Blood pressure in free-ranging gray wolves (*Canis lupus*) immobilized with tiletamine and zolazepam

Jonas Malmsten

Supervisor: Pia Funkquist
Department of Clinical Sciences
Assistant supervisor: Åsa Fahlman
Department of Wildlife, Fish & Environment
National Veterinary Institute
Assistant supervisor: Prof. Jon Arnemo
Section for Arctic Veterinary Medicine
Norwegian School of Veterinary Sciences

Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap
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SAMMANFATTNING

Studien avsåg att utvärdera blodtrycket hos immobiliserade frilevande vargar (*Canis lupus*) i Sverige. Hypotesen är att de sövda vargarna får hypertension av helikopterjakten och tiletamin. Åtta vargar (fem vuxna och tre valpar) ingick i studien. Vargarna sövdes från helikopter med bedövningspilar innehållande 250 mg tiletamin och 250 mg zolazepam, motsvarande 10,6 – 21,3 mg/kg. De vargar som under sövningen uppvisade tecken på att vakna, fick en intramuskulär injektion medetomidin motsvarande doser på 0,016 – 0,0625 mg/kg. Systoliskt och diastoliskt blodtryck mättes icke-invasivt med en oscillometrisk apparat (Memoprint, MedVet, Babenhausen, Tyskland). Rektaltemperatur, hjärtfrekvens och andningsfrekvens mättes var femtonde minut, i direkt anslutning till blodtrycksmätningen. Tre vargar var normotensiva, fyra hade måttlig diastolisk hypertension vid ett eller flera mätningstillfällen, och en varg hade kraftig diastolisk hypertension tillsammans med lindrig systolisk hypertension. Ingen av vargarna hade måttlig eller kraftig systolisk hypertension vid mättillfällena. De vargar som fick medetomidin under anestesin fick en blodtryckssänkning och en lägre andningsfrekvens. Eftersom tidpunkt för första mätning varierade mellan individerna, samt att den aldrig utfördes tidigare än 15 minuter efter pilskott, kan en möjlig initial hypertension ha inträffat. En etablering av referensvärden för blodtryck och hjärtfrekvens hos sövd frilevande varg är nödvändig i framtida studier.

INTRODUCTION

Surrounded by myth, controversy and awe, the wolf in Sweden has always been of interest to the public. The current Scandinavian population of this wild canid is estimated at approximately 145 individuals that are located in the deep forests of South-eastern Norway and South-western Sweden (Viltskadecenter 2005).

Since the year 2000, members of the Scandinavian research project called "Skandulv" have been monitoring gray wolves (*Canis lupus*) within the Scandinavian population. The project is a cooperation between the Norwegian and the Swedish Departments of Natural Resources, and three universities, and it began based on a governmental proposition in Sweden. This proposition stated that the Swedish wolf population should consist of at least 200 individuals, or that at least 20 litters of pups should be born each year in order to maintain a healthy and viable population (Regeringsproposition 2000). In order to monitor the populations, captures of wolves for radio-collaring are carried out between January and March, when the snow depth is adequate for tracking the wolves. When immobilized, individuals from the different packs are equipped with a combined radio-, GPS-, and GSM-transmitter for tracking purposes. The immobilizations also provide the opportunity to assess the health status of the wolves, through clinical examinations, and the collection of physical measurements, biopsies, fur, and blood. The capture team consists of trackers, fieldworkers, biologists, ecologists and veterinarians.

Wolf facts

The wolf is the largest member of the wild canid group, with adult males averaging 50 kg (Brainerd & Pedersen 2005). Some males from the Alaskan population can weigh up to 75 kg (Arnemo, pers. comm.). The wild canid group does not only consist of gray wolves, but 34 different species of canines. There are an estimated 100,000 – 200,000 wolves in the world with the largest populations in Alaska, northern Canada, Russia, and in some parts of Eastern Europe. Within Europe, the largest population of wolves is found in Romania and consists of an estimated 2,000 – 2,500 individuals (Rovdjursföreningen 2000). Sweden shares parts of its population with Norway. Finland has an estimated population of 135 animals (Wabakken et al 2005). Local reintroductions of wolves have been successful in some locations in North America and Europe where wolves were extinct, as is the case in Yellowstone National Park in Wyoming and in Idaho (Roecker 1997).

Wolves in Sweden

The presence of wolves in Sweden has long been a controversial issue. The species has been hunted extensively since the 1600s and a more significant decrease in the population began in the mid 1800s (Rovdjursföreningen 2000). In

1900, wolves only remained in the mountainous regions of Sweden, causing problems for the reindeer-herding native Sami people. In 1966 the hunting of wolves was officially banned and an estimated 10 wolves were believed to have escaped hunting at that time. In 1978 a litter was born in the northernmost region of Sweden, and became the first confirmed litter in 14 years. However, due to various reasons, including illegal hunting, Sweden's wolf population was presumed to be virtually non-existing until 1982 (Rovdjursföreningen 2000). The same year two wolves were tracked in the southwest province of Värmland, and in 1983 they gave birth to the first litter in southern Sweden in more than 100 years. The two adult wolves were believed to have migrated from Russia, Finland, or both. The following years at least one litter was born each year, except in 1986. As of 2005, the population on the Scandinavian Peninsula is divided into 14 so-called family groups and 15 pairs. A family group is comprised of at least one male and one female with pups (Wabakken et al 2005). The pairs have no pups. Figure 1 shows the approximate locations of the Scandinavian wolves.

One of the main concerns regarding the Scandinavian wolf population, besides illegal hunting, is the lack of genetic diversity. There is a strong need for new genetic material, preferably from the Finnish/Russian population (Liberg et al 2005). On several occasions, wolves have been observed and radiomarked heading south toward the Scandinavian population. However, incidents with Sami people, farmers, traffic, hunters, and civilians, have prevented these individuals from reaching future mates and hunting grounds. On only one occasion, in 1991, a lone male wolf from Finland or Russia reached the Scandinavian wolf population (Liberg et al 2005).

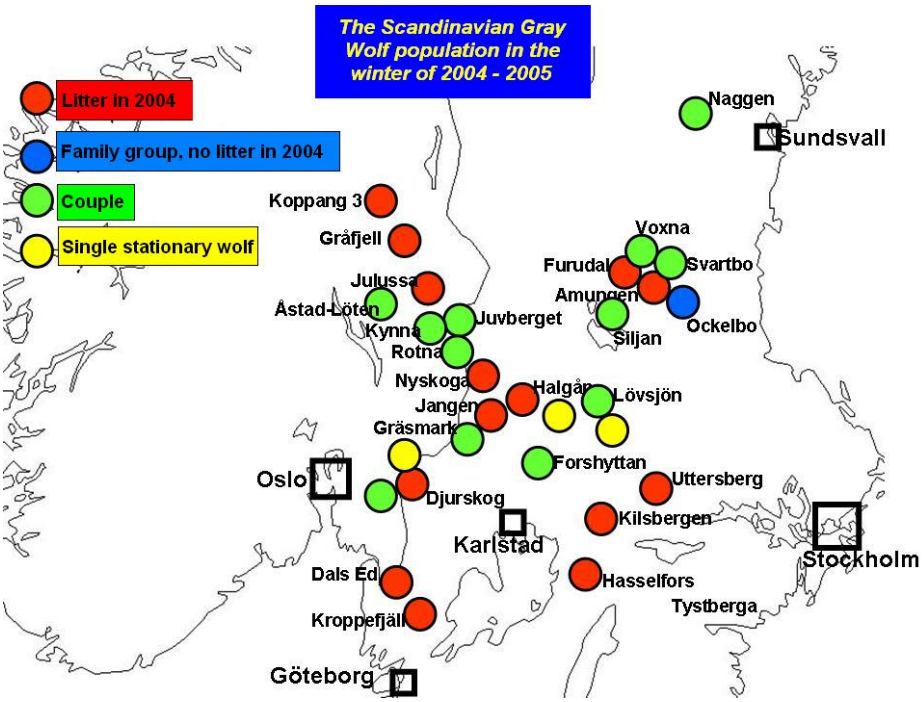


Figure 1.The Scandinavian wolf population during the winter of 2004-2005.

