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CLINICAL REPORT

Comparison of Three Stages of Chemical Immobilization and Changes in Vital Signs in Striped Hyena (*Hyaena hyaena*) under Immobilization with Ketamine-Medetomidine, Ketamine-Xylazine, and Butorphanol-Medetomidine-Midazolam

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ABSTRACT

Chemical immobilization is a fundamental aspect of wildlife conservation and can be extremely detrimental if proper protocols are not administered. This report compares the duration of different stages of anesthesia in striped hyena (*Hyaena hyaena*) immobilized with different combinations of ketamine (2.5-3 mg/kg)-medetomidine (0.035-0.04 mg/kg), ketamine (6-8 mg/kg)-xylazine (0.7-0.8 mg/kg) and butorphanol (0.2 mg/kg)-medetomidine (0.02 mg/kg)-midazolam (0.2 mg/kg) combinations. In total, 3 striped hyenas with different clinical conditions were anesthetized nine times. The time of induction, anesthesia, recovery, and physiological parameters were evaluated. Atipamezole (0.1-0.18 mg/kg), naltrexone (0.3 mg/kg), and flumazenil (0.003 mg/kg) were used to antagonize the effects of anesthetic drugs. Results revealed that both combinations of ketamine-medetomidine and butorphanol-medetomidine-midazolam were more effective and predictable in induction, anesthesia, and recovery phases as compared to ketamine-xylazine. Also, several sorts of adverse effects like vomiting, decreasing the percentage of SpO_2, and reducing rectal temperature were observed while using the ketamine-xylazine combination. The physiological parameters haven't been a noticeable difference as well as stages of chemical immobilization, in two combinations of ketamine-medetomidine and butorphanol-medetomidine-midazolam.

Introduction

Striped hyenas (*Hyaena hyaena*) are widely spread in a zone extending from east and northeast Africa, through the Middle East, the Caucasus region, and central Asia, to the Indian subcontinent. The striped hyena is categorized as near threatened by the IUCN but the scanning of literature reveals that the striped hyena is already extinct in many areas and that populations are generally declining in their entire geographical range due to persecution, poisoning, and hunting for meat or medicinal purposes.[1]

The effects of stress on the blood hematology and biochemistry of the species are directly related to the capture technique applied and are much diminished in chemical immobilization compared to physical immobilization.[2] Due to broad safety margin, low cost, high rate of absorption, lower cardiovascular and respiratory effects, and its international accessibility, ketamine hydrochloride has been widely used for the chemical immobilization of wild carnivores. Nonetheless, ketamine has some downsides, like the demand for a higher dose, excessive salivation, muscle

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rigidity and poor muscle relaxation, damage to the retina and mydriasis.[3] Xylazine was the first α2-adrenergic agonist drug used in veterinary medicine, and it found wide use in both large and small animals.[4] The side effects of xylazine comprise regurgitation mostly in carnivores, decreased blood pressure, heart rate and rectal temperature, excessive salivation, and may cause abortion if the female animal is pregnant.[2] Medetomidine is a highly specific α2-adrenergic agonist that has an alpha2: alpha1 selectivity factor of 1620, and when compared to xylazine is reportedly 10X more specific for alpha2 receptors versus alpha1 receptors.[5] Butorphanol is a blended agonist/antagonist opioid which effects vary from weak partial mu receptor agonist activity to mu antagonist activity plus a strong K-agonist effect. Although butorphanol has less respiratory depression than the stronger, mu agonist opioids.[4] Midazolam is a short-acting benzodiazepine used as a preanesthetic tranquilizer. In veterinary patients, midazolam is used principally as a premedication for general anesthesia and it is used with ketamine to reduce central excitatory effects and can be also used as an anticonvulsant.[6]

In this report, we evaluated the three different anesthetic protocol combinations of ketamine with xylazine and medetomidine and also the combination of Butorphanol with medetomidine and midazolam assessed the quantity of induction, anesthesia, and recovery stages along with the effects of these drugs on the physiological parameters through monitoring of vital signs.

