Final Report

MACS Project, Work Package 1

The effect of capture, handling and husbandry procedures on the welfare of South American Camelids in Chile

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Summary

This report describes research carried out on vicuñas (*Vicugna vicugna*) and guanacos (*Lama guanicoe*) by researchers at Oxford University, in collaboration with researchers at the Catholic University (Santiago, Chile), over a 10-month period. This work aimed to investigate the effects of capture, shearing and release on the behaviour and physiology of camelids, and evaluate the stress response to these and other handling procedures; and also contribute information for use in the preparation of a welfare and behavioural audit. There are eight distinct components to this work, summarised briefly here.

Prior to conducting any research work in Chile, Ros Clubb was given full training in herd handling techniques for farmed guanacos by Cristian Bonacic and Jose Luis Riveros (the veterinarian responsible for day to day herd management). This covered: safe practice; routine management procedures; handling techniques; collection of samples from restrained animals; as well as highlighting key considerations for recording behaviour and designing experiments.

Progress was made on the use of Automatic Blood Sampling Equipment (ABSE) with farmed guanacos. A trip was made to the Macaulay Land Use Institute where Dr Goddard trained Ros Clubb in the use of the ABSE, who in turn trained Jessica Gimpel at the Catholic University so that work could continue. This allowed all four devices held at the Catholic University to be set-up and run successfully. Work was also conducted on the design of a harness specifically for guanacos, and a prototype constructed. This work is still ongoing and aims to successfully collect blood samples from a number of guanacos, and then possibly vicuñas, using the ABSE.

A study was conducted on the use of chemical capture to gather data on wild guanacos and vicuñas in their natural habitat. A new drug combination, medetomidine-ketamine (med-ket), delivered via a projectile dart and reversed by hand-injection with the drug atipamezole, was tested on both camelid species for the first time. Preliminary data on a range of parameters were collected, including dosage regime, time to induction, quality of immobilisation and time to recovery. Notably, vicuñas were found to require a far higher dose of med-ket than expected to achieve an adequate level of sedation, and so more work is required to determine the optimal dose. Chemical capture was found to have potential as a means of gathering data that are more representative of baseline values than data collected by capturing unsedated animals.

A pilot study was conducted on the response of captive guanacos to a routine husbandry procedure, namely vaccination, at two experimental farms near Santiago, Chile. This allowed a run through of the data collection protocol and provided insights

into the relationships between behavioural and physiological measures obtained during handling. Significant differences were found between animals held at the two farms, which differed considerably in the size and composition of enclosures. Although this could be due to slightly different handling practices, it would be interesting to investigate further. Few correlations were found between the behavioural and physiological measures taken.

Another study looked at the use of analgesics in the routine surgical castration of farmed guanacos, which a previous study suggests causes pain and activation of the stress response. A dose of the non-steroidal anti-inflammatory drug phenylbutazone was administered intravenously to one group of animals immediately prior to surgery, while a control group received an injection of saline solution. Results suggest analgesia to have beneficial effects: treated animals showed marginally lower behavioural reactivity during handling; spent less time protectively covering the wound with their tails, and most strikingly, showed no significant elevation in plasma cortisol levels following surgery, in marked contrast to those treated with saline solution, in which levels doubled.

A transportation study was planned, specifically comparing farmed guanacos transported while tied-up with those left unrestrained. A pilot study of two restrained guanacos was conducted, but due to logistical problems, completion of the main study was not possible. However, a detailed protocol was prepared, allowing the work to be completed at some point in the future.

Throughout this work, digital footage of captive and wild guanacos and vicuñas was collated and categorised according to behaviour of the animals. The planned end product is a comprehensive ethogram that will be placed on a website accessible to all people wishing to study the behaviour of these species. This would not only act as an effective learning tool, but also allow some standardisation of methodology.

Finally, a range of possible future research projects on captive and wild guanacos and vicuñas is suggested, that would contribute to our understanding of how handling practices affect these animals and hence contribute more information to a welfare and behavioural audit.

