



Chemical immobilization of free-ranging and captive Sunda clouded leopards (*Neofelis diardi*) with two anesthetic protocols: medetomidine-ketamine and tiletamine-zolazepam

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ABSTRACT. There is currently no available information regarding the veterinary management of Sunda clouded leopards (*Neofelis diardi*), either in captivity or in the wild. In this study, 12 Sunda clouded leopards were anesthetized between January 2008 and February 2014 for medical exams, and/or GPS-collaring. Seven wild-caught individuals were kept in captivity and 5 free-ranging animals were captured by cage traps. Two anesthesia combinations were used: medetomidine-ketamine (M-K) or tiletamine-zolazepam (T-Z). Atipamezole (0.2 mg/kg im) was used as an antagonist for medetomidine. Medetomidine (range: 0.039–0.054 mg/kg) and ketamine (range: 3–4.39 mg/kg) were administered during 5 immobilizations, resulting in median induction times of 7 min. After a median anesthesia time of 56 min, atipamezole was injected, observing effects of antagonism at a median time of 12 min. T-Z (range: 6.8–10.8 mg/kg) was administered on 7 occasions. Median induction times observed with this combination were shorter than with M-K (4 min vs 7 min; $P=0.04$), and anesthesia and recovery times were significantly longer (244 and 35 min vs 56 and 16 min, respectively; $P=0.02$). Lower heart rates were measured in the M-K group, while lower rectal temperatures were found in the T-Z group. Both combinations resulted in safe and reliable immobilizations, although given the favorable anesthesia and recovery times of M-K, we recommend this approach over T-Z for the veterinary handling of Sunda clouded leopards.

KEY WORDS: anesthesia, medetomidine, *Neofelis diardi*, Sunda clouded leopard, tiletamine-zolazepam

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Chemical immobilization of free-ranging and captive wild felids is undertaken to perform procedures, such as physical examinations, blood collection and collaring for remote-tracking studies, as well to ensure the safety of the personnel and animals involved while decreasing the stress of the animals [3]. Combinations of medetomidine-ketamine and tiletamine-zolazepam have both been used successfully in several species of exotic carnivores [10, 13, 15, 17]. Medetomidine is a potent alpha-2 agonist, which, combined with the dissociative agent ketamine, typically results in safe anesthesia with smooth induction and good muscle relaxation [9, 10]. Furthermore, its anesthetic effects can be antagonized with the use of atipamezole [9, 10]. Tiletamine is a dissociative agent, only available commercially together with the muscle relaxant zolazepam, as Zoletil© or Telazol©. This combination has been used in certain species of Southeast Asian felids, where it has resulted in short induction times [8]. Since

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tiletamine-zolazepam is produced in a commercially available powder mixture, it can be reconstituted in reduced volumes, allowing its use in small darts [8].

The Sunda clouded leopard (*Neofelis diardi*) is a medium-sized felid that only inhabits the Sundaic islands of Borneo and Sumatra in Southeast Asia. There are no captive populations of this cat outside of its range countries, which are Brunei, Indonesia and Malaysia. The Sunda clouded leopard is considered *Vulnerable* by the International Union for Conservation of Nature [12], yet its biology is poorly known, hampering the development of conservation management efforts [11]. In addition, there is no available information regarding the veterinary management of this species, either in captivity (*ex-situ*) or in the wild (*in-situ*). The only published recommendation regarding immobilization protocols for clouded leopards [8] refers to the mainland clouded leopard (*Neofelis nebulosa*), which is a distinct species [2, 4]. Therefore, species-specific information about the response of the Sunda clouded leopards to different anesthesia protocols is of value.

In this study, we describe the chemical immobilization of free-ranging and captive Sunda clouded leopards using two anesthetic protocols: 1. a combination of medetomidine and ketamine (partially antagonized with atipamezole) and 2. tiletamine-zolazepam. We compare the physiological responses of Sunda clouded leopards to these two immobilization protocols and provide the first insights of species-specific anesthetic recommendations.