For a comparative analysis of different anesthesia protocols, we retrospectively evaluated the capture notes during the procedures, and times of different stages of chemical immobilization (induction, anesthesia maintenance time and recovery) in all three individuals. To compare the effects of different anesthesia protocols, each of the three different stages of chemical immobilization has a given definition. Induction time: Time from the injection of the medicine to the time when the animals were completely unconscious and safe to approach and handle. Anesthesia maintenance time: Time from complete induction to the time when symptoms of drug metabolism were displayed such as a change in breathing, palpebral reflex, or body jerk. Recovery: Time from the administration of the antagonist until the animal stopped exhibiting any signs of the effects of anesthetic drugs. The following procedures were accomplished for monitoring the vital signs during anesthesia at all times that chemical immobilization was performed for all third Individuals. Continuous monitoring of heart rate and hemoglobin oxygen saturation (SpO_2) was carried out using a pulse oximeter with a probe applied at the base of the tongue throughout the procedure. The rectal temperature was measured via a digital rectal thermometer. The respiration rate was recorded by direct observation of the movement of the thoracic cavity. Capillary refill time was evaluated by pressing the oral mucosa through the thumb and then counting the required seconds to regain its color.

Case Description

During a period of 14 months from December 2020 to February 2022, 3 different striped hyenas (*Hyaena hyaena*) with different histories were anesthetized. The first striped hyena had been observed for several days by locals at a landfill site 45 kilometers away and in the southeast of Shiraz city with lethargy and weakness. The second case was an adult male that had collided with a vehicle on a highway and was lying on the side of the road. The third striped hyena, an adult female, had fallen into a water storage pool that was used for agricultural purposes in one of the human settlements around the city of Shiraz.

Treatment and Outcome

The first case was immobilized with a combination of ketamine and medetomidine at doses of 2.5 mg/kg and 0.035 mg/kg respectively. The estimated body weight was 35 kg and anesthesia was performed remote delivery by a blowpipe and the dart was injected into the left hindquarters. After 9 minutes of the induction time, getting the lateral recumbency of the animal, and also making sure there is no voluntary movement, approaching was done for evaluating stages of anesthesia. The hyena was adult and male. 55 minutes after dart injection for transferring the animal to the rehabilitation center of Bamou National Park and recovery from anesthesia, 1.26ml Atipamezole (0.18 mg/kg) was injected intramuscularly. After being transferred to the rehabilitation center throughout the 5-day diagnosis and treatment process, the hyena was anesthetized three times with different protocols. All three times immobilizing was done through remote delivery and using a blowpipe. The first time we used a combination of ketamine and xylazine with doses of 8 mg/kg and 0.7 mg/kg respectively. During anesthesia, the animal was weighed and its exact weight was 39.4 kg. The second time a combination of butorphanol (0.2 mg/kg) + medetomidine (0.02 mg/kg) + midazolam (0.2 mg/kg) was injected. The last one was used combination of ketamine (2.5 mg/kg) and medetomidine (0.04 mg/kg) again. 8 minutes after using ketamine and xylazine, the hyena vomited. The antidote injected for ketamine and xylazine was atipamezole (0.14 mg/kg) and to reverse butorphanol - medetomidine – midazolam, naltrexone (0.3 mg/kg), atipamezole (0.1 mg/kg) and flumazenil (0.003 mg/kg) were used.

Figure 1. The Striped hyena No. 1 infected with rabies.

Table 1. Time variables for striped hyena number 1 immobilized with KM/KX/BMM.

Duration category	Ketamine + Medetomidine (Min)		Ketamine + Xylazine (Min)	Butorphanol + Medetomidine + Midazolam (Min)
	1st time	2nd time		
Induction Time	10	12	9	8
Anesthesia maintenance	45	33	52	65
Full recovery	11	13	14	9

Table 2. Vital signs of striped hyena number 1 immobilized with KM/KX/BMM.

