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# Transmitter-equipped darts in a protocol for chemically immobilizing free-ranging red deer (*Cervus elaphus*) in Central Italy

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## ABSTRACT

Twenty-two free-ranging red deer (*Cervus elaphus*), 9 males and 13 females (7 months to 13 years old), were captured in October-November 2006 and December 2007, along the Apenninic ridge (44°06'N, 11°00'E) between the Pistoia (Tuscany) and Bologna (Emilia-Romagna) provinces, as part of a reintroduction project in Gran Sasso and Laga's Mountains National Park (42°33'N, 13°28'E), Italy. The aim of the study was to evaluate the effectiveness of a red deer capture protocol, using equipped transmitter darts, in Apennine areas where other methods had not been successful. The red deer were darted (by an operative team of 5-7 operators during dark hours) with a mixture of Zoletil® (Z) and xylazine (X) and, whenever secondary dosages became necessary, a mixture of ketamine (K) and X were used. Twenty-five animals were shot, only 3 of which could not be approached to re-dart them. All animals were darted, during dark hours, from a vehicle at a distance of 10-30 m and then recovered, using 3-cc disposable Pneu-dart® transmitter darts, 50-280 m (median 80 m) from the dart site. These technical choices were forced by an uneven and wooded environment of the study area. Before the transport to the reintroduction site, the animals were kept in a lairage stable, arranged in single boxes, provided with water and food. This accommodation was necessary to collect a reasonable number of animals to arrange the transport. The immobilized animals were brought to the stable and haemoglobin saturation and heart rate were constantly monitored with a pulse oximeter. Oxygen was insufflated into a nostril at a rate of 10 litres/min for 20-35 min. Atipamezole was administered two thirds intramuscularly (IM) and one third intravenously, 45 to 120 min after the last IM narcotic injection, to revive the animals. No side effects other than different levels of meteorism, were displayed. In conclusion, the protocol applied, which proved safe for the animals and had optimal success in the recovery of darted subjects, can be considered effective to capture free-ranging deer in areas that are thickly wooded and difficult to traverse as areas of the Apennine.

*Key words: Cervus elaphus*, Xylazine, Zoletil®, Ketamine, Atipamezole.

## RIASSUNTO

### UTILIZZO DI DARDI MUNITI DI RADIOTRASMITTENTE IN UN PROTOCOLLO PER LA TELENARCOSI DI CERVI (*CERVUS ELAPHUS*) ALLO STATO LIBERO IN ITALIA CENTRALE

*Nei mesi di Ottobre-Novembre 2006 e Dicembre 2007, lungo la dorsale Appenninica (44°06'N, 11°00'E) fra le province di Pistoia e Bologna sono stati catturati 22 esemplari di cervo (*Cervus elaphus*), 9 maschi e 13 femmine, di età compresa fra 7 mesi e 13 anni. Tali operazioni erano parte di un programma di reintroduzione della specie nel Parco Nazionale del Gran Sasso e Monti della Laga (42°33'N, 13°28'E). Lo scopo del lavoro era quello di valutare l'efficacia di un protocollo di cattura di cervi mediante dardi muniti di radio trasmittente, in un ambiente Appenninico, altamente boscato, in cui precedenti esperienze con la telenarcosi e/o reti a caduta non avevano dato esito positivo. Gli animali sono stati contattati durante le ore notturne, lungo strade carrozzabili (fra 400 e 1000 m sul livello del mare) percorse su un veicolo fuoristrada e con l'impiego costante di 5-7 operatori. Per la telenarcosi sono stati utilizzati "transmitter darts" contenenti una miscela costituita da Zoletil® (Z) e Xilazina (X) nel rapporto di 1:1. Nei casi in cui sia stato necessario somministrare una dose aggiuntiva, quando l'animale non era sufficientemente immobilizzato, la miscela impiegata prevedeva Ketamina (K) al posto dello Z. Le ricerche degli animali colpiti, sono durate 1-8 min. per una distanza percorsa di 50-280 m (mediana di 80 m). Venticinque sono stati gli animali colpiti, ma 3 non sono stati recuperati in quanto il dardo non aveva avuto un corretto impatto sul bersaglio (regione anatomica non ottimale, impatto angolato con iniezione verosimilmente sottocutanea). Una volta sull'animale narcotizzato, questo veniva immediatamente bendato, posto sul fianco destro e legato per ridurre eventuali rischi per lo stesso e gli operatori. Dopo essere stato caricato in un veicolo appositamente adibito, veniva monitorato mediante pulsossimetro ed introdotto un sondino endonasale (4 mm di diametro) nel meato ventrale fino all'altezza del canto mediale dell'occhio per un apporto di ossigeno di ca. 10 litri/min per 20-35 minuti. Gli animali venivano portati in una stalla appositamente organizzata in "boxes" singoli all'interno dei quali veniva somministrato loro Atipamezole (un terzo della dose in vena e due terzi intramuscolo) da 45 a 120 minuti dalla ultima somministrazione di narcotico. La sosta nella stalla era resa indispensabile in quanto era necessario radunare un certo numero di animali per organizzare il trasporto per la reintroduzione, che avrebbe avuto una durata di 6 ore. Il protocollo descritto, avendo registrato una ottima tollerabilità senza effetti collaterali da parte degli animali ed un ottimo successo di recupero dei soggetti colpiti, si è rivelato molto efficace in un ambiente e per densità di popolazione (2,5-3 capi/100 ha) tipici delle aree oggetto di studio.*


