

## PRODUCTION NOTES

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### **Remote chemical immobilisation method for free-ranging Australian cattle**

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**Background** Many situations are encountered in Australia where the capture and restraint of free-ranging cattle (*Bos taurus*/*Bos indicus*) is required. Chemical immobilisation via darting is a potentially useful tool for managing and researching large wild herbivores; however, there is no reliable method for its application to Australian cattle. The aim of this study was to develop an efficacious, humane, cost-effective ground darting method for free-ranging cattle.

**Methods** The 30 female cattle were darted and captured on a pastoral station in north-west Australia from a vehicle. Xylazine (0.59 mg/kg) and ketamine (3.59 mg/kg) were used to capture animals and yohimbine (0.10 mg/kg) was used as an antagonist to xylazine to reduce recumbent time.

**Results** Cattle became recumbent at a mean time of 8 min and a mean distance of 260 m from darting. The mortality rate was zero on the day of capture and 7% at 14 days post-capture.

**Conclusions** The majority of darted cattle were successfully immobilised with one dart and recovered within 30 min, with consumables costing approximately A\$30 per captured animal. The technique developed represents a rapid and humane method for capturing free-ranging cattle and, with consideration for legislation surrounding use of veterinary chemicals, could be applied in many contexts across Australia.

**Keywords** anaesthesia; cattle; darting; ketamine; xylazine; yohimbine

Cattle (*Bos taurus* and *B. indicus*) are often encountered under free-ranging conditions in Australia, particularly with extensive pastoral livestock<sup>1</sup> and 'feral' cattle<sup>2</sup> in the rangelands

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of Australia. Free-ranging domestic cattle are also sporadically encountered in cases of escapees from loading yards or saleyards,<sup>3</sup> transport vehicle rollovers<sup>4</sup> or on landholdings lacking appropriate yard facilities.<sup>5</sup> Capture of cattle in situ (i.e. without mustering into yards) is often required for veterinary intervention of sick animals,<sup>6</sup> management of feral cattle<sup>2</sup> or for research purposes.<sup>7</sup> As such, reliable cattle capture methods are increasingly required when traditional physical capture methods (yarding) are unsuitable. Remote delivery of chemical immobilisation ('darting') is commonly used for the capture of wildlife species,<sup>8,9</sup> as well as domestic species, including aggressive dogs,<sup>10</sup> unbroken horses<sup>11</sup> and free-ranging livestock.<sup>12</sup> Despite the widespread use of darting, relatively little is known of its application to cattle,<sup>6,13,14</sup> particularly in Australia. Factors to consider when developing any chemical immobilisation regimen include choosing chemicals that allow a low dart volume, produce a short induction time and ideally can be reversed by an antagonist drug.<sup>9,15</sup> This task is made more challenging for any food-producing animals in Australia because immobilising chemicals must also be legally allowed under food safety and veterinary prescribing legislation at the state and national levels. At the national level, this legislation consists of the *Agriculture and Veterinary Chemicals (Administration) Act 1992* and the *Agriculture and Veterinary Chemicals (Administration) Regulations 1995*. At the state level, in Western Australia (the site of this study) the legislation consists of the *Veterinary Preparations and Animal Feeding Stuffs Act 1976* and *Veterinary Chemical Control Regulations 2006*.

Legislation regarding the administration of veterinary chemicals to livestock is complicated in Australia,<sup>16</sup> with legislative interpretation differing between jurisdictions.<sup>17</sup> In some jurisdictions of Australia, it is prohibited to administer any product that has a label claim stating that it should not be used in food-producing animals and prescribing veterinarians may be prosecuted if they ignore these claims. Similarly, the use of unregistered or compounded pharmaceutical products in food-producing animals is restricted to individual animals in some states and prohibited in Australian territories without specific permits.<sup>17</sup> In Western Australia the legislation does not restrict the use of unregistered or compounded pharmaceutical products to individual food-producing animals.<sup>17</sup>

The Australian Pesticides and Veterinary Medicines Authority is the Australian Government regulatory body that oversees the registration of all veterinary chemicals and products in Australia. The Authority provides a list of products that are registered for use in Australian jurisdictions and outlines details relating to each specific veterinary chemical product, such as withholding periods for meat consumption or export purposes.<sup>18</sup> Most of the common preparations of drugs used in modern wildlife immobilisation are not registered for use in food-producing animals in Australia (Table 1).