MATERIALS AND METHODS

Since 2008, The Bornean Wild Cats Veterinary Project has provided veterinary assistance for ecological studies of Sunda clouded leopards in Sabah, Malaysian Borneo, carried out by researchers from Wildlife Conservation Research Unit (WildCRU, University of Oxford), Sabah Wildlife Department and Danau Girang Field Centre (Cardiff University). Moreover, the project aimed to improve knowledge regarding the veterinary management of the captive population of this species in Malaysia and Indonesia. The capture and handling protocol was approved by the Sabah Biodiversity Council and the Sabah Wildlife Department; the research methodology was reviewed and approved by the University Complutense's Committee on Animal Care (Department of Animal Physiology, Faculty of Veterinary Medicine).

This study reports the anesthesia effects of two protocols in free-ranging and captive Sunda clouded leopards. The free-ranging animals ($n=5$) were captured in Sabah, Malaysian Borneo, between January 2008 and March 2014 during intermittent live-trapping operation periods. Steel mesh cage traps ($2.5 \times 1 \times 1$ m) were deployed in the Danum Valley Conservation Area/Ulu Segama Forest Reserve ($4^{\circ} 58' N$, $117^{\circ} 46' E$ / $4^{\circ} 59' N$, $117^{\circ} 52' E$) [11] and the Lower Kinabatangan Wildlife Sanctuary ($5^{\circ} 24' N$, $118^{\circ} 02' E$). Cage traps were located in areas where previous activity of clouded leopards had been recorded via camera-traps, and traps were camouflaged and protected from direct sunlight and rain whenever possible. Cage traps were monitored daily at first light, to reduce holding times. Once a Sunda clouded leopard was trapped, two researchers approached the trap to estimate each individual's body weight in order to calculate the doses of drugs to administer. Drug mixtures and darts were prepared out of sight of the captured clouded leopard to avoid additional stress. Drugs were injected intramuscularly to the hindquarters via blow pipe with 5 ml darts (Telinject, Dudenhofen, Germany) fitted with 1.2×38.1 mm needles (Sterile Monoject, Telinject, Germany).

We followed two anesthesia protocols: medetomidine-ketamine (M-K) and tiletamine-zolazepam (T-Z). We aimed to administer dosages of medetomidine (Dorbene® 1 mg/ml, Pfizer, Madrid, Spain) between 0.04–0.05 mg/kg and doses of ketamine (Imalgene 1000® 100 mg/ml, Rhone Merieux, Lyon, France) between 3–5 mg/kg. To antagonize the effects of medetomidine, we used atipamezole (Alzane® 5 mg/ml, Pfizer, Spain), at 0.2 mg/kg. We administered atipamezole intramuscularly via hand-held syringe to avoid cardiovascular side effects or super excitement occasionally documented with intravenous administration [13, 20]. For the tiletamine-zolazepam mixture, we used Zoletil 100® (Virbac SA, Carros, France). Zoletil 100® contains 250 mg tiletamine in powder (as hydrochloride) and 250 mg zolazepam in powder (as hydrochloride) per vial, and 5 ml of solvent is sterile water for injection. After the addition of these 5 ml of sterile liquid to the powder, the solution contains 50 mg tiletamine and 50 mg zolazepam /ml. We aimed for dosages of tiletamine-zolazepam between 6.5–10 mg/kg, beginning by the higher dose.

All captive animals ($n=7$) were wild-born, then confiscated by local authorities (from illegal trade) and finally housed in wildlife rescue centers ($n=5$) and local zoos ($n=2$) in Malaysian Borneo and Indonesia. Captive animals were fed whole chickens or chicken carcasses. Anesthesia and sampling of captive animals took place between January 2011 and November 2013. Animals were fasted 12–14 hr prior to immobilization. Drug administration followed the same protocol as for the free-ranging individuals.