Wolf capture

Over the years, different methods have been used to capture free-ranging wolves, such as live trapping (in foothold or boxtraps), net gun and chemical immobilization. Chemical immobilization is usually performed after trapping or netting the wolves (Sillero-Zubiri 1996, Kuzyk 2001), or directly from a helicopter after pursuing the wolves (Ballard et al 1982, Constable et al 1998, Spence et al 1999, Kreeger et al 1995, Arnemo et al 2007). Due to legislative regulations that prohibit trapping of wolves in Sweden, chemical immobilization from a helicopter is the preferred method used by the Skandulv project (Arnemo and Fahlman 2007). Several different anesthetic combinations for chemical immobilization of free-ranging wolves have been used over the world. To my knowledge, the earliest published report is from 1982 and describes the use of phencyclidine and promazine in combination, and etorphine alone. These drugs were used to immobilize a total of 103 wolves in Alaska (Ballard et al 1982). Current drugs used in the field include opioids, α_2-agonists, benzodiazepines, cyclohexamines, and combinations thereof. A xylazine-tiletamine-zolazepam combination has been evaluated (Kreeger et al 1995), as has medetomidine-ketamine (Arnemo et al 2007), etorphine (Ballard et al 1992), and phencyclidine (Ballard et al 1992). The optimal drug, or combination of drugs, should provide the following: a small total volume in order to fit in the dart, a short induction time, sufficient muscle relaxation, an adequate anesthetic depth without negative physiological effects (cardiovascular, gastro-intestinal etc.), predictability, and availability of reversal agents for the drug(s). After anesthesia, the animal should ideally resume normal physiological and social functioning.

Presently the drugs of choice for the Scandinavian wolf project are tiletamine and zolazepam (Arnemo et al 2007). Tiletamine is a dissociative anesthetic belonging to the group cyclohexamines. Other drugs in this group of anesthetics are ketamine and phencyclidine. The complete acting mechanism of cyxlohexamines is not entirely known, but they block NMDA-receptors as well as depressing the thalamocortical system and at the same time activating the limbic system (Plumb 2005). When used alone, cyclohexamines usually fail to produce good skeletal muscle relaxation and frequently cause convulsions in dogs (Lumb & Jones 1984). The effects on the animal include mild cardiac stimulation and respiratory depression, increased blood pressure, active pharyngeal and laryngeal reflexes and salivation. Further, tonic-clonic spasms can occur during anesthesia and the recovery phase (Lumb & Jones 1984). There are no known chemical reversal agents for cyklohexamines.

Zolazepam belongs to a group called benzodiazepines. It acts as an anticonvulsant, muscle relaxant and calming agent (Lumb & Jones 1984). Although acting mechanisms are not fully understood, zolazepam seems to increase or facilitate the release of gammaaminobutyric-acid, a transmitter substance in the central nervous system, as well as antagonize serotonin release. (Plumb 2005).

Tiletamine and zolazepam are only available in a combined product. This product is sold under the trade names Zoletil 100®, Zoletil 50®, Zoletil forte® (Virbac S.A, Carros Cedex, France), and Telazol® (Fort Dodge Laboratories, Iowa, USA). When combined, tiletamine and zolazepam cause a mild to moderate analgesia and muscle relaxation in dogs and cats. Although extensively used, the combination has not been approved for use in exotic and wild species (Plumb 2005). Adverse effects include tachycardia, athetoid movements, excessive salivation, transient apnea, and erratic behaviour during recovery, prolonged recovery, hypertonia, cardiac arrest, pulmonary edema, muscle rigidity, and either hypotension or hypertension (Plumb 2005).

Project background

Little research has been reported regarding how free-ranging wolves are responding physiologically to the pursuit and anesthesia described above. The recent deaths of two wolves in the Scandinavian project, due to gastro-intestinal problems following chemical immobilization, raises concerns regarding the physiological status of wolves during and after capture (Arnemo, personal communication). Preliminary studies on blood gases and acid-base balances during immobilization of free-ranging gray wolves show that they sometimes develop high blood-lactate levels, a sign of metabolic acidosis (Fahlman, unpublished data). This occurrence is possibly due to exertion during the induction period when using helicopter capture (Fahlman, personal communication). The stress and activation of the sympatic nervous system during pursuit by a helicopter, in conjunction with sympatic nerve stimulation from tiletamine, may cause a substantial physiological effect on the wolves. Besides

metabolic acidosis described above, tachycardia, tachypnea and hypertension are believed to be other results of the exertion during helicopter capture and the chemicals used.

Hypertension is primarily a pathological state, which can be caused by disease, or cause disease. Reasons for developing hypertension can be heart disease (congenital or acquired), hormonal imbalances (stress, hypothyroideism, hyperadrenocortisism), anemia, or drug-induced (Egner et al 2003). Furthermore, hypertension can increase the risk of developing cerebral encephalopathies, retinal hemorrhages, pulmonary edema and heart failure (Egner et al 2003). It is well known that a systolic hypertension is more harmful to an individual that a diastolic hypertension (Egner et al 2003). In order to further investigate the physiological response of wolves to helicopter capture, this project was initiated. The purpose of this study was to measure and evaluate blood pressure of free-ranging gray wolves immobilized with tiletamine and zolazepam from a helicopter. The hypothesis is that free-ranging wolves develop hypertension as a result of being immobilized with tiletamine, and being pursued by a helicopter.

MATERIALS AND METHODS

This study included a total of eight free-ranging wolves captured between March 14-19 2005, and January 27-30 2006, around the Grimsö Research Station, and in the province of Värmland in southwest Sweden. All eight wolves (two juvenile females, one juvenile male, two adult females and three adult males) were captured for the first time. Their packs were tracked in all areas seen in Figure 1. On the day of planned capture, the coordinates of the location of the packs were submitted to the project manager, and a helicopter (Eurocopter EC-120, Eurocopter, Marignane, France) was airborne within two hours. Upon reaching the location of the pack, the helicopter hovered over the pack briefly, until the wolves started moving. When the moving wolf or wolves entered an opening, such as a frozen lake or a field, the helicopter closed in, and pursuit was initiated. As rapidly as possible, in order not to pursue the wolf for too long, one or more darts (Dan-Inject® 3.0 ml, Dan-Inject, Børkop, Denmark) were fired from a CO_2-powered rifle (Dan-Inject® model IM, Dan-Inject, Børkop, Denmark). The darts contained 250 mg of tiletamine, and 250 mg of zolazepam (Zoletil 100®, Virbac S.A, Carros Cedex, France), and were fired from the helicopter at a distance from 3 – 10 meters, and at a ground speed of up to 65 km/h. Once a hit was confirmed visually by the shooter, the helicopter immediately increased the distance from the animal in order to minimize further stress. From a distance, the shooter and the pilot estimated the distance traveled. If the animal was not recumbent within 10 minutes, another approach was initated and a second dart was fired. When successfully immobilized, the helicopter brought the animal to a location where the ground crew were waiting.