The  $\pm$ -2 agonist xylazine has traditionally been the drug of choice for cattle immobilisation via hand injection for 40 years,<sup>19</sup> but the requirement for higher drug doses with dart administration dictates that mortality risks can be very high when xylazine is used alone.<sup>5</sup> For this reason, very few modern wildlife darting methods use a single drug<sup>20,21</sup> and  $\pm$ -2 agonists are commonly combined with dissociative anaesthetics (ketamine and/or tiletamine) to achieve anaesthesia.<sup>6,22,23</sup> In this study, xylazine was combined with ketamine and the combination was partially reversed with the  $\pm$ -2 antagonist yohimbine.

The aim of the present study was to develop a practical, cost-effective method for the darting and capture of free-ranging Australian cattle, using inexpensive drugs and a vehicle-based

darting method. As there have been few published methods for cattle darting using drugs that are accessible in Australia (i.e. not prohibited for use in food-producing animals), we conducted a preliminary trial of a novel chemical immobilisation technique on a small group of free-ranging pastoral cattle.

**Comment [NS1]:** Is it more a novel regimen rather than a technique?

### Materials and methods

All animal procedures used in this study were approved by the Department of Parks and Wildlife Animal Ethics Committee (approval no. 2014–22) and all animal observations were approved by the Murdoch University Animal Ethics Committee (approval no. 02674/14).

#### Study area

This study was conducted on an extensive grazing pastoral lease in the Kimberley region of north-western Australia (16°50'S and 125°28'E) over 6 days in April 2015. Maximum air temperatures in the shade were high at the field site on all days of the study (mean 38°C; Bureau of Meteorology, 2015). Immobilisation attempts were planned to avoid the hottest times of day to reduce the hyperthermia risk for captured animals.

#### Animals

We targeted adult female cattle (*B. taurus*, *B. indicus* and mixed-breed animals; Figure 1) that were standing close to station roads, in areas of open vegetation and in the presence of other cows. These criteria were selected to minimise the proportion of animals that would express agitated escape behaviour and also minimise our likelihood of losing escaping animals in thick vegetation. Selected cattle were healthy in appearance with an estimated body weight of 300–500 kg based on visual appraisal. Bulls were not targeted and only stationary animals standing broadside to the darting vehicle (Figure 1A) were darted. The cattle were being captured to facilitate fitting of telemetry collars as part of a larger study investigating cattle movements around fire scars run by Rangelands NRM and the Australian Wildlife Conservancy, and funded by the Australian Government's National Landcare Programme.

#### Drug regimen

All cattle were immobilised with xylazine (100 mg/mL; Ilium Xylazil-100®; Troy Laboratories, NSW, Aust) and an unregistered ketamine preparation (2 g freeze-dried powder; Mavlab Animal Health, QLD, Aust). The xylazine portion of the anaesthetic combination was reversed with the  $\pm$ -2 antagonist drug yohimbine (10 mg/mL; Reverzine®; Bayer Animal Health, NSW, Aust).

Most cattle received one of two drug dosages: one designed for larger animals (estimated weight > 400 kg; 230 mg xylazine, 1333 mg ketamine) and one designed for smaller animals (estimated weight < 400 kg; 220 mg xylazine, 1333 mg ketamine). Xylazine was mixed with freeze-dried ketamine and combined with sterile water. Remote injection of the immobilising agents was facilitated by 6-mL Pneu-Dart® type U explosive-powered darts, fitted with 1.25 inch (3.8 cm) needles that were either collared or had wire barbs to prevent them from falling out (Figure 2; Pneu-Dart, PA, USA). The dart rifle used was a Pneu-Dart® X-Calibre CO<sub>2</sub>-powered dart rifle (Pneu-Dart) fitted with telescopic sights (Figure 1A). The power settings of the rifle were as per manufacturer recommendations (www.pneudart.com). Darting and drug handling were performed by a veterinarian.