Variables	Ketamine + Medetomidine		Ketamine + Xylazine	Butorphanol + Medetomidine + Midazolam
	1st time	2nd time		
Rectal Temperature (°C)	37.3-37.5	37.3-37.4	36.5-36.9	37.5-37.7
Heart Rate (beats/min)	53-55	52-57	55-57	50-52
Respiration Rate (breaths/min)	27-28	28	22-24	25-26
SpO_2 (%)	88-90	87-91	78-81	84-86

Some details of the all chemical immobilizations of this case (number 1) (Figure 1) were mentioned in Tables 1 and 2. Rabies was confirmed as the final diagnosis of the disease after the death of this individual.[7]

In the second case which had a vehicle collision, Chemical immobilization was done by injecting a combination of butorphanol (0.2 mg/kg) + medetomidine (0.02 mg/kg) + midazolam (0.2 mg/kg) in the hindquarters. After the induction period and initial consciousness assessment, the primary examination was done. The hyena was male and no obvious fracture was detected but the animal seemed to be blind. To prepare a radiograph and confirm the presence or absence of any bone fractures, the animal was first transferred to the diagnostic imaging center. After the radiology was done, the animal was referred to the rehabilitation center. During the entire time of moving and taking radiographs for 2 hours and 20 minutes, the animal was maintained at stage 3 of anesthesia by adding 2 dose combination of butorphanol (0.1 mg/kg) + medetomidine (0.02 mg/kg) + midazolam (0.1 mg/kg) intravenously. After transferring to the rehabilitation center and 40 minutes after the last injection, for recovering from anesthesia, naltrexone (0.2 mg/kg), atipamezole (0.1 mg/kg) and flumazenil (0.002 mg/kg) were injected intramuscularly. During the 10-day treatment process, the hyena was immobilized twice with different protocols. In the first immobilization, we used a combination of ketamine (6 mg/kg) and xylazine (0.8 mg/kg) and atropine (0.05 mg/kg). The second time was done with a combination of ketamine (3 mg/kg) and medetomidine (0.04 mg/kg). In this case, like Hyena number 1, vomiting as a side effect of anesthesia occurred 13 minutes after ketamine and xylazine were injected. After 10 days of supportive treatment with a stable general condition but without any signs of improvement in the animal's vision, the hyena was transferred to the Shiraz Zoo for long-term keeping. For transferring, the animal was immobilized with a combination of BMM once more time with the same doses as the first time (butorphanol: 0.2 mg/kg + medetomidine: 0.02 mg/kg + midazolam: 0.2 mg/kg). The antagonists were administered in the zoo 42 minutes after the administration of induction agents.

Some details of the chemical immobilizations of the hyena (number 2) (Figure 2) were written in Tables 3 and 4. The main cause of the animal's blindness was not determined, but based on the results of toxicology, the use of organophosphorus pesticides in agricultural fields in human settlements in the area where the animal was found and also reports of conflicts between hyenas and farmers in that region, a possible diagnosis of pesticide intoxication was made.

Table 3. Time variables for striped hyena number 2 immobilized with BMM /KXA/KM.

Duration category	Ketamine + Medetomidine (Min)	Ketamine + Xylazine +Atropine (Min)	Butorphanol + Medetomidine + Midazolam (Min)	
			1st time	2nd time
Induction Time	8	11	7	9
Anesthesia maintenance	40	72	140	40
Full recovery	12	15	14	10

Table 4. Vital signs of striped hyena number 2 immobilized with BMM /KXA/KM.

Variables	Ketamine + Medetomidine	Ketamine + Xylazine + Atropine	Butorphanol + Medetomidine + Midazolam	
			1st time	2nd time
Rectal Temperature (°C)	37.8- 37.9	37.1	37.7	37.3
Heart Rate (beats/min)	49-51	61-64	46-49	48-50
Respiration Rate (breaths/min)	30-32	26-31	25-28	28-30
SpO2 (%)	89-91	83-85	85-87	89-92