Parole chiave: Cervus elaphus*, Xilazina, Zoletil®, Ketamina, Atipamezolo.*



## Introduction

During the last twenty years many studies have reported the efficacy and safety of alpha-2-adrenoceptor agonists in combination with general anaesthetic agents such as ketamine (K) or tiletamine-zolazepam (T-Z) in many species of deer: red deer (*Cervus elaphus*) in Europe (Jalanka and Roeken, 1990; Janovsky *et al.*, 2000) and other countries (Millspaugh *et al.*, 1995; Read *et al.*, 2001; Walter *et al.*, 2005), and white-tailed deer (*Odocoileus virginianus*) in the USA (Kilpatrick and Spohr, 1999; Miller *et al.*, 2004). These combinations, alpha-2-adrenoceptor agonists and K or T-Z, are very well known and used, probably because they have shown the closest characteristics to the "ideal" immobilization drugs to date (McKenzie, 1993). Moreover, significant improvements have been made in radio transmitter technology. Radio-transmitters in darts, when used in this field, allowed quick and effective recovery of the darted animals (Kilpatrick *et al.*, 1996; Kilpatrick *et al.*, 1997; Walter *et al.*, 2005) in a difficult environment. This aspect is very important and decisive in the Apennine districts where most territory is wooded (over 70%), has an inclination between 15 and 42 degrees (over

40%) (Gaggioli, 2004) and the red deer density is 2.5-3 deer/square kilometre (Mattioli and Nicoloso, 2003). Moreover, by the study of the territories and the observations of the red deer population, we became conscious of the possibility of approaching these animals only during dark hours. In previous studies carried out in the National Park of Casentinesi Forest, Falterona Montains and Campigna (43°52'N, 11°42'E) (1993-94) and in the same area of this study (1998-99), the use of chemical-darting immobilization without radio transmitters proved to be unsuccessful because the recovery of shot animals was very difficult or a failure (unpublished data). Negative results were obtained as well in 2002 in the study area using dropping-nets or a combination of dropping-nets and chemical immobilization when, despite the participation of many workers, up to 2 out of 8 captured animals died (unpublished data). These results proved that the wooded areas of the Apennines, unlike results reported in recent studies carried out in open or high altitude areas (Dematteis *et al.*, 2006), do not permit observers to visually trace the shot animals. Finally, food trapping was thought to be ineffective in the Apennines where brief and mild winters do not prevent the animals from finding food.