Contents

1. Background

The work presented in this report forms part of Work Package 1 of the MACS project. This work package aimed to:

- investigate the effects of capture, shearing and release on the behaviour and physiology of camelids, and evaluate the stress response to these and other handling procedures;
- investigate the effects of capture and shearing on reproduction, comparing different management systems on pregnancy and abortion rates, birth rates and mortality;
- prepare a welfare and behavioural audit, including guidelines on the best management practice, handling and shearing techniques.

The work presented here involves aims one and three of WP1, above. This report describes the work done with vicuñas (*Vicugna vicugna*) and guanacos (*Lama guanicoe*) over a 10-month period, five of which were spent in Chile working with Cristian Bonacic and Jessica Gimpel at the Catholic University, Santiago. This report is broken into several components:

1. Training in the herd handling techniques for farmed guanacos;
2. Training in the use of the Automatic Blood Sampling Equipment (ABSE);
3. A study of the chemical capture of wild guanacos and vicuñas in the altiplano;
4. A pilot study on the response of farmed guanacos to a routine husbandry procedure: vaccination;
5. A study on the effect of analgesia on the behaviour and physiology of farmed guanacos following surgical castration;
6. Transportation: effects on behaviour, physiology and stress hormone output of farmed guanacos;
7. Digital catalogue of guanaco behaviour;
8. Suggested future research projects.

2. Training in the herd handling techniques for farmed guanacos

Prior to conducting any research work, Ros Clubb was given full training in herd handling techniques for farmed guanacos by Cristian Bonacic and Jose Luis Riveros (the veterinarian responsible for day to day herd management). This covered the following topics:

- Safety measures employed while working in areas with guanacos, including behaviour around the animals to minimise disturbance; the use of a herding stick; maintaining a position around the edge of the enclosure at all times, etc.
- Movement of animals from the home pen to the holding pens, and between areas of the management area.
- Use of the restraint device, and the position of personnel during its use.
- Handling techniques when the animal was restrained in the chute.
- Collection of samples from restrained animals.
- Collection of physiological measures from restrained animals.
- General information about husbandry and veterinary procedures.
- Observation of the entire handling process and routine husbandry procedures, including shearing and vaccinations.
- Observation of the behaviour of guanacos during herding and handling, highlighting the practical difficulties that had to be overcome during experimental design.

3. Automatic Blood Sampling Equipment (ABSE)

The handling and restraint of animals, which are generally required to collect blood samples, can invoke a significant stress response, particularly in non-domestic species. This obviously makes it extremely difficult to collect good baseline data for various haematological, clinical biochemistry and hormone concentration measures from the blood and so interferes with the evaluation of how particular practices affect these measures. The Automatic Blood Sampling Equipment (ABSE) is a device that can overcome these problems (Goddard *et al.* 1998), and has already been used successfully in red deer (Diverio *et al.* 1996), sheep (Ferre *et al.* 1998), cattle and reindeer (Peter Goddard, pers. comm.).

The ABSE consists of a small unit controlled by a microprocessor mounted on the back of the animal with a harness (Goddard *et al.* 1998). A line runs from the unit to a catheter in the animal's neck, from which blood is drawn by a simple pump. Sampling can be programmed to occur at set time intervals, collecting up to 14 samples. Collection of the first sample can be delayed for a period of up to 48 hours to allow the animal to recover from the initial fitting of the harness and ABSE (Goddard *et al.* 1998).

The ABSE would be an extremely useful tool for studying the effects of various treatments in guanacos and vicuñas, as well as being invaluable for the collection of good baseline data, perhaps even from free-ranging animals in the wild. The Catholic University in Santiago, Chile, has four such devices to try on the farmed guanacos held at their research farm. Attempts have already been made to fit the device to two guanacos, using a modified harness, under the supervision of Dr Peter Goddard, who has extensive experience in its use on a number of species. The result was extreme inactivity in one animal, probably as a result of the harness with which it had no prior experience, and damage to the device on the other animal. Further progress on this project has stalled due to a lack of personnel, as well as a lack of knowledge concerning the setting-up and operating of this rather complicated piece of equipment. With this in mind, I made a visit to Dr Peter Goddard at the Macauley Land Use Institute to gain training in the use of the ABSE. During this visit, Dr Goddard and a colleague familiarised RC with all the equipment; demonstrated how to set-up the device and run various programmes with different sampling schedules. On returning to Chile, this training enabled the equipment to be successfully set-up and run, after some complications with the software had been resolved. RC then demonstrated all the necessary procedures to Jessica Gimpel so that the group in the Catholic University could carry on the work.