#### Immobilisation procedure

All cattle in the study were darted from the open window of a stationary vehicle, using the wing mirrors as a shooting rest (Figure 1A). Cattle more than 40 m away from the shooter, as

measured by a Leupold® RX™ II digital range finder (Leupold and Stevens Inc., Beaverton, OR, USA), were not darted. For all animals, the intended dart administration site was the rump (Figure 1B) or the neck and the intended injection route was IM. As soon as a cow was darted, two operators pursued the cow ( $\pm$  accompanying mob) in an attempt to maintain visual contact without encouraging further flight behaviour. Induction time was measured as the time from the darts impact to the time the cow first became recumbent.<sup>23</sup> Induction distance was calculated as the linear distance between the darting and recumbency sites, as measured with a hand-held GPS unit.<sup>22</sup> Once recumbent, two operators waited a minimum of 3 min before quietly approaching the cow from behind and applying physical restraint of the cow's head (Figure 1B), as is common field practice for capturing large wild herbivores.<sup>22–24</sup> Cows were restrained on whichever side they fell on and were blindfolded with a blanket to reduce visual stimulation and to diminish potential injury to their eyes (Figure 1B). Darts, if present (e.g. Figure 1B), were removed and antiseptic spray was applied to all dart sites (Figure 1C). Attempts were made to cool recumbent animals by pouring water over the inguinal and axillary regions.<sup>23</sup>

Anaesthetic monitoring of physiological parameters was performed immediately after lateral recumbency was achieved and then every 5 min until the animal was standing. Heart rate was recorded by cardiac auscultation; respiratory rate by counting chest excursions; pulse strength by digital pressure on the facial artery and body temperature by a thermometer placed in the rectum. Haemoglobin saturation, measured as oxygenation, was measured by a battery-powered hand-held pulse oximeter (Newtech, Guangdong, China), placed on the tongue. Body weight was estimated from morphometric measurements of recumbent animals, using the 'weigh tape' approach.<sup>25</sup>

The only procedure performed beyond anaesthetic observation in our study was the fitting of telemetry collars (Figure 1C). As soon as this was achieved, cows were given an IM injection of yohimbine (40 mg per animal), not less than 20 min after darting.<sup>23</sup> It was not administered to cows with a very light plane of anaesthesia, as antagonism was not considered necessary to allow a rapid recovery for these cows. Once animals began to show signs of anaesthetic recovery, their blindfold was removed and they were released from physical restraint. If animals remained in lateral recumbency after the removal of the blindfold, they were placed into sternal recumbency to aid rumination and reduce the likelihood of hypoventilation, bloat and aspiration of rumen contents<sup>26</sup> (Figure 1C). Once animals had risen to their feet, further observations were performed from a safe distance to ensure recovery, defined as sufficient muscular strength and coordination of movements to remain standing, was complete (Figure 1D).

#### ***Monitoring of captured animals***

Cows were opportunistically observed 18–24 h post-capture through VHF telemetry, if they were sufficiently close to their capture site (< 500 m). Locations where cattle were captured were revisited, either within the 6-day darting period or 2 weeks later, and the movement patterns of cattle were monitored opportunistically over the next 3 months using VHF tracking, to determine whether individuals were alive and mobile. For individuals that were not sighted during radio-tracking (because of the terrain or vegetation), the VHF signal to detect signs of movement (changes in signal direction, as well as fluctuations in signal strength associated with head movements when grazing) was carefully monitored. We report two mortality rates: at the time of capture to account for acute traumatic injuries and at 14

days post-capture to account for chronic metabolic diseases (e.g. capture myopathy).<sup>27</sup> Results are presented as mean  $\pm$  SEM and range.