To study the anesthesia effects of both drug combinations, we registered the following times: induction time (time from drug's injection until the animal's head rests on the floor), anesthesia time (time from when animal's head is on the floor until it is able to lift it) and recovery time (time from when the animal holds its head up until it is able to stand). For clouded leopards anesthetized with M-K, anesthesia time was defined as the time since the animal's head rests on the floor until atipamezole was injected. In the M-K group, we also recorded antagonist time, which was the time from atipamezole administration until the animal was able to lift its head from the floor. Once a clouded leopard's head was down and it displayed no auricular reflex, it was placed in lateral recumbency to obtain the biological samples and to fit the GPS-collars (free-ranging individuals). We placed clouded leopards in a shady area (free-ranging animals) or inside a well ventilated room (captive animals). Eye lubricating ointment was applied to all animals, and individuals were subsequently blindfolded to minimize visual stimuli during anesthesia.

The following physiological parameters were measured during anesthesia: respiratory rate, heart rate, rectal temperature and oxygen saturation. To measure the respiratory rate, we observed the thoracic wall's movements for 30 sec. We used a hand-held pulse oximeter (Nellcor® Oximax N-65, Nellcor Inc., Pleasanton, CA, U.S.A.) with a veterinary probe attached to the animal's tongue (Vetsat, Nellcor Inc.) to measure oxygen saturation (SpO₂). To measure heart rate, we used a stethoscope, palpation of the



Fig. 1. Free-ranging Sunda clouded leopard (*Neofelis diardi*) anesthetized and fitted with a GPS-collar.

femoral pulse and/or a pulse-oximeter. We used a digital thermometer to measure rectal temperature. We recorded all parameters every ten min. Muscular relaxation was subjectively measured by the control of the muscular tone in one of the hind limbs [5]. We evaluated the pain perception by withdrawal reflex during venipuncture [24]. Capillary refill time was evaluated for each individual. Any lesions on the body or teeth fractures found during the medical exam were recorded. Study animals were weighed, and their body condition assessed according to the following criteria: 1/5 was defined as cachexia, 2/5 as underweight, 3/5 as ideal, 4/5 as overweight and 5/5 as obese. Standard body measures were taken, hair was collected for genetic studies, blood samples, feces and urine were collected whenever possible for disease studies, and a medical exam was performed on each individual. All free-ranging clouded leopards were fit with a GPS-collar (Fig. 1), with the exception of one female, whose weight and overall body condition precluded attachment. Upon completion of the medical exam and/or GPS-collar fitting, individuals were returned to the cages and atipamezole administered as relevant. Animals were released only after they demonstrated the ability to stand with no wobbling.

The anesthesia data were analyzed using the software SPSS (IBM SPSS Statistics for Windows, Version 21.0. IBM Corp., Armonk, NY, U.S.A.). Two test groups were considered (M-K and T-Z), and non-parametric Mann-Whitney *U*-tests were used to evaluate differences between the groups. Values are expressed as median, 10th percentile (10thPercent.) and 90th percentile (90thPercent.). *P*-values ≤ 0.05 were considered statistically significant. Due to the small number of individuals within the study, we did not distinguish between captive and free-ranging animals. During the medical exam, all individuals were considered healthy, and no abnormalities that could affect the anesthesia process were observed.

No mortality was associated in any animal after 6 months of follow ups. Follow up in free-ranging clouded leopards was performed by camera-trap derived photographs and/or GPS telemetry.

RESULTS

We collected data from 12 different Sunda clouded leopard individuals (Table 1). Males' weight ranged between 15 to 25.2 kg, and females' weight ranged between 9.1 to 18 kg. According to the tooth wear and body weight of the animals, every individual was considered adult (>2 years old) [21].