The technique allows a rapid release of immobilized animals (Kilpatrick *et al.*, 1996; Kilpatrick *et al.*, 1997; Walter *et al.*, 2005), depending on the choice of the drug. A critical aspect of chemical immobilization may cause hypoventilation and hypoxemia resulting from depression of central respiratory centres (Caulkett, 1997; Caulkett *et al.*, 2000; Janovsky *et al.*, 2000; Murray *et al.*, 2000; Matt, 2001; Read *et al.*, 2001). In addition the animals may assume positions that prevent complete pulmonary ventilation (Doherty *et al.*, 1986; Doherty *et al.*, 1987) and/or show quick ruminal meteorism (van Miert, 1994; DeRossi *et al.*, 2005) deriving from ruminal atony and/or incorrect recumbence. Hence, it is of the utmost importance to consider that hypoxemia can result in a predisposition to tissue hypoxia, myocardial ischemia, and organ failure similar to capture myopathy (Spreaker, 1993). Recently, positive effects against hypoxemia in wapiti (*Cervus elaphus*, North American elk) were reported by Read *et al.* (2001) by using 10 litres/min per 5 min nasal insufflation of oxygen.

The aim of the present study was to investigate the applicability and the use of a protocol of chemical-darting immobilization in red deer, followed by recovery of animals using radio transmitters, in central Italy, where environmental characteristics of the Apennines have been proven to render other techniques less safe and/or effective.

## Material and methods

Twenty-two free-ranging red deer were immobilized during three capturing sessions in the Tuscan-Emilian Apennines along the Apenninic ridge (44°06'N, 11°00'E) between the Pistoia (Tuscany) and Bologna (Emilia-Romagna) provinces in Italy. Two sessions were organized in October and November 2006, the third in December 2007. The animals were part of a study of red deer reintroduction in Gran Sasso and Laga's Mountains National Park (42°33'N, 13°28'E), Italy. The above-described environmental characteristics made the arrangement for lairage necessary. In fact, each of the three capturing sessions lasted at least twelve days and within each session one animal was captured on alternate days. That time was necessary to collect a reasonable number of animals for the organization of the translocation travel to be completed within six hours. The animals used for the study were 9 males and 12 females, aged from 7 months to about 8 years, plus one 13 –year-old female. An operative team of 5-7 opera-

tors searched for the animals during dark hours (from 8.00 p.m. to 4.00 a.m.) and while driving along wooded roads (from 400 to 1,000 m above sea level). The red deer were primarily darted using 500 mg of Zoletil®100 (Virbac) (Z) (250 mg of tiletamine and 250 mg of zolazepam) reconstituted with 5 ml of 100 mg/ml xylazine HCl (X) (Rompun® dry substance, Bayer). Thus, a solution of 100 mgX/ml+100 mgZ/ml was prepared. Whenever secondary dosages were necessary, a mixture of ketamine (K) and X (100 mgK/ml+125 mgX/ml) was used to avoid a zolazepam accumulation and enable a quicker recovery (Fahlman, 2005). The first mixture was put into a Pneu-dart® (Pneu-dart Inc., Williamsport, Pennsylvania, USA) 3.0 cc, double-barded and 1 1/4 inch long needled, transmitter-equipped darts to make the quick recovery of the immobilized animals possible. Darts were blown by Injection Rifle Model JM Special® (Dan-Inject, Børkop, Denmark) at a distance ranging from 10 to 30m from the vehicle. The secondary dosage was also administered (23-28 min after the first dose) by rifle from about 10-20 meters, using Pneu-dart® 3.0 cc darts with gel-collared, 1 1/4 inch long needles.

For the sessions during October-November 2006, three different standard doses were preventively considered for filling the dart: for calves, for females and 1.5-3.5 years old males, and for males over 3.5 years old. This choice was based on the knowledge that the deer population had three weight ranges: calves in October-November, median 55 kg; females and 1.5-3.5 years old males, median range 95 and 122 kg, respectively; and males over 3.5 years, median 179 kg (Nicoloso, unpublished data). For the session during December 2007, based on the high level of safety of the drugs experienced during the previous year and as also reported by Janosky *et al.* (2001), a first standard dosage of 2.8 ml of 100 mgX/ml+100 mgZ/ml solution per dart was used for all age classes.

To allow a good level of sedation and reduce the stress of handling, the search with a receiving radio Televilt RX98® (Televilt, Lindesberg, Sweden) never began before 15-20 min after darting (Kilpatrik *et al.*, 1996). Upon capture, the animals were immediately blindfolded, legs tied, turned on the right side and the dart was removed with a sterile scalpel, and oxytetracycline spray was administered on the wound (Walter *et al.*, 2005). All subjects were laid on a vehicle arranged for transport to a stable, except for the 13-year-old female, judged too old for the transport/reintroduction and chemically revived on the capture site.