### Results

The darting method described was used to capture 30 cattle, with 28 cattle becoming laterally recumbent after administration of one dart and 2 requiring two darts to become recumbent because of initial under-dosing. Shooting distance ranged from 14 to 31 m, with a mean ( $\pm$ SE) of 21 ( $\pm$  1) m. Induction time ranged from 3 to 18 min with a mean of 8 ( $\pm$  1) min. All cows displayed some form of flight behaviour upon being struck by a dart<sup>28</sup> and induction distance ranged from 36 to 794 m with a mean of 260 ( $\pm$  32) m. Recumbency time ranged from 8 to 71 min with a mean of 27 ( $\pm$  3) min (Table 3). One other animal was lost during induction (escaped), as the capture team unable to track the animal through thick vegetation (e.g. Figure 1D).

Drug doses were calculated retrospectively after estimation of body weight post-darting. The mean dose for xylazine was 0.59 ( $\pm$  0.02) mg/kg and for ketamine was 3.59 ( $\pm$  0.012) mg/kg. Yohimbine was administered to 87% (26/30) of cows, at a mean dose of 0.10 ( $\pm$  0.009) mg/kg. All barbed darts (n = ?) and 15% (n = ?) of collared darts were found still embedded in the animal's skin when the cows were approached in lateral recumbency (Figure 1B). All measured physiological parameters were within normal ranges for the duration of the anaesthesia in all cows, with no animals displaying a body temperature  $>$  40°C, which constitutes hyperthermia<sup>8</sup> (Table 3). On average, all cattle were laterally recumbent for 27 ( $\pm$  3) min.

No cows died during anaesthesia; however, one cow was found dead 18 h after capture near her capture site. Postmortem examination suggested aspiration had occurred secondary to regurgitation. The remains of another cow were located 3 months after capture (hence postmortem examination was not possible) and analysis of movement data from her telemetry collar confirmed that she had died within 2 days of capture. Both cows that died received a single dart to the rump, had rapid inductions (5 and 8 min, respectively) and did not display hyperthermia during anaesthesia. Most of the cows (n = 25) were confirmed to be alive and mobile via either visual confirmation (n = 13) or continued movement of VHF transmission (n = 12) at 1 month and 3 months post-capture. The remaining 3 cows had moved outside of the general study area 1 month post-capture and therefore were presumed to be alive and mobile. Hence, the mortality rate at the time of capture was zero, but the mortality rate at 14 days post-capture was 7%.

### Discussion

The immobilisation regimen described in this study was effective, allowing reliable capture of free-ranging cattle. The method produced a mortality rate of zero at the time of capture, but 7% at 14 days post-capture. Induction times and distances travelled during induction were sufficiently short to allow the pursuit and capture of 97% of darted animals within a landscape that featured thick vegetation. Recumbency periods were short in duration (27 min) through the use of partially reversible, multiple agent anaesthesia.

The drug combination used was chosen for several reasons. Firstly, the drugs are registered for use in food-producing animals in Australia. Although the preparation of freeze-dried ketamine used in this study is not registered, an alternative formulation of the same drug (Ketamil®; 100 mg/mL ketamine), registered in Australia and with a 28-day meat withholding period, is available (Table 1).<sup>18</sup> The dart size limitation (6 mL) made the use of

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Ketamil® impractical in the single-dart technique used of our study. However, the dose rate of xylazine used for darting the cattle was much higher than that traditionally given through hand injection to domestic cattle.<sup>29</sup> Free-ranging, excited or aggressive cattle have an increased resistance to the effects of xylazine; hence doses of up to 1.6 mg/kg of xylazine have previously been used for the capture and immobilisation of such individuals.<sup>5</sup> However, high doses of xylazine in cattle are associated with high mortality rates and should be used with extreme caution.<sup>5</sup> Furthermore, the xylazine–ketamine combination interferes with thermoregulation and hot conditions are known to make cattle more prone to xylazine-induced physiological distress,<sup>29</sup> which was a concern in our study because high mean daily maximum temperatures (38°C) were encountered. By operating at cooler times of day, minimising induction and recumbent times, actively cooling recumbent animals and early use of a reversing agent, yohimbine, we were able to avoid any animals experiencing hyperthermia.

