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Abstract

Nine wild Bornean leopard cats were anesthetized using a combination of tiletamine and zolazepam (Zoletil®) after being captured in humanely-designed live traps in Sabah, Malaysian Borneo, for the purpose of fitting radio-collars. For five leopard cats (group 1) a single dose of 6.92±1.06 mg/kg of Zoletil® was administered. The mean induction time from the initial Zoletil® dose was 7.9 ± 1.77 minutes, and the mean anesthesia time was 47.2 ± 25.1. For 4 leopard cats (group 2) after an initial mean dose of 6.92±1.06 mg/kg of Zoletil®, it was necessary to administer a second dose (or booster) of Zoletil® (mean dose 2.6±0.33 mg/kg) or ketamine (mean dose 3.5± 0.05mg / kg) to achieve complete immobilization. There were differences between the periods of anesthesia resulting from these boosters, which were 43.5 ± 2.1 minutes for ketamine and 89.5 ± 6.36 minutes for Zoletil®. We conclude that an initial dose of Zoletil® of 6.92 mg/kg can produce an adequate plane of anaesthesia without needing additional or booster injections of anaesthetic; if a booster is required, the use of ketamine in preference to Zoletil® has the benefit of shorter release times (245 minutes for ketamine booster compared to 350 minutes for Zoletil® booster) whilst providing adequate anesthetic times (mean 43.5 minutes for ketamine booster).

Keywords: Chemical immobilization, Ketamine, leopard cat, *Prionailurus bengalensis borneonensis*, Tiletamine, Zolazepam

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บทคัดย่อ

การใช้ยา Tiletamine และ Zolazepam ในการควบคุมแมวดาวสายพันธุ์บอร์เนียว (*Prionailurus bengalensis borneoensis*) ในการปฏิบัติงานในท้องที่เกาะบอร์เนียว

Nájera Fernando^{1,2} Cediél-Algovia Rafael² Hearn Andrew^{1,3} Ross Jo^{1,3} Dench Rosalie¹ Alcázar Paloma¹ Nathan Senthivel³ de Gaspar Iñaki² Revuelta Luis²

การศึกษานี้ได้วางยาสลบแมวดาว สายพันธุ์บอร์เนียวจำนวน 9 ตัว ด้วยการให้ Zoletil[®] ซึ่งเป็นตัวยาที่ผสมระหว่างไทล์ทามีน (tiletamine) กับโซลาซีแพม (zolazepam) หลังจากที่จับได้จากการวางกับดักที่สร้างขึ้นเองในรัฐซาบาห์ บนเกาะบอร์เนียวของมาเลเซีย เพื่อติดตั้งวิทยุติดตามตัวแบบปลอกคอ โดยกลุ่มที่ 1 ประกอบด้วยแมวดาวจำนวน 5 ตัว วางยาด้วยการให้ Zoletil[®] ขนาดยา 6.92±1.06 มก./กก. เพียงขนานเดียว ค่าเฉลี่ยการเหนียวนำ (ทำให้สลบ) นับตั้งแต่เริ่มให้ Zoletil[®] คือ 7.9±1.77 นาที และค่าเฉลี่ยระยะเวลาการสลบ คือ 47.2±25.1 นาที กลุ่มที่ 2 ประกอบด้วยแมวดาวจำนวน 4 ตัว หลังจากที่มีการให้ Zoletil[®] ในปริมาณ 6.92±1.06 มก./กก. ไปในครั้งแรกแล้ว จำเป็นต้องมีการเติมยาเสริมเข้าไป ด้วยการให้ Zoletil[®] (ค่าเฉลี่ยขนาดยา 2.6±0.33 มก./กก.) หรือ ketamine (ค่าเฉลี่ยขนาดยา 3.5±0.05 มก./กก.) เพื่อให้เกิดการสลบโดยสมบูรณ์ พบว่ามีความแตกต่างกันของระยะเวลาสลบ โดยการใช้ ketamine ทำให้สลบนาน 43.5±2 นาที และ Zoletil[®] ทำให้สลบนาน 89.5±6.36 นาที ผู้ศึกษาจึงมีความเห็นว่า การใช้ Zoletil[®] ในครั้งแรกที่ระดับ 6.92±1.06 มก./กก. ก็เพียงพอสำหรับการทำให้สลบโดยไม่ต้องมีการเติมยาเพื่อการสลบอีก ถ้าจำเป็นต้องมีการเติมยา ควรใช้ ketamine แทนการใช้ Zoletil[®] เพราะสามารถทำให้สัตว์ฟื้นตัวและปล่อยได้เร็วกว่า (245 นาที เมื่อเติมยาด้วย ketamine เปรียบเทียบกับ 350 นาที เมื่อใช้ Zoletil[®] เป็น booster) ขณะที่ยังคงสามารถทำให้เกิดการสลบได้อย่างสมบูรณ์ (ค่าเฉลี่ย 43.5 นาที เมื่อใช้ ketamine เป็น booster)