Upon arrival at the meeting location, the animal was placed in lateral recumbency on a blanket. Physiological parameters were recorded every 15 minutes, starting as soon as possible after recumbency. Rectal temperature was monitored with a

digital thermometer (Welch Allyn Diatec 600, Welch Allyn, Inc., Skaneateles Falls, New York, USA) with a measurement range from 28.9 – 42.2 °C. Respiratory rate was measured visually by observation of chest movement, heart rate was measured by cardiac auscultation, and capillary refill time was measured. A cuff, designed for medium and large breed dogs, was applied proximal to the carpus of the upper front leg. Arterial blood pressure was measured non-invasively with an oscillometric device (Memoprint, MedVet, Babenhausen, Germany). Blood pressure was measured three times in one series, and the average of these three measurements were calculated. Anesthetic depth was evaluated by assessing the following parameters: palpebral reflex, degree of general muscle relaxation (jaw tone and stretching of the upper hind leg), and pain sensitivity (reaction to toe pinch). If the animal started to show signs of arousal, a supplemental intramuscular injection of medetomidine (0,25 – 1 mg) was administered in order to induce complete immobilization and to enable completion of the procedure.

Outdoor temperature was also measured, as well as snow depth.

Reference values for heart rate is 84 ± 4 beats per minute, after a study of resting heart rates in captive wolves by means of telemetry (Kreeger et al 1990).

Reference values regarding blood pressure are taken from studies of domestic dogs, and strenously exercised deerhounds in particular. These dogs have, according to a study, resting heart rates of 92 beats per minute (Bodey & Michell 1996), and represent a group of dogs similar in size, and with a resting heart rate most similar to that of captive wolves. The average arterial blood pressure of deerhounds in this group, measured non-invasively by oscillometry is 146 mmHg (systolic) and 85 mmHg (diastolic) (Bodey & Michell 1996). With these values used as a base reference, mild hypertension was calculated as blood pressure exceeding 165/108 mmHg (systolic/diastolic). Moderate hypertension exceeds 176/115 mmHg, and severe hypertension exceeds 196/136 mmHg. It will also be noticed if the wolf has a diastolic, systolic, or a mixed hypertension. Mixed hypertension is a combination of diastolic and systolic hypertension.

RESULTS

All captures and following anaesthetic monitoring were successful. The distance the wolves traveled before and during pursuit ranged from 500 – 10,000 m, and the distance traveled after darting ranged from 300 – 1,500 m (Table 1). Before pursuit of the wolf, the helicopter had a distance of around 40 – 50 meters to the animal, in order not to induce too much stress. This differed from the distance to the animal during pursuit, which was an estimated 3 – 10 meters. Induction times varied from 2 – 9 minutes (Table 1). Six of eight wolves were darted on the first attempt. One was hit but the dart released its contents in the fur, and not intramuscularly, which was discovered upon retrieval of the wolf. The wolf had to be darted a second time since no sign of drug effect 10 minutes after the first dart, which resulted in a longer time of pursuit. One wolf needed two darts since the

first one missed, and a second dart was fired within a minute after the first one. Since all eight wolves were darted with the same drug dose, drug dosages based on body weight varied from 10.6 – 21.3 mg/kg (Table 1) Body weights are listed in Table 1. The outside temperature ranged between -7ºC and +3ºC (average about -1ºC), snow depth was 0.3 meters for the 2005 captures, and approximately 0.6 m for the 2006 captures.

Anesthetic monitoring started from 15 to 45 minutes after darting, as seen in Table 1. Wolf 1 started showing signs of waking up at 75 minutes after darting, and recieved one intramuscular injection of 1 mg medetomidine (0.025 mg/kg) immediately after data was recorded at this point of time. Wolves 6, 7 and 8 recieved 0.25, 0.50 and 0.50 mg medetomidine intramuscularly (0.0625, 0.016, and 0.021 mg/kg respectively) as seen in Table 3 and 2.

Systolic arterial blood pressure for all wolves varied between 111 – 170 mmHg, mean arterial blood pressure 84 – 140 mmHg, and diastolic arterial blood pressure 67 – 127 mmHg (Table 3). Heart rates varied between 44 – 220 beats per minute, and respiratory rates between 11 – 44 breaths per minute. Rectal temperatures varied between 36.0 ºC and 41.2 ºC.

According to the three level scale of hypertension described previously, Wolf number 1 had some variations in both systolic and diastolic blood pressure during the anesthesia. As seen in Table 3 and Figure 2, at 45 minutes after darting, it had a mild to moderate diastolic hypertension, which at 60 minutes had dropped to a normotension. At the following three measuring points its diastolic blood pressure never passed the limit moderate hypertension. The blood pressure of this wolf never went above the limit set for a mild systolic hypertension. The medetomidine injection given shortly after the recording at 75 minutes decreased the blood pressure. In conclusion, Wolf 1 fluctuated from diastolic hypertension, down to normotension, and then back to diastolic hypertension. The wolf had a high heart rate throughout the duration of the anesthesia.

Wolf number 2 was normotensive and had a high heart rate at every measuring point.

Wolf number 3 was normotensive at 30 minutes after darting and had a mild to moderate diastolic hypertension at 45 minutes. The following recordings at 60 and 75 minutes were within the normal range. Heart rate in this wolf was high throughout the monitoring period.

Wolf 4 was normotensive at the 15, 45, and 60-minute mark. At the 75-minute mark, it had a moderate diastolic hypertension. Heart rate remained high through out the monitoring perriod.

Wolf 5 was normotensive throughout the monitoring.

Wolf 6 had a moderate diastolic hypertension at the 45-minute mark, and a mild diastolic hypertension after 60 minutes. The wolf was systolically normotensive at both measuring points.

Wolf 7 suffered from an increasing mixed hypertension over the course of the anesthesia. At the 45-minute mark it was at a mild hypertensive level, which increased to a moderate diastolic with a mild systolic hypertension after 60 minutes. After 75 minutes the wolf had a severe diastolic hypertension with a mild systolic hypertension. Heart rate decreased after receiving medetomidine.

The last wolf (no. 8) was normotensive throughout the anesthetic monitoring.

When summarized, three wolves were normotensive, four wolves had a moderate diastolic hypertenstion at one or more measuring points, and one wolf had a severe diastolic hypertension together with a mild systolic hypertension. None of the wolves had a moderate or a severe systolic hypertension.

Table 1. Distances traveled, body weights, drug doses, induction times, and arousal times in free-ranging gray wolves immobilized with tiletamine and zolazepam

	Dist. [a] before succesf. darting (m)	Dist. [a] after succesf. darting (m)	Total dist. (m)	Number of darts	Body weight (kg)	Dose (mg/kg)	Ind. time (min)	Arousal time (hrs)
Wolf 1 (Ad. ♂)	200	400	600	1	47.0	10.6	2	6.5
Wolf 2 (Ad. ♂)	300	1,500	1,800	1	46.0	10.8	8	3.4
Wolf 3 (Ad. ♂)	300	300	600	1	44.5	11.2	3	2.8
Wolf 4 (Ad. ♀	9,700	300	10,000	2[b]	27.0	18.5	2	2.6
Wolf 5 (Ad. ♀)	200	300	500	1	32.5	15.4	9	4.5
Wolf 6 (Juv. ♀)	300	700	1,000	1	40.0	12.5	7	2.0
Wolf 7 (Juv. ♂)	200	300	500	1	32.0	15.6	2	2.0
Wolf 8 (Juv. ♀)	200	300	500	1	23.5	21.3	4	3.0