Without adequate caution and veterinary care, high mortality rates may occur with xylazine anaesthesia, especially in *B. indicus* cattle, which have a known sensitivity to xylazine compared with other breeds of cattle.<sup>30</sup> We strongly discourage the use of a xylazine-only drug regimen for the darting of excited free-ranging cattle, as the dosages required (up to 1.0 mg/kg) are likely to result in a high number of deaths and anaesthetic injuries.<sup>5</sup>

Yohimbine has been shown to be an inferior antagonist to xylazine than tolazoline or atipamezole in many ungulate species,<sup>31,32</sup> including white-tailed deer (*Odocoileus virginianus*)<sup>33</sup> and cattle.<sup>34</sup> However, it is the only  $\pm$ -2 antagonist drug currently licensed in Australia for food-producing animals.<sup>18</sup> We acknowledge that there are inherent operator safety risks associated with the use of potent immobilisation chemicals in remote settings, but we argue that these risks can be managed by following best practice for dart handling.<sup>9,23</sup>

The frequency of animals that escaped in our study (3%) was low compared with many wild ungulate darting studies (e.g. 48%; white-tailed deer).<sup>35</sup> Transmitter darts could be used in thicker vegetation if there was concern over high escape rates.<sup>22,35</sup> We recommend the use of collared needles if there is a high likelihood of animals escaping, as we found in our study that only 15% of collared needles remained embedded in animal's tissues at capture compared with 100% of the barbed needles. The implication is that darts with barbed needles will remain embedded in the tissues of animals that escape, causing ongoing pain and trauma and likely leading to secondary infections. With the use of collared needles, darts are very likely to fall out within 15 min of darting, reducing the likelihood of ongoing health issues in escaped animals. The mean induction time we report (8 min) was slightly less than that reported by other cattle darting studies (e.g. 10 min).<sup>5</sup>

The mortality rates of zero at the time of capture and 7% at 14 days post-capture were comparable to those for many other large mammals subjected to darting. It has been suggested that a mortality rate target of 2% at the time of capture<sup>8</sup> and 5% at 14 days post-capture<sup>27</sup> should be aspired to for all long-term large mammal chemical immobilisation programs. However, mortality rates are typically highest for newly developed regimens before drug doses are adjusted and capture methods are refined.<sup>8</sup> Higher mortality rates have been demonstrated for many ungulate species subjected to chemical immobilisation, particularly in hot climates. For example, mortality rates of between 6% and 38% have been reported during darting of feral horses<sup>11,36,37</sup> and often exceed 10% for the darting of kangaroo species.<sup>28</sup>

The total cost of the consumable component of the darting method (excluding labour and vehicle use) was approximately A\$30 per animal (Table 2). This cost is relatively low when compared with other darting regimens using more expensive drugs such as medetomidine and atipamezole (e.g. \$154 per animal for feral donkeys, *Equus asinus*).<sup>23</sup> Our aim in this study (fitting of telemetry collars) allowed us to release animals as quickly as possible, as per standard practice in wildlife capture.<sup>27</sup> However, our chemical immobilisation technique could be easily combined with physical restraint or transport of captured animals<sup>38</sup> to allow the trucking of escaped cattle or cattle that cannot be mustered. We recognise that the economic realities of pastoral production in Australia dictate that this method may not be practical for broadscale capture of free-ranging cattle, but may be useful for the capture of specific animals, such as cattle escaping from handling facilities.<sup>3</sup>

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### Conclusion

We describe a simple method of chemical immobilisation of free-ranging cattle that may be of use in many Australian contexts. Physical capture methods for cattle, which have been relied upon heavily in extensive agriculture settings, could be complemented by chemical immobilisation regimens for the capture of individual animals. In some circumstances, particularly for cattle escape scenarios in urban or peri-urban contexts, chemical immobilisation represents a safer and more reliable option than physical methods for capturing individual animals. We encourage application of the method described to capture Australian cattle when physical capture methods are not available or deemed unsafe or unreliable.