Chemical immobilization

M-K Group: This group ($n=5$; four free-ranging individuals and one captive individual) received doses of medetomidine ranging between 0.039–0.054 mg/kg (median: 0.04 mg/kg; 10th Percent.: 0.03; and 90th Percent.: 0.05) plus a dose of ketamine ranging between 2.42–4.39 mg/kg (median: 3.17 mg/kg; 10th Percent.: 2.65; and 90th Percent.: 4.23). Atipamezole doses ranged from 0.19–0.27 mg/kg (median: 0.2 mg/kg; 10th Percent.: 0.19; and 90th Percent.: 0.25). The first sign of drug effect was ataxia. All individuals experienced a smooth induction, laying in sternal recumbency. Capillary refill time was <2 sec for each animal. Muscle relaxation was considered good given the lack of both muscular tone in the hind limbs and resistance while opening the mouth [5]. Withdrawal reflex was not perceived in any animal during venipuncture. From this group, one individual's body condition was

Table 1. Sunda Clouded leopard (*Neofelis diardi*) examination results

ID	Sex	Weight (kg)	Corporal condition	Fractured canines	Estimated age (years)
M3-Fr	Male	25.0	3/5	Left lower canine	5–6
M4-Fr	Male	25.2	3/5	-	2–3
F1-C	Female	18.0	5/5	-	3–4
F3-Fr	Female	9.1	2/5	Both lower canines	7–8
F2-C	Female	13.4	4/5	-	3–4
M2-C	Male	21.8	3/5	-	4–5
M1-Fr	Male	24.0	3/5	-	3–4
M1-C	Male	15.0	2/5	-	4–5
M2-C	Male	21.5	3/5	Left upper canine; both lower canines	6–7
M3-C	Male	18.0	2/5	-	2–3
F3-C	Female	16.0	5/5	4 canines	8–9
F1-Fr	Female	12.0	3/5	-	2–3

ID: (Fr, Free Ranging; C, Captive), Corporal Condition: (2/5 Underweight, 3/5 Ideal, 4/5 Overweight, 5/5 Obese).

Table 2. Physiological parameters of Sunda clouded leopards (*Neofelis diardi*) anesthetized with a combination of medetomidine-ketamine (M-K) and tiletamine-zolacepam (T-Z)

Physiological parameter	M-K					T-Z				
	Median	P10th	P90th	Range	n	Median	P10th	P90th	Range	n
Estimated age (years)	4.5	2.7	7.3	2–8	5	4	3	7.7	2–9	7
Female weight (kg)	11.25	9.53	12.97	9.1–13.4	2	16	12.8	17.6	12–18	3
Male weight (kg)	25	24.2	25.16	24–25.2	3	19.75	15.9	21.71	15–21.8	4
Heart beats per min	87	78.4	107.8	74–109	5	142	126	174.4	126–196	7
Respirations per min	14	10.4	32.2	8–36	5	14	12	23	10–26	7
Oxygen saturation (%)	94	89.4	96.6	87–99	4	94	92.8	95.4	91–99	3
Temperature (°C)	38.65	37.7	38.96	37.7–39.8	5	37.8	36.78	38.2	36.6–38.2	7

P10th, 10th percentile; P90th, 90th percentile.

Table 3. Anesthesia results of Sunda clouded leopards (*Neofelis diardi*) anesthetized with a combination of medetomidine-ketamine (M-K) and tiletamine and zolacepam (T-Z)

	M-K					T-Z					P
	Median	P10th	P90th	Range	n	Median	P10th	P90th	Range	n	
Dose (mg/kg)	0.04 (M) 3.17 (K) 0.20 (A)	0.03 (M) 2.65 (K) 0.19 (A)	0.05 (M) 4.23 (K) 0.25 (A)	0.03–0.05 (M) 2.42–4.39 (K) 0.19–0.27 (A)	5	7.62	6.9	9.3	6.8–10.8	7	
Induction time (min)	7	4.2	11.2	3–14	5	4	2.6	10	2–10	7	0.04 ^{a)}
Anesthesia time (min)	56	52.8	81.8	52–93	5	244	224.8	510.4	220–577	7	0.02 ^{a)}
Antagonist time (min)	12	9.4	12.6	9–13	5	NA	NA	NA	NA		NA
Recovery time (min)	16	13.2	33.6	12–44	5	35	25.4	35.8	23–36	6	0.02 ^{a)}

(M), Medetomidine; (K), Ketamine; (A), Atipamezole; P10th, 10th percentile; P90th, 90th percentile; NA, Not applicable. a) $P < 0.05$, statistically different.

considered 2/5 and another as 4/5; all other study animals were considered to be in ideal body condition (Table 1). Two individuals presented with old and mild fractures of the lower canines. No animals displayed injuries related to trap confinement. One individual was found to have a shotgun pellet embedded in the subcutaneous tissue of the right forelimb.