[a] *Dist. = distance, succesf. = succesful*

[b] *dart no. 1 emptied its contents in the fur, dart no. 2 intramuscularly*

Table 2. Body temperature, heart rate, and respiratory rate in free-ranging gray wolves immobilized with tiletamine and zolazepam

	Time (min after darting)	15	30	45	60	75	90	105	120	135
Wolf 1	T[a]			38.1	38.1	38.0 [d]	37.8	37.2	37.2	36.0
(Adult ♂)	HR[b]			172	172	178 [d]	136	80	50	44
	RR[c]			44	42	26 [d]	30	20	18	11
Wolf 2	T			38.6	38.3	38.3	38.2			
(Adult ♂)	HR			182	168	172	180			
	RR			30	26	30	32			
Wolf 3	T		39.7	38.9	38.7	38.6				
(Adult ♂)	HR		190	188	188	164				
	RR		28	30	26	28				
Wolf 4	T	41.2	39.7	38.8	38.0					
(Juv. ♀)	HR	220	176	180	188					
	RR	24	18	46[e]	34 [e]					
Wolf 5	T		39.0	38.2	37.8	37.2				
(Adult ♀)	HR		150	136	136	130				
	RR		28	26	30	32				
Wolf 6	T			40.3[f]	40.0					
(Adult ♀)	HR			170 [f]	164					
	RR			42 [f]	32					
Wolf 7	T			39.0[g]	38.6	38.5				
(Juv. ♂)	HR			196 [g]	180	78				
	RR			40 [g]	36	28				
Wolf 8	T	37.2	36.7	36.3						
(Juv. ♀)	HR	162	144	128						
	RR	42	24	26						

[a] *T = rectal temperature in ˚C*

[b] *HR = heart rate in beats/minute*

[c] *RR = respiratory rate in breaths/minute*

[d] *Received 1 mg medetomidine i.m. immediately after point of measuring*

[e] *Shallow breathing pattern*

[f] *Received 0.25 mg medetomidine i.m. 9 minutes before this measuring point*

[g] *= received 0.50 mg medetomidine i.m. immediately after this measuring point*

Table 3. Arterial blood pressure (mmHg) in free-ranging gray wolves immobilized with tiletamine and zolazepam

	Time (min after darting)	15	30	45	60	75	90	105	120	135
Wolf 1 (Adult ♂)	SABP [a]			153	142	163[d]	142	146	139	151
	MABP [b]			127	101	139 [d]	123	129	122	130
	DABP[c]			114	80	127 [d]	113	121	114	114
Wolf 2 (Adult ♂)	SABP			131	128	144	147			
	MABP			97	100	113	119			
	DABP			80	87	82	105			
Wolf 3 (Adult ♂)	SABP		152	163	153	158				
	MABP		105	130	110	101				
	DABP		82	114	88	73				
Wolf 4 (Juv. ♀)	SABP	145	136	142	155					
	MABP	111	95	109	136					
	DABP	79	75	93	127					
Wolf 5 (Adult ♀)	SABP		118	143	129	136				
	MABP		84	104	104	101				
	DABP		67	85	91	84				
Wolf 6 (Adult ♀)	SABP			141[e]	161					
	MABP			124[e]	128					
	DABP			116[e]	112					
Wolf 7 (Juv. ♂)	SABP			158[f]	167	170				
	MABP			113[f]	118	140				
	DABP			90 [f]	93	125				
Wolf 8 (Juv. ♀)	SABP	111	123							
	MABP	86	102							
	DABP	73	91							

[a] *SABP = systolic arterial blood pressure*

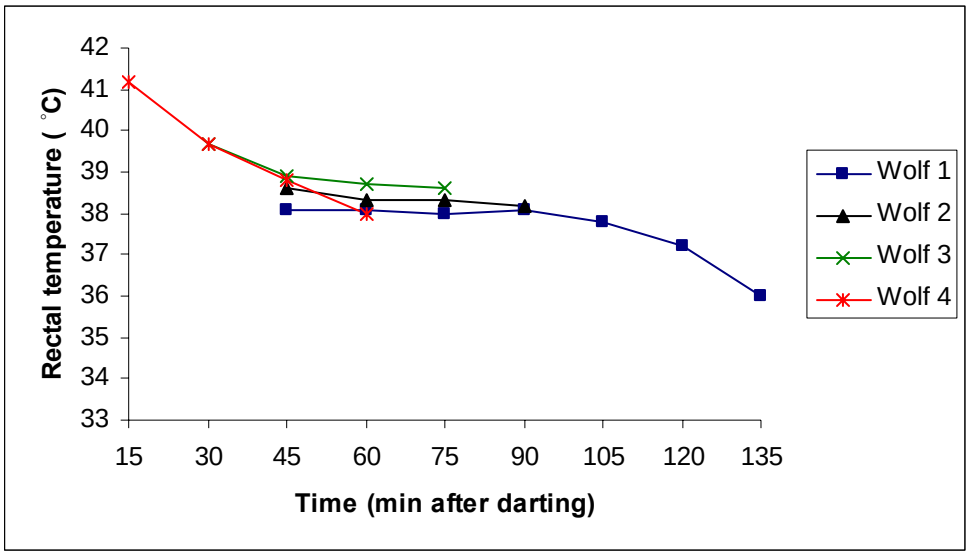
[b] *MABP = mean arterial blood pressure*

[c] *DABP = diastolic arterial blood pressure*

[d] *Received 1 mg medetomidine i.m. immediately after this measuring point*

[e] *Received 0.25 mg medetomidine i.m. 9 minutes before this measuring point*

[f] *Received 0.50 mg medetomidine i.m. immediately after this measuring point*



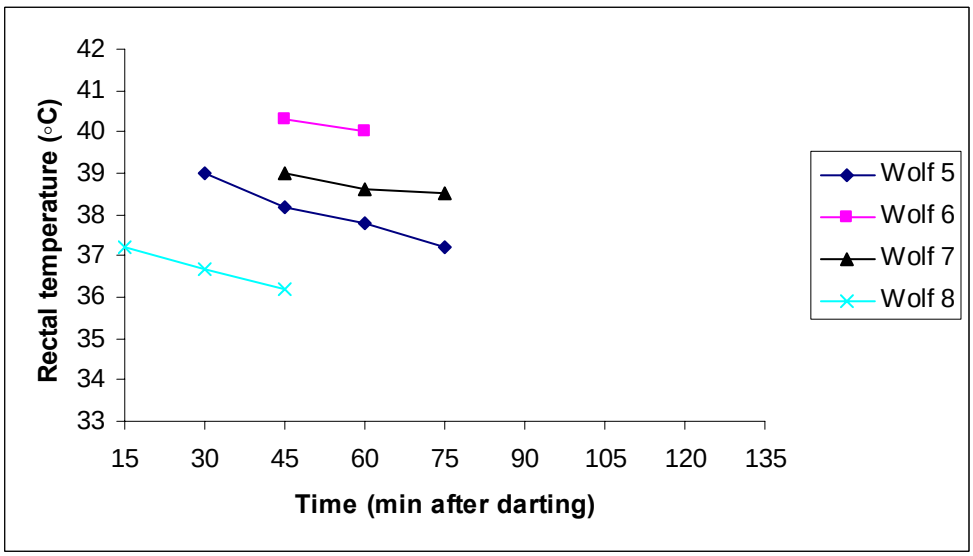
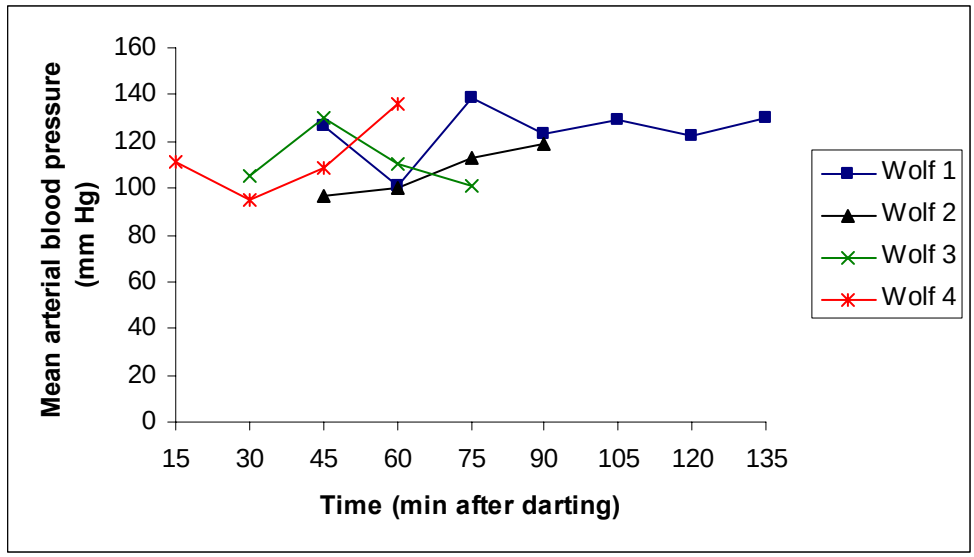


