.

The apparent lack of a significant difference in recovery time or postrecovery residual effects at a 1:1 ratio of diprenorphine to etorphine is contrary to manufacturer recommendations and current usage of the drugs in equids and other species in the United States (Alford et al., 1974; Seal et al., 1985a). In Europe, however, etorphine administered as LA Immobilin® (Reckitt & Colman Pharmaceutical Division, Hull HU8 7EL, United Kingdom) is reversed commonly with diprenorphine at a ratio of 1.2:1 (Chapman, 1973; Cox and Meese, 1973; Hillidge and Lees, 1974). The desirability of using the minimum dose of an antagonist necessary for safe recovery of the animal and the cost savings that could result support further study of the use of a lower dose ratio of diprenorphine to etorphine.

The animals captured from the Beaty's Butte region were evaluated consistently as being in good condition on the basis of appearance whereas those from the Flanigan area were recorded as very poor to fair in condition. Hematology values for the Beaty's Butte stallions were similar to those reported for feral mares, judged in good condition, collected from the Pah Rah Mustang Area of Nevada in 1982 (Seal et al., 1985a). The physiological and hematological data support the observation that the stallions from the Flanigan area were in poorer condition than those from Beaty's Butte.

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