Haemoglobin saturation ($SpO_2$) and heart rate were monitored by a pulse oximeter Nellcor NPB-40® (Nellcor, Hayward, California, USA) with a transmission probe placed on the tongue. A 4mm nasal insufflation catheter was inserted into a nostril in the ventral nasal meatus and advanced to the level of the medial canthus of the eye, insufflating humidified oxygen ($O_2$) at a rate of 10 litres/min (Read *et al.*, 2001) for 20-35 min. Once arrived at the stable, each animal was weighed and 0.2 mg/kg of ivermectin were subcutaneously administered (Foreyt *et al.*, 2004). The deer were provided with closed and single boxes (2x1.5x2.5 m for young, females and 1.5 years old males, and 2x2x2.5 m for older males). The walls of boxes were built into a stable with window illumination by assembling wood 2.7 cm thick panels (the same panels used in the building industry to make reinforced-concrete walls). Straw and zootechnical wood-shavings were used as litter. Before to introducing the deer inside, every box was carefully washed using a washing machine with bleach and then scattering burnt lime.

Antagonist treatment consisted in mean ±DS dose 0.13±0.03 mg/kg atipamezole (At) (15.2±4.56 mg/deer) and it was injected as soon as the animals were in their boxes: two

thirds IM (gluteus) and then a third intravenously (IV) (jugular vein), 45 to 120 min after the last IM narcotic injection.

Before the transport to the reintroduction site, the animals were kept in a stable with water and food availability (leafed boughs, chestnuts, seasonal fruits such as apples, pears, grapes, as well as vegetables and pellets, although a constant preference for ivy (*Hedera helix*) was shown. The lairage time was a median of 13 days (minimum 3 days and maximum 20).

Statistical analysis was performed by Student's t-test for comparison of mg/kg dosages between A and B group (once and twice darted animals, respectively), and by Fisher's exact test for the comparison of anatomic dart sites in the two different groups.

## Results and discussion

The 13 deer of the 2006 sessions, shot with initial standard darts prepared for the three animal classes mentioned above, had a final mean ±DS dose of 1.95±0.4 mg/kg of both Z and X. For five out of 13 cases, a secondary dosage became necessary to complete the sedation. In these cases, a mixture of X-K at a mean ±DS doses of 1.81±1.08 mg/kg/X and 1.46±0.84 mg/kg/K were used, respectively. The 9 subjects of the 2007 session had a final mean ±DS dose of 2.4±0.56 mg/kg of both Z and X, and in this case only one deer was re-darted with the above-mentioned X-K dosage. Dividing all animals into two groups (group A animals darted once, and group B animals darted twice), the subjects of group B received a first standard X-Z dose lower than animals of group A (1.88±0.51 mg/kg *vs* 2.34±0.53 mg/kg), but the difference was not statistically significant (P>0.05, by Student's t-test).

The red deer were found within 1-8 min of search (20 to 28 min after the shot) and at 50-280 m (median 80 m) from the dart site. The darts had entered a hind limb in 10 cases, a shoulder in 8 cases, and the remaining 4 in the back, perineum, flank (in front of left knee) and the sacrum-iliac, respectively. Seven of the group A animals were hit in the shoulder, 8 in the hind limb and the last one in the flank. Two of the group B animals were shot in the hind limb and the remaining 4 cases in the shoulder, back, sacrum-iliac region, and perineum, respectively. Moreover, three darted animals were approached, enough to see the dart on the subjects but not to be re-shot and recovered. In one case the deer was shot in the tibial region. The other two moved during the moment of shooting and the dart hit aslant the animal, probably administering the drugs subcutaneously. Table 1 summarises all the shots, goals, and recovered animals.