Physiological parameters monitored during M-K anesthesia are shown in Table 2, and times recorded during the anesthesia are shown in Table 3. Antagonist time ranged between 9–13 min (median: 12 min.; 10th Percent.: 9.4; and 90th Percent.: 12.6). After atipamezole administration, animals first showed parpebral reflex, then auricular reflex and finally lifted their heads. Within 30 min (median 16 min.; 10th Percent.: 13.2; and 90th Percent.: 33.6), every animal was standing. Recovery was smooth and during this time, and whenever possible, animals remained in dark and quiet areas.

T-Z Group: Seven individuals (one free-ranging individual and six captive individuals) received doses of T-Z ranging between 6.8–10.8 mg/kg (median: 7.62 mg/kg; 10th Percent.: 6.9; and 90th Percent.: 9.3). As with the M-K group, the first drug effect observed was ataxia. Induction was smooth, and all animals were extracted from the trap or cage in less than 10 min. Five individuals were found in sternal recumbency and two in lateral recumbency. Capillary refill time was <2 sec for each animal. Muscular relaxation was considered good in almost all anesthesia events. One male (M1-C), which received a dose of 6.8 mg/

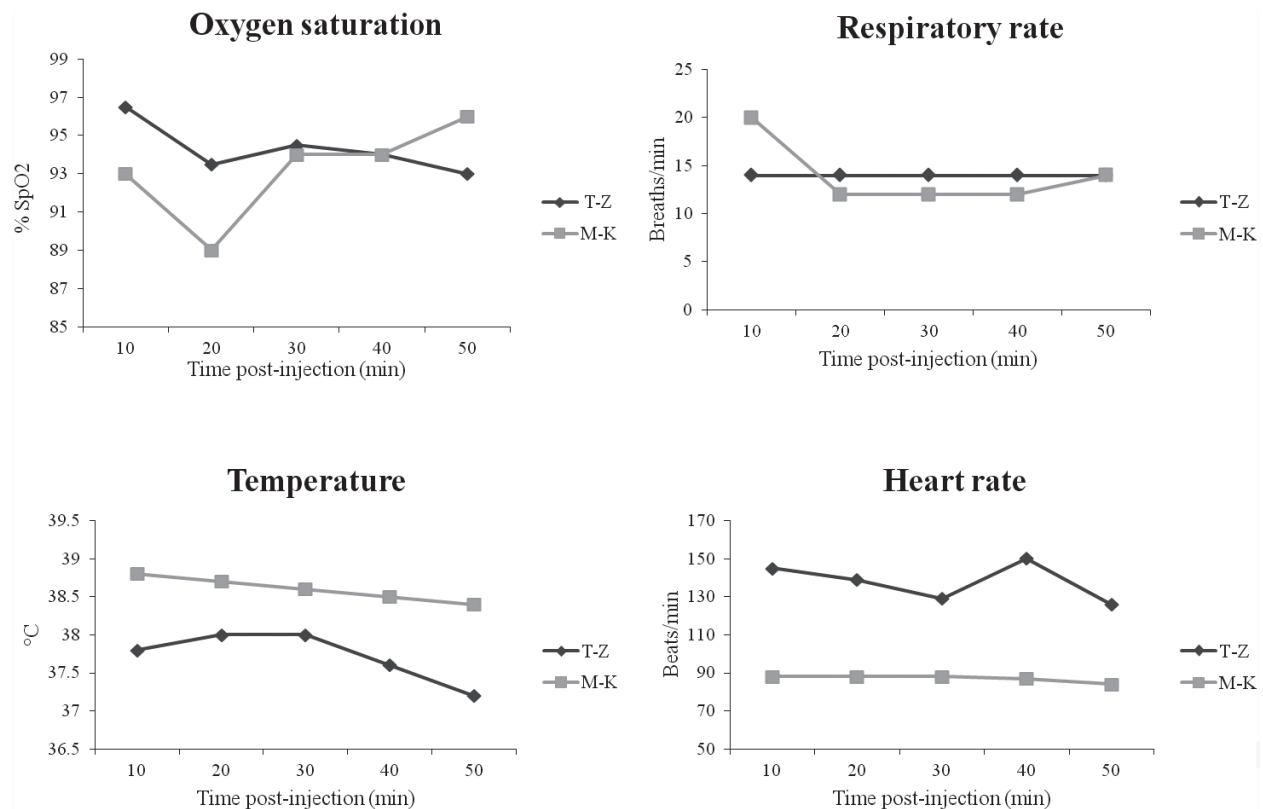


Fig. 2. Heart rates, respiratory rates, SpO2 and rectal temperatures in Sunda clouded leopards (*Neofelis diardi*) anesthetized with medetomidine-ketamine (M-K) and tiletamine-zolazepam (T-Z). Values presented as median.

kg, displayed muscular rigidity in the hind limbs. No clouded leopards showed pain perception during the venipuncture. The physical exam revealed 2 individuals had old canine fractures. The body conditions of two individuals were considered as 2/5, two were considered as 5/5, and three were considered to have an ideal corporal condition (Table 1). Physiological parameters registered during T-Z anesthesia are shown Table 2, and times recorded during the anesthesia are shown in Table 3. In this group, samples were obtained and/or GPS collars were fitted in less than 90 min, although anesthesia times ranged between 220 and 577 min. This upper anesthesia time was recorded from the first trapped female that received a dose of 10.8 mg/kg [11], following recommendations found in the literature for free-ranging mainland clouded leopards [8]. In order to decrease anesthesia times while using T-Z, lower doses were given to the individuals subsequently immobilized.