คำสำคัญ: การใช้สารเคมีในการบังคับ เคนตามีน แมวดาว *Prionailurus bengalensis borneonensis* ไทล์ทามีน โซลาซีแพม

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Introduction

Tiletamine is an anesthetic agent chemically related to ketamine, both are considered dissociative agents. Zolazepam is a diazepamone minor tranquilizer. Pharmacology of this drug combination is similar to that shown by the combination of ketamine and diazepam (Plumb, 2005). Tiletamine and zolazepam have been used extensively for chemical restraint of several species of non-domestic felines (Deem et al., 1998; Shindle and Tewes, 2000; Kreeger, 2002; Grassman et al., 2004). Among the advantages reported by authors using Zoletil[®] to perform anesthesia in wild cats in field conditions, two important features are its wide safety margin and the short induction period (Shindle and Tewes, 2000; Grassman et al., 2004). Recovery time when using this drug can be decreased using flumazenil (Spelman et al., 1997). Bornean leopard cat (*Prionailurus bengalensis borneoensis*) is the only species of the Bornean felids which remains listed as *Least Concern* by the IUCN (IUCN, 2011). Within the techniques to study the

ecology of leopard cats in the wild, radiotelemetry is widely used, by means of attaching a collar with a radio transmitter to the target animal after its chemical immobilization (Sunquist and Sunquist, 2002).

There are few publications with focus on the field anesthesia of leopard cats in the wild (Grassman et al., 2004). In this study we assessed the use of Zoletil[®] to achieve chemical immobilization of wild leopard cats and compared the effects of booster doses of Zoletil[®] or ketamine on both anesthesia and release times.

Materials and Methods

The capture and chemical immobilization of leopard cats was carried out as part of an ecological study of this species in the Ulu Segama Forest Reserve, Sabah, Malaysian Borneo between May 2008 and March 2009. We used live-traps of various sizes, all were cage-style and triggered by a treadle

mechanism at the back of the trap which closed the single sliding door once the cat had stepped on the treadle. A sheet of plywood was used as a roof to give protection from the rain. We baited the traps with live bait (rats or chickens) or electronic sound lure or a combination of both. The live bait was housed in a separate compartment attached to the rear of the trap and was protected from the elements. Food and water was provided daily. We selected trap sites based on previous observations of leopard cats, from actual sightings and/or camera-trap photography. The traps were completely or partially camouflaged with local vegetation and were visited daily between 08:30 am and 10:30 am to check for trapped animals, assess trap condition and feed the live bait.

Once a leopard cat was trapped, we covered the cage with a cloth to reduce stress while we assessed the animal's suitability for anesthesia, estimated the animal's weight and determined the volume of drug to inject. Zoletil 100® (Virbac SA, Carros, France) intramuscular route, an estimated dose of 7 mg/kg, was used for the anesthesia of the leopard cats trapped, based on previous publications in the species (Grassman et al., 2004; Rajaratnam et al., 2007) and other publications related to medium-sized cats (Shindle and Tewes, 2000).

Booster doses of ketamine and Zoletil® have been used by other authors in wild carnivores following an initial dose of Zoletil® (Kreeger et al., 1990). Therefore, we were prepared with additional Zoletil® and or ketamine (Imalgene® 1000, Merial, Barcelona, Spain) in case booster anesthetic doses were required. If the animal did not reach the desired plane of anesthesia within 14 min, we administered booster injections of either Zoletil® or ketamine (depending on availability) at a dose of 3 mg/kg (Kreeger et al, 2002) intramuscular.

Leopard cats were injected into the hindquarters by hand. We recorded the induction time (time from injection of the drug until the head rests on the floor), anesthesia time (time from the head resting on the floor until the animal is able to lift it again) and the release time (time from the animal lifting its head after anaesthetic until full normal behavior returns with no evidence of drug action, and the animal is able to be released). We also recorded the handling time during which the animals were measured and weighed, radio-collars were fitted, body temperature, respiratory rate, heart rate were recorded, samples of hair were taken for genetic studies and a blood sample was taken for hematology and biochemistry analysis. Once the captured leopard cats were weighed, we completed the calculation of the actual dose received by the animal given in

Table 2 Induction, anesthesia, release and effective working times for animals in each of the two study groups. † Animals group 1;

*Animals group 2.

	Zoletil†	Zoletil + Zoletil*	Zoletil + Ketamine*
Zoletil® (mg / kg)	6.92 ± 1.06	6.92 ± 1.06	6.92 ± 1.06
Booster (Zoletil or Ketamine) (mg / kg)		2.6 ± 0.33	3.05 ± 0.05
Induction Time (min)	7.9 ± 1.77	9.5 ± 2.12	7.5 ± 2.12
Anesthesia time (min)	47.2 ± 25.1	89.5 ± 6.36	43.5 ± 2.1
Release Time (min)	236.2 ± 19.69	350.0 ± 70.7	245.0 ± 91.9
Effective Working Time (min)	36.0 ± 6.52	42.5 ± 3.54	40 ± 0.00

mg/kg.