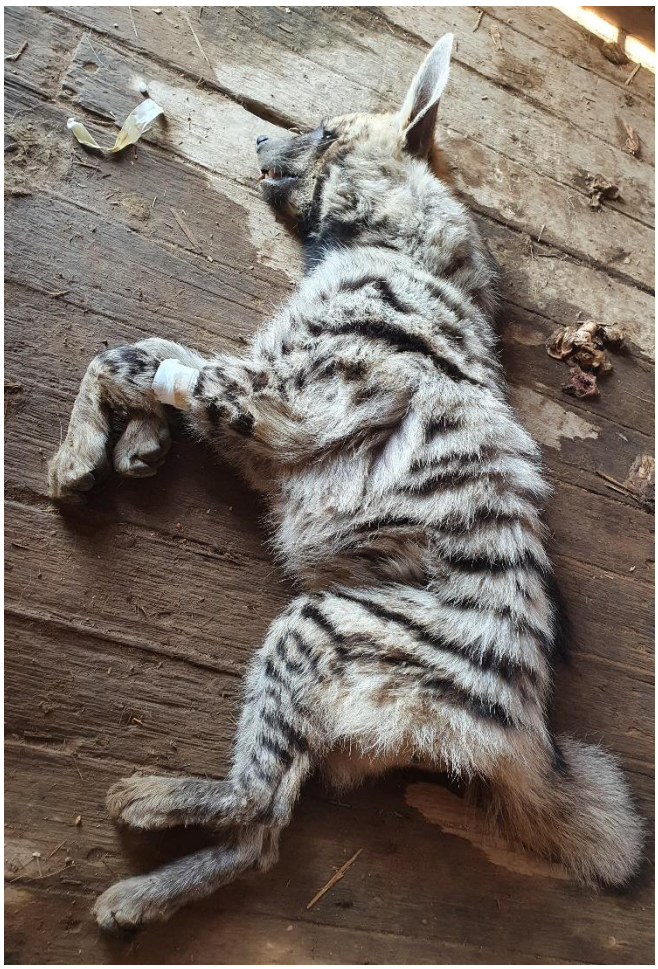

Figure 2. The Striped hyena No. 2, suspected of pesticide intoxication.

The third individual was immobilized with a combination of ketamine (3 mg/kg) and medetomidine (0.04 mg/kg) by remote drug delivery using a blowpipe and injecting the anesthetic compound into the right hindquarters. In this case, because a vast number of people gathered around the hyena and the pool before injecting immobilizing drugs, the anesthesia induction period was prolonged and took 18 minutes. Afterwards, the animal was taken out of the pool and after receiving fluid therapy and supportive treatment, it was transferred to the carrying cage. The hyena was an adult female and after a physical examination and ensuring the health of the animal and injecting atipamezole (0.2 mg/kg) to reverse anesthesia, the hyena was released in a protected region that was just 20 minutes away from the area where the animal had been found. Some details of the chemical immobilizations of the hyena (number 3) (Figure 3) were mentioned in Tables 5 and 6.

Clinical Relevance

The most commonly used agent for immobilization in the field, however, is a combination of tiletamine hydrochloride and zolazepam hydrochloride,[8] but it is very tough to access this combination in Iran. Besides tiletamine/zolazepam, using a dissociative agent like ketamine + α2-adrenergic agonists like medetomidine or xylazine for immobilization in carnivores is common. Moreover, combinations of medetomidine, butorphanol and midazolam or diazepam have been used successfully to immobilize domestic and exotic carnivore species as well, such as the red wolf (*Canis rufus*), African wild dog (*Lycaon pictus*), Cheetah (*Acinonyx jubatus*) and California sea lion but using this protocol in hyenas especially striped hyena anesthesia is not common. Spotted hyaena has been anesthetized successfully with ketamine at a dosage rate of 7–15 mg/kg,[9] Medetomidine 0.04 mg/kg with 3 mg/kg ketamine,[10] zoletil 4 mg/kg,[11] and ketamine 4-6 mg/kg with xylazine 1 mg/kg,[8] in many studies. However, references for the anesthesia of striped hyenas are scarce. In one study the effective chemical immobilization of Arabian striped hyena by two protocols of ketamine (2.27 ± 0.04 mg/kg) -medetomidine (0.04 mg/kg) and ketamine (4.95 ± 0.11 mg/kg) -xylazine (0.99 ± 0.02 mg/kg) has been compared.[2]

Table 5. Time variables for striped hyena Number 3 immobilized with KM.