Figure 5a and 5b. Rectal temperature in free-ranging gray wolves immobilized with tiletamine and zolazepam.



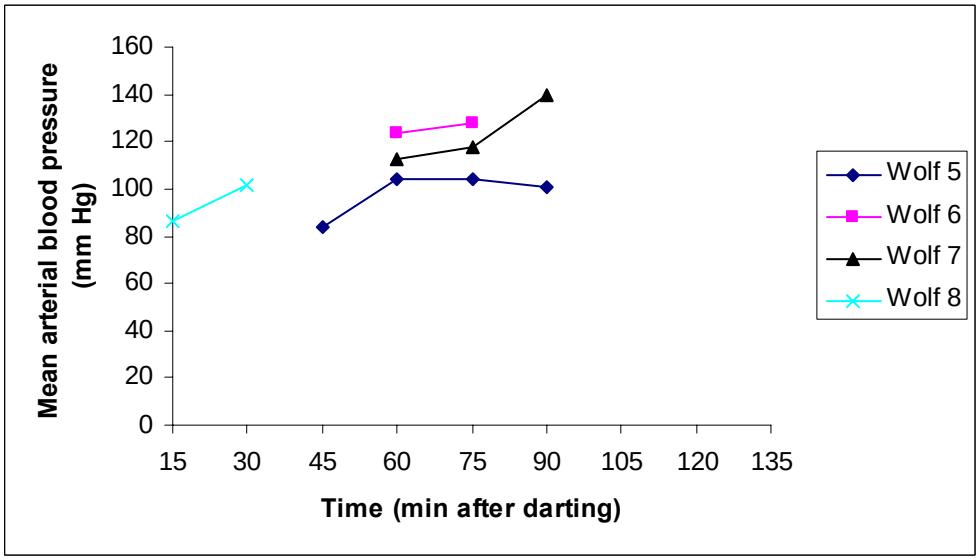
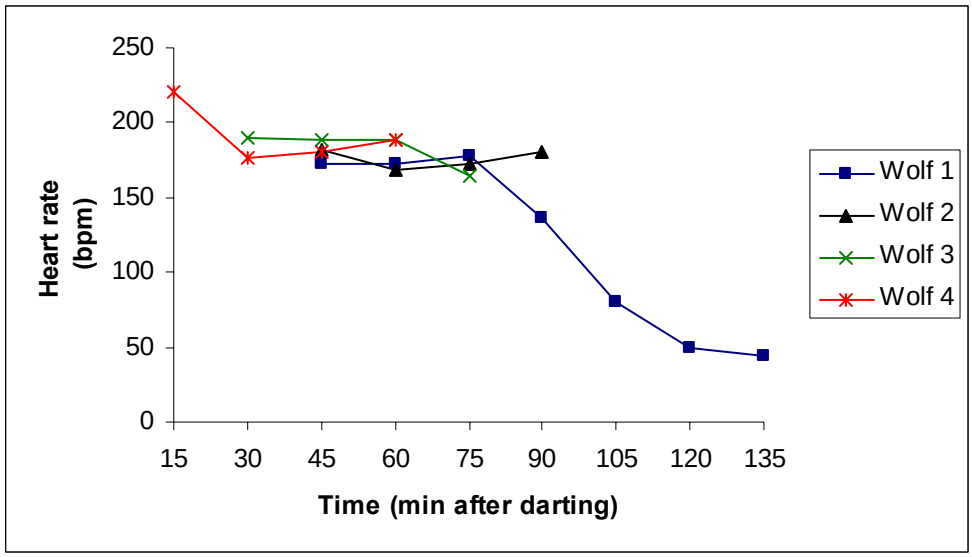


Figure 2a and 2b. Mean arterial blood pressure in free-ranging gray wolves immobilized with tiletamine and zolazepam.



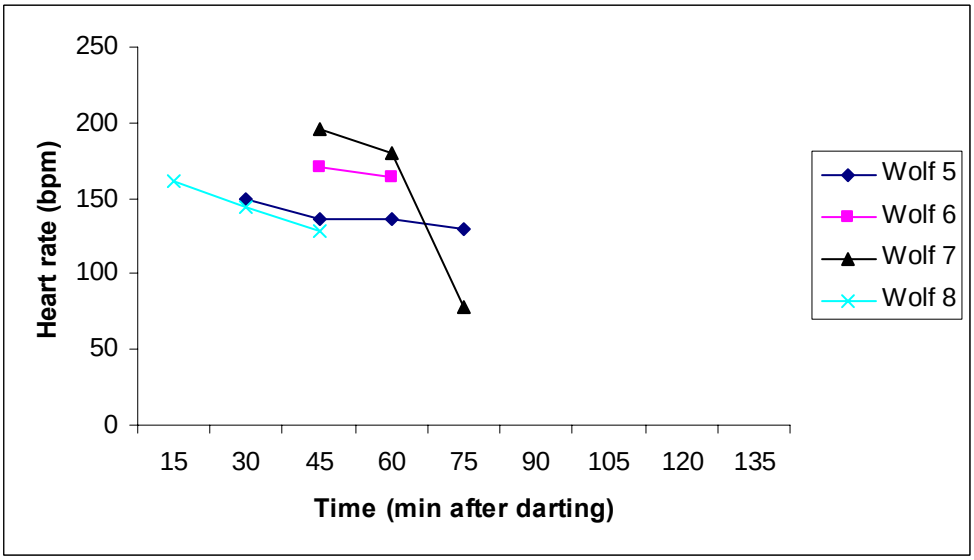


Figure 3a and 3b. Heart rates (beats/min) in free-ranging gray wolves immobilized with tiletamine and zolazepam.