Comparison between M-K and T-Z groups: Induction, anesthesia and recovery times were significantly different between the two groups. T-Z group showed a shorter induction time and longer anesthesia and recovery times, although anesthesia times in the M-K group followed administration of an antagonist. Lower heart rates were measured in the M-K group ($P=0.02$), and lower rectal temperatures were found in the T-Z group ($P=0.04$) (Fig. 2), but no differences in the other physiological parameters were found between groups.

DISCUSSION

This is the first study to investigate Sunda clouded leopard responses to chemical immobilization protocols. Given the results, both anesthetic combinations were considered safe and reliable for the chemical immobilization of Sunda clouded leopards. T-Z anesthesia resulted in shorter induction times than M-K anesthesia. In a previous study of free-ranging mainland clouded leopards in Thailand, T-Z combinations were preferred over xylazine and ketamine due to shorter induction times and smaller required drug volumes [8]. However, in our study, anesthetic times using T-Z at the dosage recommended by Grassman *et al.* [8] (10 mg/kg) resulted in prolonged anesthesia and recovery times. One of the free-ranging Sunda clouded leopards (F1-Fr) received a total dose of 10.8 mg/kg of T-Z resulting in 577 min of anesthesia time. Such a long anesthesia time is unacceptable for short procedures and presents unnecessary risks to the animal. Our small sample size prevented us from establishing whether the lengthy anesthesia and recovery time was a species-specific or individual response to this drug combination. Nevertheless, in subsequent immobilizations, T-Z doses were decreased, and acceptable recovery times documented. Long T-Z recovery times in felids are due to the metabolism of the zolazepam, which has a plasma half-life of 4.5 hr, which is longer than that of tiletamine [5, 16]. Nevertheless, T-Z combinations have a wide safety margin, as shown in previous research with other wild felids [6]. Thus, in Sunda clouded leopards, we recommend T-Z doses of 8 mg/kg, as this results in adequate anesthesia depth and times for typical purposes, such as sampling

and radio-collaring.