All statistical analyses were performed with the software program SPSS program for Windows (SPSS 15.0; SPSS Inc., Chicago, IL, USA). Relationships between measures of drug effect and drug dosages were tested with a one-way ANOVA test and confirmed with a Welch and Brown-Forsythe Tests. Significance was accepted at $p \leq 0.05$

Results and Discussion

We successfully trapped 9 leopard cats. The estimated weights ranged between 2.0-2.45 kg in males (n = 6) and 1.70-1.90 kg in females (n = 3). The mean actual weight for males was 2.1±0.12 kg and 1.72±0.10 kg for females.

Table 1 Range of selected physiological parameters measured during anesthesia time

	Group 1 (n = 4)	Group 2 (n = 3)
Cardiac Rate	201-212	204-224
Respiratory Rate	20-24	20-22
Body Temperature (°C)	37.6-37.8	38.3-38.8

In five leopard cats (group 1), the average Zoletil® dose used was 6.92±1.06 mg/kg. This dose was enough to manage and perform all the required procedures in the animals. In four leopard cats (group 2), however, an initial mean dosage of Zoletil® of 6.92±1.06 mg/kg was insufficient to produce the complete muscle relaxation and loss of consciousness necessary for the planned procedures within 14 minutes post-injection (Kreeger, 2002). We, therefore, decided to inject an extra dose of 3 mg/kg of Zoletil® in two animals and 3 mg/kg of ketamine in another two animals. The results are shown in Table 2. For group 2, the induction time started since the animal rested its head on the floor after the booster was injected.

In all cases the signs of drug effects were observed during the induction time and were similar to those previously reported for ocelots (Shindle and Tewes, 2000) such as licking the nose and lips, loss of control of head and neck and limb paralysis. However, in group 2 up to 14 min after the injection of Zoletil®, the leopard cats were still responsive to low levels of environmental stimulation (e.g. slight noise), indicating that the level of anesthesia was not adequate for their safe removal from the trap and subsequent handling. Therefore, we injected the extra dose of 2.6±0.33 mg/kg of Zoletil® or 3.05±0.051 mg/kg ketamine (both intramuscular).

The ketamine boosters did not cause seizures in any cats. Seizures have been reported in wild cats (Kreeger, 2002; Grassman, 2004) and non-domestic cats in captivity with the use of ketamine. Of the cases in which a booster anesthetic injection was administered, we found a statistically significant difference ($p < 0.001$) in the anesthesia and release times in those leopard cats immobilized with Zoletil® followed by a booster of the same drug compared to those receiving a booster of ketamine. Although the induction times obtained in our study differ from other previous research with free-ranging leopard cats, we find major differences in the anesthesia times, where using Zoletil® at a higher dose or Zoletil® plus a booster of Zoletil® increases the immobilization times.

In view of these results, we conclude that a relationship exists between the dose of Zoletil® and the times of anesthesia and release, being significantly longer when Zoletil® is used in higher doses or in those cases where a booster of Zoletil® is administered. For non-painful procedures in which animal handling is minimal, the estimated dose to use of 6.92 mg/kg appears to be adequate. In situations where the Zoletil® primary dose does not achieve an adequate anesthetic plane, we recommend a booster dose of 3 mg/kg of ketamine after the initial estimated dose of Zoletil® of 6.92 mg/kg if the planned procedure can be performed within the anesthesia time (mean 43.5 min). This protocol does not significantly lengthen the time of anesthesia nor

the release time of the animal. Due to the extended anesthesia and release times resulting from administration of a booster dose of Zoletil®, we recommend the use of ketamine in preference to Zoletil® for booster doses after an initial dose of Zoletil®. In this study Zoletil® proved to be a useful and safe drug for chemical restraint of free-ranging Bornean leopard cats.

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Table 3 Anesthesia and induction times in groups 1 and 2 in comparison with previous studies. ¹Grassman et al., 2004; ²Group 1; ³ Group 2: ZH + ZH (Zoletil + Zoletil); ZH + KH (Zoletil + ketamine)

	Mean Dose (mg/kg)	Induction Time (min)	Anesthesia time (min)
Zoletil® ¹	12.3 ± 2.8	4.2 ± 2.8	67.0 ± 30.06
Zoletil® ²	6.92 ± 1.06	7.9 ± 1.77	47.2 ± 25.1
ZH + ZH ³	6.92 ± 2.6	9.5 ± 2.12	89.5 ± 6.36
ZH + KH ³	6.92 ± 3.05	7.5 ± 2.12	43.5 ± 2.12

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