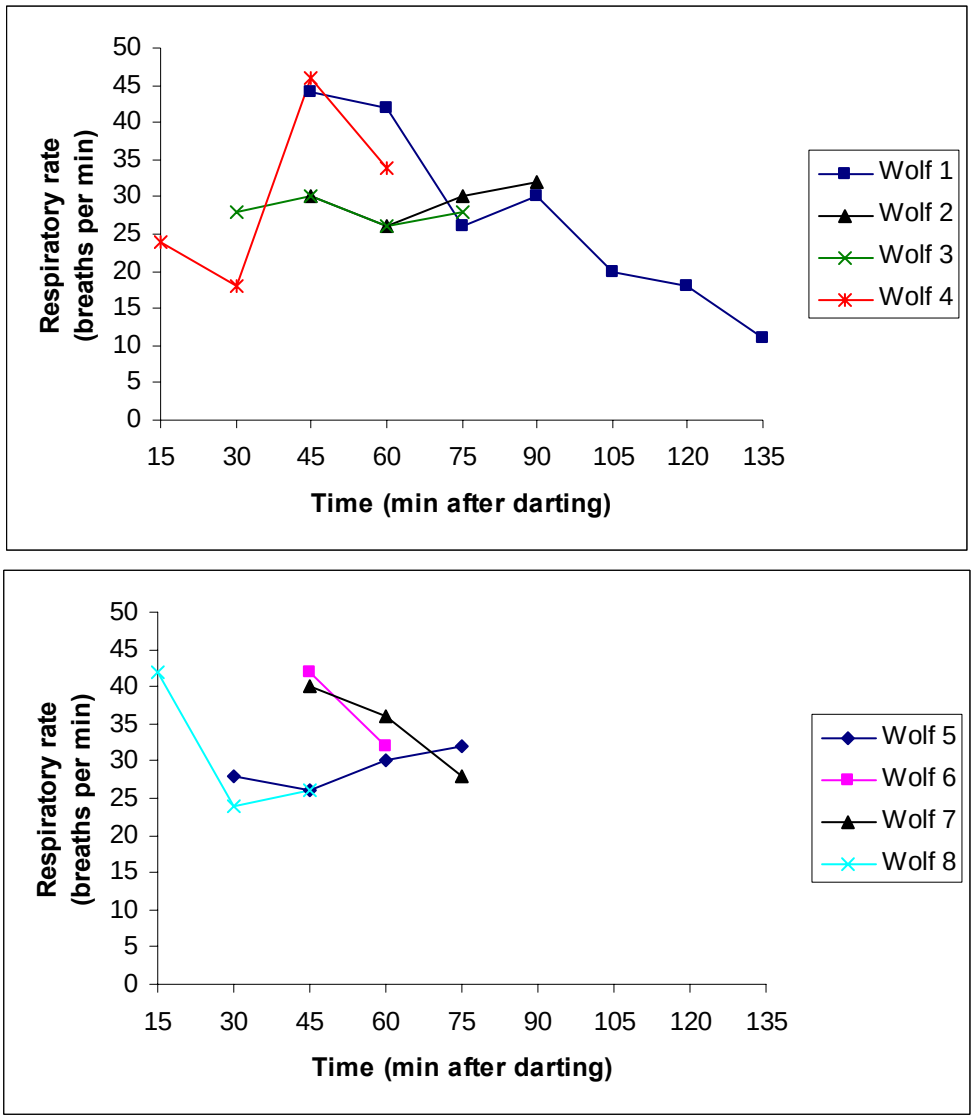
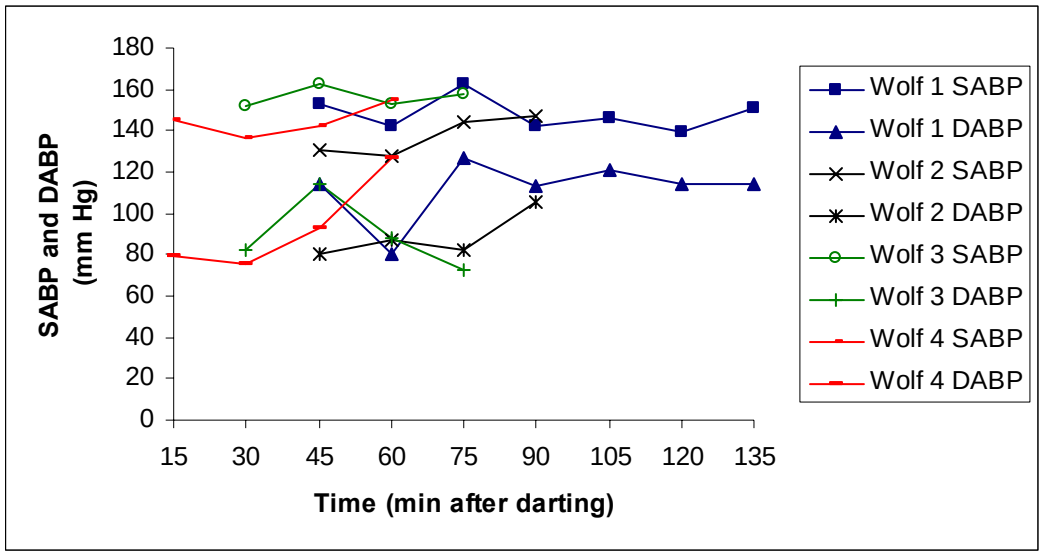


Figure 4a and 4b. Respiratory rate (breaths/min) in free-ranging gray wolves immobilized with tiletamine and zolazepam.



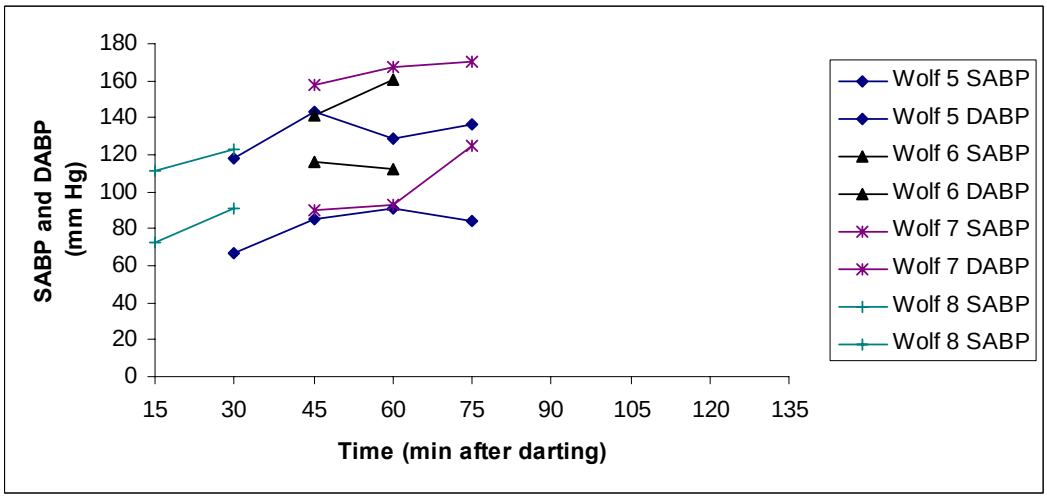


Figure 6a and 6b. Systolic and diastolic arterial blood pressure (SABP and DABP) in free-ranging gray wolves immobilized with tiletamine and zolazepam.

DISCUSSION

In this study, minor blood pressure changes in the captured wolves were identified. They are not considered to be harmful to the wolves. In addition, the study shows that a supplemental dose of medetomidine might have a positive physiological effect on wolves by decreasing the heart rate, respiratory rate and blood pressure. This was seen in one case. Reference values for blood pressure in free-ranging gray wolves need to be established.

There is more published data on physiological responses to anesthesia of captive wolves than of free-ranging wolves. The responses also vary with with the anesthetics, and the doses that are being used. Captive gray wolves that are immobilized with xylazine at a dose of 2.2 mg/kg and ketamine at a dose of 6.6 mg/kg develop bradycardia and hypertension for the entire duration of the anesthesia (Kreeger et al 1987). The limit for hypertension was set at a mean arterial blood pressure exceeding approximately 120 mmHg (Kreeger et al 1987).