When administering M-K, we used lower dosages than those described by Fletchall [7] for captive mainland clouded leopards, who recommend doses of 0.05–0.08 mg/kg. In our study, a dose of medetomidine of 0.04 mg/kg combined with ketamine at 3 mg/kg was very effective and as such are recommended for future practitioners. Using atipamezole as an antagonist agent was advantageous as it shortened both anesthesia and recovery times. While using M-K, induction times were statistically longer ($P=0.04$) than those for T-Z. Longer induction times have been described with an alpha-2 agonist combined with ketamine than when using T-Z [8].

Although in both groups, the processing time (time to collect samples, take body measures or fit the GPS-collar) never exceeded 90 min, while working with the M-K group, we attempted to inject the atipamezole approximately one hr after the administration of the drug combination. Atipamezole was administered intramuscularly at 0.2 mg/kg, which is 5 times the dose of medetomidine used. Although this dosage has been used in felid species with no reported side effects [14, 15], in domestic cats and lions (*Panthera leo*), it has been linked to tachycardia [1, 13, 22, 23]. Even though that dose was considered effective, we were unable to monitor heart rate during the recovery time to assess if the Sunda clouded leopards presented tachycardia, and thus we recommend the use of lower doses in future research involving this species. Once atipamezole was administered, clouded leopards showed antagonist effects in less than 13 min, such as blinking, ear twitching and/or head movements. The shorter recovery times allowed us to release the clouded leopards back into the wild sooner than when using T-Z.

To date, there is no available literature regarding the physiological parameters observed during anesthesia in either Sunda clouded leopards or mainland clouded leopards.

In this study, we found significant differences between some of the physiological parameters measured during the chemical immobilization with both drug combinations. Heart rates were lower ($P=0.02$) in the M-K group. This difference may primarily be due to the side effects of medetomidine in the cardiovascular system [13, 20]. Nonetheless, we did not register bradycardia in any of the heart rates obtained during the M-K anesthesia, as heart rates ranged from 74 to 109 heart-beats per min. However, heart rate baselines in Sunda clouded leopards are not available for comparison. Medetomidine can result in peripheral vasoconstriction and hypotension, which in our case made vein visualization difficult for blood extraction [6, 15, 18]. Lower rectal temperatures were measured in the T-Z group ($P=0.04$), although this difference may be caused by environmental factors. Most (4 of 5 cases) of the immobilizations carried out with M-K were carried out under field conditions. In humid/hot areas, felids pant as a mechanism to regulate the corporal temperature and dissipate heat, but during anesthesia, this response is compromised, resulting in increased body temperatures [6]. In our study, one of the clouded leopards (M1-Fr) was immobilized during the middle of the day, and its temperature rose up to 39.8°C. We found no evidence of differences in respiratory rates between groups, and rates were similar to those reported during the chemical immobilization with T-Z or medetomidine and T-Z combinations in other wild felids [6]. We found no difference in partial oxygen saturation between groups, and it was considered adequate during all anesthesia events, although peripheral vasoconstriction or hypotension induced by medetomidine may affect the normal functioning of pulse oximeters [6, 19]. For further studies, we recommend the measurement of arterial blood gases as a more accurate measure of oxygen saturation [14].

We conclude that both T-Z and M-K anesthetic combinations are safe for the *in-situ* and *ex-situ* chemical immobilization of Sunda clouded leopards, and both result in smooth inductions. We suggest administering dosages of T-Z at 8 mg/kg dosages when longer procedures, such as GPS collaring, are planned. Medetomidine (0.04 mg/kg) and ketamine (3 mg/kg) produce acceptable anesthetic depth to carry out routine procedures in captive settings or under field conditions without cardiovascular side effects. Although atipamezole administered at 5 times the dose of medetomidine was adequate to reverse the effects of medetomidine in Sunda clouded leopards, reduced doses would need to be evaluated to avoid possible side effects like tachycardia. It is highly recommended to record the physiological parameters during the chemical immobilization procedure to avoid any potential abnormalities that could arise.

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