The animals showed physiologic ranges of heart and respiratory rate and rectal tem-

Table 1. Comparison between shot darts and recovered animals.

| | 2006 | 2007 | Total |
|---|---|---|---|
| Shot darts | 29 | 28 | 57 |
| Recovered animals | 13 | 9 | 22 |
| Darts plunged in the animals | 15 | 10 | 25 |
| Wrong shots | 14* | 18* | 32 |
| Percentage of success | 44.8% | 32.1% | 38.6% |

**in 4 cases dart touched the target but it did not penetrate.*

perature. During transport, $SpO_2$ measured by the pulse-oximeter ranged from 68% to 90% and after the insufflation of $O_2$ the measure suddenly increased and stabilized between 88% and 95%. A 6-year old female was the only one showing slow and superficial breathing ($SpO_2$ constantly between 40% and 52%), seeming not to respond to the oxygen-therapy. In four cases (two 3year-old females and two 6-7year-old males), it was necessary to use a 1 cm diameter endo-esophageal latex tube to evacuate gas and reduce ruminal meteorism, which was present to a lesser extent in all the other animals without group prevalence.

The atipamezole was administered 45 to 120 min after the last IM narcotic injection at a mean ±DS dose of 0.13±0.03 mg/kg with a mean ±DS At:X dose ratios of 1:22.16±6.59. The mean ±DS times from antagonist injection until standing were 3.7±1.5 min.

The use of "transmitter-equipped darts" allowed us to wait for 15-20 min before beginning the search of the darted animals that were recovered within 1-8 min, in a median of 80 m from the darting site. The three failures in this study seemed due to casual events but not to the transmitter technique. These outcomes confirmed the effectiveness and quickness of both techniques together, i.e. chemical and by transmitter (Kilpatrick *et al.*, 1996; Walter *et al.*, 2005). It also proved successful in the Apennine environment where visibility is reduced at short distances, and in particular when the behaviour of the animal population forced the researchers to work during dark hours. Thus, the radio technique assured a complete and safe effectiveness of the narcotic, when the injection was made correctly, a calm sedation and optimum success in the recovery of all the darted animals as also reported by Kilpatrick *et al.* (1996) and Walter *et al.* (2005), in a heavily wooded environment, as well. Furthermore, the approach was made as slowly and carefully as possible, and the workers were always informed about movements of the deer. These aspects yielded good results with respect to the times and means for a suitable approach.

The general average doses of X (2.95±1.09 mg/kg) used with success in this study were slightly higher than those reported by other authors (Janovsky *et al.*, 2000; Read *et al.*, 2001; Miller *et al.*, 2004; Walter *et al.*, 2005). The same applies to the X and Z dosing range. In the present study, the ranges were 1.63 to 5.7 mg/kg and 1.21 to 3.00 mg/kg, respectively, whereas Walter *et al.* (2005) reported 1-3 mg/kg and Janovsky *et al.* (2000) 0.7-4 mg/kg. The known wide safety margins of X and Z (McKenzie, 1993; Walter *et al.* 2005) have been confirmed showing no side-effects other than meteorism, even in the third session where the same standard dose for every age class were used. When we considered the two groups of animals (A and B), group B (deer darted twice) received a first lower mean dose than group A (deer darted once). This difference was not statistically significant probably due to several reasons. One of those, even though not statistically significant because of the small number of animals, was the anatomic shooting goal. Fifteen animals of group A received the first dose in an optimal anatomic region (hindquarters and shoulder) (Janz *et al.*, 1980), whereas 3 out of 6 (50%) animals of group B received the mixture in less suitable anatomic regions: namely the perineum (too much adipose tissue), back and sacrum-iliac. In these latter cases, it is possible that the impact of the needle was not perpendicular to the body and part of the drug was injected in the subcutaneous tissue. It is known that an injection in a non-muscular or damaged tissue, associated with the impact of the dart, may cause incomplete drug absorption (Meuleman *et al.*, 1984; Walter *et al.*, 2005). Moreover, 50% (3 out of 6) of group B became 66.6% (4 out of 6) if we consider that a subject lost the dart shortly after

shooting and 77.7% (7 out of 9) if we consider the three non-recovered subjects. Even though these differences in events (first dose, impact site and angle of impact) were not singly enough or statistically significant, these observations and individual variability (Smith, 1993) could altogether give an explanation to the second necessary sedative dose. Furthermore, our results (two darting doses in 6 out of 22 cases, 27.3%) are consistent with those reported by Walter *et al.* (2005). In a similar study on USA free-ranging elk, an additional narcotic dose was administered to 5 out of 14 deer (35.7%).