### Acknowledgments

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**Comment [KB7]:** Refs were not cited in strict numerical order in text so have been renumbered please check carefully. Original ref 38 not cited at all so has been deleted

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**Figure 1.** Process of darting and capturing free-ranging cattle in north-west Australia, April 2015, using a novel regimen of xylazine, ketamine and yohimbine. Using the wing mirror as a darting platform (A), hindlimb darting site and lateral recumbency physical restraint position (B), recovery sternal recumbency position (C) and complete anaesthetic recovery (D).

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**Figure 2.** Two types of needles used on charge-powered darts in a novel chemical immobilisation regimen for capturing free-ranging cattle (Left) Wire barbed needle and (Right) gel collared needle.

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**Table 1.** Veterinary chemicals suitable for darting free-ranging cattle and the legal status of their common preparations for use in food-producing animals in Australia

Chemical class	Drug	Preparation	Status for use in food-producing animals		
			Registered (meat WHP)	Label claim prohibiting use	No information
±-2 agonist	<b>Xylazine</b>	<b>Xylazil-100®</b>	✓(28 days)		
		Xylazil-20®	✓(28 days)		
	Medetomidine	Domitor®		✓	
		Compounded			✓
	Detomidine	Dormosedan®		✓	
Dissociative	<b>Ketamine</b>	Ketamil-100®	✓(28 days)		
		<b>Freeze-dried</b>			✓*
		Tiletamine-zolazepam	Zoletil®		✓
±-2 antagonist	<b>Yohimbine</b>	<b>Reverzine®</b>	✓(28 days)		
	Atipamezole	Antisedan®			✓

Tolazoline	Tolazine®	Horses only (28 days)	✓
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\*Unregistered preparation in Australia. Chemicals and preparations used in this study are shown in bold.  
WHP, withholding period.<sup>18</sup>

**Comment [KB10]:** asterisk not apparent in table.

**Table 2.** Mean costs (incl. GST) per animal of each consumable component of the novel darting protocol developed for capture of free-ranging cattle in north-west Australia

Component	Cost per unit (A\$)	Mean cost per animal (A\$)
6-mL charge-powered dart	\$9	\$9
Freeze-dried ketamine 2000 mg	\$15	\$10
Xylazine 50 mL (100 mg/mL)	\$38	\$2
Yohimbine 20 mL (100 mg/mL)	\$39	\$9
Total		\$30

GST, goods and services tax (10%).

**Table 3.** Darting and physiological parameters for 30 free-ranging cattle captured in north-west Australia, April 2015

Parameter	Range (min.–max)	Mean $\pm$ SE
Shooting distance (m)	14–31	21 $\pm$ 1
Animal weight (kg)	255–564	384 $\pm$ 13
Xylazine dosage (mg/kg)	0.34–0.86	0.59 $\pm$ 0.02
Ketamine dosage (mg/kg)	2.36–5.23	3.59 $\pm$ 0.1
Yohimbine dosage (mg/kg)	0.0–0.24	0.10 $\pm$ 0.01
Induction time (min)	3–18	8 $\pm$ 1
Induction distance (m)	36–794	260 $\pm$ 32
Recumbent time (min)	8–71	27 $\pm$ 3
Body temperature ( $^{\circ}$ C)	38.1–39.8	39.0 $\pm$ 0.07
Heart rate (beats/min)	28–60	44 $\pm$ 2
Respiratory rate (breaths/min)	15–36	23 $\pm$ 1
Oxygen saturation (%)	76–98	90 $\pm$ 1
Mortality rate at capture	0	–
Mortality rate 14 days post-capture	7%	–

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