Duration category	Ketamine + Medetomidine (Min)
Induction Time	18
Anesthesia maintenance	48
Full recovery	10

Table 6. Vital signs o striped hyena number 3 immobilized with KM.

Variables	Ketamine + Medetomidine
Rectal Temperature (°C)	37.5
Heart Rate (beats/min)	56-57
Respiration Rate (breaths/min)	34-35
SpO2 (%)	90-91

Figure 3. The Striped hyena No. 3 rescued from a water storage pool.

The combination of ketamine and medetomidine was the only protocol used in all three individuals. In the first case, this protocol was used twice. The mean induction time of anesthesia with this protocol in the first two cases was about 10 minutes. In the third case, due to the stress caused by making a lot of noise and the presence of people around the hyena, the activity of the sympathetic nervous system has probably increased and as a result, the release of epinephrine in the blood before darting the animal has been enhanced. Since extremely agitated individuals may have a decreased response to medetomidine [5] and α2 adrenergic agonists are not able to have optimal effects in the presence of epinephrine,[12] the induction time took 18 minutes. The combination of ketamine-xylazine was used in two cases. The mean induction time with this protocol is 10 minutes. Induction of anesthesia with the combination of butorphanol-medetomidine-midazolam has shown a shorter time compared to the other two protocols.

Rectal temperature with the use of ketamine-xylazine dropped below 37 °C. In combination with ketamine, xylazine and atropine the rectal temperature was around 37.1 °C. The rectal temperature was 37.3-37.9 in all other cases using two other protocols. Since it has been shown that xylazine causes a decrease in body temperature,[13] this reduction in body temperature, compared to the other two anesthetic protocols, was probably one of the side effects of xylazine.

The heart rate as a result of using a combination of ketamine, xylazine and atropine was higher than in all other protocols. As xylazine decreases the heart rate and cardiac output,[14] sometimes atropine is added to the immobilization protocol to reduce the cardiovascular side effects of xylazine.[4] Therefore, it can be concluded that one of the reasons for the increasing heart rate in combination with ketamine-xylazine-atropine compared to ketamine-xylazine without atropine was probably the result of adding atropine. The respiratory rate and percentage of SpO_2 in immobilization with ketamine and xylazine were lower than in other protocols. Respiratory suppression is one of the side effects of xylazine,[4] and it seems respiratory suppression in both cases has been caused by using xylazine in an anesthetic cocktail.

Moreover, xylazine causes vomiting in many animal species presumably through its effect on the chemoreceptor trigger zone (CTZ).[6,9] We used xylazine in anesthetic protocols for two different individuals with different histories and health conditions. Both individuals vomited after anesthesia. In another study, the occurrence of vomiting in Arabian striped hyenas after immobilization with xylazine has been reported.[2]

The effects of medetomidine and xylazine can be reversed by using selective α2-adrenoceptor antagonists such as atipamezole hydrochloride. To reverse the effects of the combination of BMM (butorphanol, medetomidine, midazolam) three different antidotes naltrexone, atipamezole and flumazenil have been used. All antidotes were injected intramuscularly. The full recovery time in two combinations of ketamine-medetomidine and BMM (butorphanol, medetomidine, and midazolam) has not had any significant differences. But in combination with ketamine-xylazine (also with the addition of atropine) in both cases of using this combination, full recovery took a little longer (14-15 minutes).

In conclusion, all three combinations resulted in reliable levels of immobilization but the side effects driven by the combination of ketamine and xylazine significantly outnumbered those of the other two combinations. Vomiting, decreasing the percentage of SpO_2, having a longer recovery time and mitigating body temperature were some of the most important adverse effects of using xylazine in a chemical immobilization cocktail. Both combinations of ketamine + medetomidine and butorphanol + medetomidine + midazolam have been safe with minimal side effects and favorable recovery times. Despite the fact that the difficult availability of newer immobilization agents is a limiting factor in some countries and parts of the world, it seems that due to the presence of newer and more specific α2-adrenergic agonists, using xylazine as the oldest α2-adrenergic agonist is not logical in many conditions and species.

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Conflict of Interest

None to declare.

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