That which is proposed in this paper is probably the best combination of chemistry and physics in the capture of wild ungulates, also in difficult environments. It was also confirmed by the physiologic heart rate, respiratory rate or rectal temperature shown by the subjects, even in highest dosage (5.7 mg/kg X and 3.0 mg/kg Z). Moreover, the $O_2$ insufflation suddenly increased $SpO_2$ from 87% to 95%. Depression of the central respiratory centre caused by anaesthetic agents in different ruminant species (Lin *et al.*, 1993; Caulkett *et al.*, 2000) and the $O_2$ therapy to reduce the chemically inducted hypoxemia (Read *et al.*, 2001) is well known. Prolonged hypoxemia of increased metabolic request may predispose the animals to the development of capture myopathy (Spreaker, 1993). In the only case in which the $SpO_2$ did not increase after $O_2$ therapy, it did not even improve after intramuscular administration of a third of the complete antagonist dose on the vehicle (25 minutes after darting). In this specific case $SpO_2$ had not even increased after a sub-lingual administration of 1.3 ml doxapram HCl. Nevertheless, the animal was reversed from sedation with a normal standing without experiencing other side effects. Probably, this was a case of individual sensitivity to drugs (Smith, 1993) or possibly a sensitivity connected to some unknown metabolic and/or physiologic condition.

The alpha-2 adrenergic drugs promote sympathetic nervous system depression and a consequent increase in the parasympa-

Table 2. Costs/benefits of the two methods.

| | Captured animals | Working-days/man | Preparing site of capture | Number of working days | Dead animals | Cost of capture tools (€) | Costs of drugs per captured animal (€) | Other aspects |
|---|---|---|---|---|---|---|---|---|
| Transmitter-equipped darts technique (2006-2007) | 22 | 5-7 people | - | 39 | - | 6,000* | 224 | Quick possibility to move to different capturing areas |
| Dropping-net/chemical immobilizing technique (2002) | 8 | 10-15 people | 2 | 11 | 2 | 14,200§ | 65 | The possibility to work only where the nets are spread |

**Considering rifle, darts, transmitters, receiving radio, pulse-oximeter.*
*§Considering nets, darts, rifle.*

thetic activity with many physiologic effects and ruminal-reticular atony (van Miert, 1994; DeRossi *et al.*, 2005). This aspect, recumbence and a mix of seasonal foods may explain the described ruminal meteorism.

The choice of atipamezole as alpha2-antagonist to reverse the X effects is in accordance with many studies reporting this drug to be effective as an antagonist to X in wild animals (Anremo *et al.*, 1993; Acrenaz, 1994; Caulkett 2001; Miller *et al.*, 2004; Janicki *et al.*, 2006). The difference in handling time (45 to 120 min) regarded the place in which the animals had been darted and the distance between same and the lairage stable. The differences in time of antagonist administrations and single or adjunctive darted animals did not show discrepancies in mean ±DS times (3.7±1.5 min) compared to standing after reversal-agent injection (Walter *et al.*, 2005).

In this study, no zolazepam antagonist was used, thus adjunctive doses of Z could lengthen the recovery times (Schobert, 1987; Fahkman, 2005). Our standing times were a bit shorter than those of other authors (Miller *et al.*, 2003; Miller *et al.*, 2004; Walter *et al.*, 2005), probably because of the administrated formula we used: two thirds IM and a third IV. This means of administration allows the animals reversed under wild conditions to immediately have good reactions and flight towards external stimuli (Schobert, 1987).

## Conclusions

The present study proved the effectiveness and safety of the wild animal capture protocol presented, in particular when compared with the other experiences in the same area (Table 2). Moreover, it was the first real success in Apennine districts where the environmental, deer-population characteristics and the forced night-time work make the use of transmitter-equipped darts to find immobilized animals essential.

*Part of the results were presented during the 1st International Conference on Genus Cervus, Trento, Italy (Natural Park of Paneveggio-Pale di San Martino, September 2007).*

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