In this study, five of eight wolves pass that limit, but only one of eight is surpass the limit for mild systolic hypertension. It is the systolic hypertension that is considered to be harmful (Egner 2003). If xylazine (2.0 mg/kg) and butorphanol (0.4 mg/kg) is used for captive gray wolves, normotension and bradycardia is observed (Kreeger et al 1989). One study was designed to compare effects of immobilization with four different combinations on captive red wolves (*Canis rufus*): medetomidine and ketamine (0.04 and 2 mg/kg, resepectively), medetomidine, butorphanol and ketamine (at doses of 0.02, 0.2, and 2 mg/kg, respectively), xylazine, and ketamine (2.0 and 8.0 mg/kg, respectively), and medetomidine, ketamine, and acepromazine (0.04, 2.0, and 0.01 mg/kg, respectively). It was observed that the systolic, mean and diastolic blood pressure is highest in all four combinations immediately after induction. Blood pressure remained high in three of the combinations used, with the exception of the group of wolves that were immobilized with medetomidine, butorphanol, and ketamine, where it decreased steadily over time (Sladky et al 2000). The four-combination study defined a diastolic blood pressure of 116 mmHg or more to be hypertension. A blood pressure of 116 mmHg would correspond to a moderate diastolic hypertension in the present study. A decrease in blood pressure over time was also observed in captive gray wolves that were immobilized with medetomidine, butorphanol, ketamine, and acepromazine. The wolves received doses of 0.03 mg/kg (medetomidine), 0.2 mg/kg (butorphanol), 2 mg/kg (ketamine), and 0.02 mg/kg (acepromazine) (Valerio et al 2005). If used on red wolves (*Canis rufus*), that four-component combination generates a similar decrease in blood pressure (Larsen et al 2002).

Captive gray wolves that are immobilized with tiletamine and zolazepam (at doses of 2.5 + 2.5 mg/kg, and 5 + 5 mg/kg) are hypertensive from 1 – 30 minutes after induction (Kreeger et al 1990). The mean arterial blood pressure ranged between 120 – 130 mmHg for wolves receiving the higher dose (5 mg/kg of tiletamine + 5 mg/kg of zolazepam). Wolves that recieved the lower dose had mean arterial blood pressures between 110 – 120 mmHg through the entire duration of the anesthesia.

All studies reported above have measured blood pressure non-invasively and by means of oscillometry. It has been reported that compared to direct blood pressure measurements obtained using invasive techniques, oscillometric blood pressure measurements in the extremely hypertensive range appears to be somewhat low. At the same time, values in the extremely hypotensive range are somewhat high (Egner 2003). In the study presented in this paper, no extreme values were measured.

Dogs that are anesthetized with tiletamine and zolazepam intravenously initially develop an increase in heart rate and cardiac output, as well as fluctuations in blood pressure (measured invasively). A rapid onset of hypotension, which is followed by normotension, is observed. The normotension soon changes into a significant hypertension (Helleyer et al 1989). Dogs receiving tiletamine and zolazepam intramuscularly at a dose of 5.5 mg/kg, show variable and relatively slight cardiovascular effects (Ward et al 1972). The only common finding in that study was tachycardia, whereas blood pressure changes (measured invasively) were not consistent, nor very large.

Due to the lack of publications on blood pressure in unanesthetized wolves, captive or free ranging, it is necessary to extrapolate blood pressure references from studies of domestic dogs. Several studies on blood pressure have been conducted in dogs over the years, and blood pressure varies mostly with age and breed (Bodey & Michell 1996). Blood pressure was measured in 1,267 clinically healthy dogs and the average systolic, mean, and diastolic blood pressures were estimated at 131, 97, and 74 mmHg, respectively. The average heart rate in all dogs was 110 beats per minute.

Recent studies on blood pressure in wolves have used Bodey and Michell (1996) as a reference, but seem to have extrapolated the values from a normal healthy dog, meaning using 131/75 mmHg, and 97 mmHg (systolic/diastolic and mean arterial blood pressure) as normal references (Sladky et al 2002). Egner (2003) sets the average systolic, diastolic arterial blood pressure at 133/75 mmHg, which corresponds well with Bodey and Michell (2003). Further, Egner divides hypertension into three grades; a mild, moderate and severe hypertension. The limits for these grades are set at 150/95 mmHg for a mild hypertension, 160/100 for a moderate hypertension, and 180/120 mmHg for severe hypertension (Egner 2003).

The limits for hypertension in wolves in the present study were set after extrapolating data from strenuously exercised deerhounds (Bodey and Michell 2003). There are similarities between wolves and deerhounds in resting heart rates (Kreeger 1990), size, and condition. An adult male deerhound has a weight of around 45 kg, roughly the same as an adult male gray wolf (Brainerd & Pedersen 2005, Scottish Deerhound Club of America). Free-ranging wolves are animals that roam free over large areas, and presumably have good stamina, fitness and health. Average blood pressure in deerhounds is 146/85 mmHg. The limit for mild hypertension in deerhounds, and subsequentley gray wolves is 165/108 mmHg, when using a three level scale designed by Egner. Moderate hypertension for deerhounds and wolves is a blood pressure exceeding 176/115 mmHg, and severe hypertension is a blood pressure over 196/136 mmHg. This is calculated by multiplying the blood pressures from the average domestic dogs with a factor corresponding to the limits set for mild, moderate, and severe hypertension. This scale has served as a reference for the wolves in this study.

Monitoring starting later than 15 minutes after darting is attributed to the distance between where the wolf was captured and where the ground crew was waiting. This distance was longer in instances where there were no roads.

When evaluating the distances travelled by the wolves, the difference between active pursuit of a wolf, and the “moving” and “pushing” of one into an opening suitable for a closer approach, has to be considered. As stated in the results section, moving of the wolves was performed at an approximate distance of 40 – 50 meters from the wolf. According to the pilot and the shooter, most wolves did not seem to find this very stressful. Instead of galloping, the wolves walked or trotted away from the helicopter. Contrary to this, when pursuit was initiated, all wolves were travelling at full speed.

The pursuit, capture, and induction time was satisfying for Wolf 1. The relatively short pursuit, only one dart fired, and 2 minute induction time was ideal for this sort of capture. Those three factors might have limited some negative physiological effects on the wolf, and blood pressure and body temperature in particular. No severe systolic, or diastolic hypertension was observed, although it was mildly or moderately diastolically hypertensive for most of the anesthesia. The signs of arousal observed at this time can explain the marked elevation in blood pressure at 75 minutes. After receiving medetomidine intramuscularly, muscle relaxation improved, and heart rate and respiratory rate decreased substantially, and blood pressure decreased slightly. The decrease in heart rate and respiratory rate is a common effect of an α_2-agonist (Plumb 2005).

Wolf 2, which had a similar distance of pursuit as Wolf 1, but a longer induction time (8 minutes), was normotensive for the entire duration of anesthesia. The slight increase in blood pressure after 90 minutes could have been a result of being close to arousal. The high heart rate observed could have been caused by the tiletamine, the pursuit of the wolf, or both. When considering the short distance traveled before recumbency, there is a possibility that the tachycardia was caused by a sympatic nervous stimulation of tiletamine. An injection of an α_2-agonist might have decreased the hear rate to normal levels.

Wolf 3 was, as Wolf 1, a satisfying capture in terms of pursuit, darting, induction time, and blood pressure. The wolf had a high heart rate throughout the entire duration of the anesthesia, which could be attributed to the tiletamine used.

Wolf 4, the juvenile female, was between 0.5 and 1 year old. As mentioned before, age is one of the most significant factors that affect blood pressure in dogs (Bodey & Michell 1996). The average blood pressure for all dogs (average age 5.2 years) in that study is 133/76 mmHg. When comparing those values with the blood pressure measured in dogs age 0.5-1 year old (121/68), the 5.2-year-old average dog has a 10% higher systolic blood pressure. The diastolic blood pressure is 12% higher in the average 5.2-year-old dog than in a 0.5-1 year old dog. The difference in blood pressures should also be applied for wolves, which results in a lower threshold for when a juvenile wolf has hypertension. If applying the calculated differences on the juvenile wolf, it was normotensive at 15, 30 and 45 minutes. At 60 minutes, this normotension turned into a mild to moderate systolic hypertension, and a severe diastolic hypertension. At 15 minutes, this wolf also had hyperthermia with a body temperature of 41.2 ºC. Such an elevated body temperature might have facilitated a peripheral vasodilatation, in order to cool the animal down. This vasodilatation could have been a factor that affected the blood pressure. Later, a cooling effect by the outer temperature on the body temperature, which could have caused a peripheral vasoconstriction, might have increased the blood pressure. As seen in Table 2, at 60 minutes, the wolf had a normal body temperature in a cool environment, and tachycardia. These two factors could have been playing major roles in the severe diastolic hypertension observed at this point. Additionally, the stress of a long pursuit, and the drugs used, should also be taken into consideration. The normotension recorded at 15, 30 and 45 minutes after darting is hard to explain.

Wolf 5 had a satisfying distance of pursuit and the longest induction time. Administration of drugs in subcutaneous fat is possible, which usually prolongs induction times. An interesting observation is that the wolf traveled a mere 300 m in the 9 minutes it took before it became recumbent. The wolf was normotensive according to the reference values discussed regarding Wolf 4. Body temperature and respiratory rate was satisfying.

Wolf 6 had a relatively long induction time but did not travel very far. The medetomidine administered after 15 minutes did not seem to have any immediate effects on blood pressure or heart rate. However, the respiratory rate decreased, most probably due to the effect of the α_2-agonist. The wolf recovering from exercise can also explain this decrease, or a combined effect as the wolf was recovering from the exercise during pursuit.

Wolf 7 had a satisfying induction time and distances traveled before and during the induction. A shorter distance seems to have less negative effects on the cardiovascular system. The blood pressure never passed the previously set level for a severe systolic or diastolic hypertension, but was steadily increasing from normotension, to a moderate hypertension. The medetomidine administered 45 minutes after darting did not seem to have an immediate effect, although at 75 minutes a decreased heart and respiratory rate was noted. The increase in blood pressure after 75 minutes does not follow the same pattern that other wolves that were given medetomidine, have shown.

Wolf 8 did not show any specific physiological alterations except for the substantial drop in respiratory rate seen at the 30 minute mark. The induction time was short as was the distance traveled before and during the induction.

The blood pressure seems to rise when the wolf is showing signs of arousal. During the procedure, the measuring stopped as soon as the animal started to show these signs, except for Wolf 1, 6 and 7, who all received injections of medetomidine.

Distance traveled and blood pressure does not seem to follow a pattern. Wolf 2 traveled three times the distance of Wolf 1 and 3 (Table 1), but did not show a substantially higher blood pressure compared to the other two (Table 3). Wolf 4 traveled almost seven times the distance of Wolf 2, and was the only wolf where a severe hypertension was recorded. The distance traveled, the tachycardia, hyperthermia, severe hypertension, and large dose of tiletamine and zolazepam, probably resulted in some degree of psychological and physiological stress to Wolf number 2. Snow depth and terrain was similar for all wolves.

The use of medetomidine to prolong immobilization for Wolf 1, 6, and 7 showed some noteworthy effects. Heart rate, respiratory rate, and blood pressure

decreased, and muscle relaxation improved. This is something that should be taken into consideration when discussing whether to add an α_2-agonist to the tiletamine and the zolazepam used to induce anesthesia. The combination of tiletamine, zolazepam and medetomidine has been used in other species with satisfying results. With the addition of medetomidine, doses of tiletamine and zolazepam can be lowered several times in immobilization of African lions (*Panthera leo)*, than when used the latter components alone (Fahlman 2005). In addition, induction times are short, anesthesia is good, there are no substantial adverse physiological effects, and the anesthesia is reversible with a smooth recovery.

There is no clear pattern in the results observed. The high heart rates are presumably a result of running during the helicopter pursuit and tiletamine. The hypertension seen in the majority of the wolves was expected to be higher, but is probably not harmful, since no moderate or severe systolic hypertension was seen. Nor were extreme fluctuations in blood pressure seen. Hence, the hypothesis described earlier has not been proven. However, it was not possible to measure blood pressure during or immediately after induction. There is a possibility that negative variations in blood pressure are seen during and shortly after induction.

Let it be noted that new reference values have been calculated and used, which are not the same as values used in older studies. If reference values are taken from the average domestic dog (Bodey and Michell 1996), the hypertension could be interpreted otherwise and probably considered to be more harmful to the wolves. However, not suffering from hypertension at all, may it be of a mild or severe diastolic or systolic type, would be optimal. It is the author's opinion that reference values should not be taken from the average domestic dog, but from a breed more similar to gray wolves in terms of size, condition and stamina, since reference values from free-ranging gray wolves are hard to obtain. The addition of medetomidine could be of use when investigating physiological responses of wolf immobilization, and trying to find an optimal drug combination for gray wolves.

In conclusion, the effect of helicopter pursuit on the wolves should be further investigated with a larger sample of animals. A control group of healthy captive wolves immobilized with the same anesthetics and dose, and no helicopter pursuit, will provide important data on how the pursuit afftects the wolves.

Although only mild physiological changes in blood pressure were identified in this study, there is a need to develop an optimal drug combination for safe capture and immobilization of free-ranging gray wolves. The addition of medetomidine to tiletamine and zolazepam could provide a more suitable anesthesia. The effects of the α_2-agonist could complement the effects of tiletamine and zolazepam, and drug doses would presumably not be as high as when only using tiletamine and zolazepam. In addition, it would be ideal to start monitoring as soon as the animal is recumbent in order to try and detect early fluctuations in blood pressure.

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ABSTRACT

This study aims to evaluate blood pressure measurements in immobilized free-ranging gray wolves (*Canis lupus*) in Sweden. Eight immobilized wolves (five adults and three juveniles) were included in this study. All wolves were darted from a helicopter with darts containing 250 milligrams of tiletamine and 250 milligrams of zolazepam. Immobilized wolves that showed signs of arousal, recieved a supplemental intramuscular injection of medetomidine. Systolic and diastolic blood pressure was measured non-invasively with an oscillometric device (Memoprint®, MediVet, Babenhausen, Germany) designed for dogs and cats. Rectal temperature, heart rate and respiratory rate were also measured. The hypothesis is that the wolves develop hypertension as a result of helicopter pursuit and the drug used. Three wolves were normotensive, four wolves had a moderate diastolic hypertenstion at one or more measuring point, and one wolf had a severe diastolic hypertension together with a mild systolic hypertension. None of the wolves had a moderate or a severe systolic hypertension. In conclusion, the effect on blood pressure was not as severe as previously thought. It was also noted that wolves who recieved an injection of medetomidine had a better anesthesia. In addition to this, other reference values for blood pressure and heart rates for free-ranging gray wolves should be used in future studies.

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