Dr Goddard believed that the key to successfully using the ABSE on a guanaco lay in the harness design, which would have to be completely secure and afford a good level of protection to the device. On returning to Chile, RC worked with Jessica Gimpel to try and modify the typical ABSE harness (designed for red deer) to provide a better fit for a guanaco. The harness was designed using measurements from a restrained adult female guanaco of average size. This design was then taken to a rucksack manufacturer with the aim of having a robust harness constructed out of durable material.

This topic is currently being pursued further by the group at the Catholic University. Future work will include: a) refinement of the harness design to ensure that no slippage occurs, and that the device remains secure during all forms of behaviour; b) desensitisation of the animals to wearing the harness, by leaving them on animals for varying periods of time and observing their behaviour; c) fitting a suitably weighting 'dummy' device to the harness before trying the ABSE itself, to minimise the potential for damage; d) practice in fitting catheters to the neck of the guanacos; e) practice running the device prior to fitting it to the animal to ensure it is working as programmed; f) finally, fitting the device itself to a catheterised animal and collecting samples.

4. Chemical capture of wild guanacos and vicuñas in the altiplano

Introduction

Data on physiological and haematological measures previously collected from wild guanacos and vicuñas have involved chase, capture and restraint of animals. This process is know to cause significant activation of the stress response (e.g. Bonacic & Macdonald 2003) and hence does not provide true baseline values for some of the blood measurements taken (e.g. glucocorticoid concentration). An alternative means of capturing wild animals is by chemical sedation administered via a projectile dart, this negating the need for chase, and to some extent, restraint. This has not yet been attempted in South American Camelids (SACs). In this study, vicuñas and guanacos living in their natural habitat (the Chilean altiplano) were captured using darts loaded with a combination of medetomidine and ketamine. This mixture has been used successfully to induce reliable anaesthesia in a range of hoofstock species (e.g. sika deer, *Cervus Nippon*: Tsurga *et al.* 1999; giraffes, *Giraffa camelopardalis*: Bush *et al.* 2001; Thomson's gazelles, *Gazella thomsoni*: Chittick *et al.* 2001; reindeer, *Rangifer tarandus tarandus*: Ryeng *et al.* 2002) and is effectively reversed with atipamezole (Jalanka & Roeken 1990).

The aim of the work presented here was twofold: to record the effectiveness of medetomidine-ketamine in sedating these animals (including the quality of immobilisation and time to induction), as well as the effect of the reversal drug atipamezole (time to recovery); and to obtain physiological data from sedated animals to compare with values obtained by chase and capture methods. The data presented here were collected during one field trip. Future expeditions will allow additional data to collected and thus the data presented here represent preliminary findings.

Methods

Study site

The study was carried out in the areas surrounding the town of Putre in the Chilean pre-altiplano (capture of guanacos) and in the altiplano in and around Lauca National Park (capture of vicuñas). For more details of the study site see Bonacic & Macdonald (2003).

Anaesthesia protocol

The anaesthesia used was a combination of medetomidine and ketamine (med-ket) administered intramuscularly via a projectile dart fired from a Dan-Inject rifle. This was an experimental method at the time and was chosen because med-ket is a recently developed drug combination that replaces older chemicals with longer induction periods. Dosages were prepared prior to sightings based on average body weights of 120 kg for a fully-grown guanaco and 50 kg for a vicuña. Dosages were then adjusted on site, if necessary, according to the size of the animal. Anaesthesia was reversed by means of an intra-muscular injection of atipamezole.

Darting protocol

The animal was approached as closely as possible before a darting attempt was made.

Darting of wild populations took place over a period of four days between September 24th and 28th, 2002. Searches took place from a four-wheel drive vehicle along minor roads that ran through suitable habitats. When an animal was spotted, a range finder was used to accurately estimate the distance of the target animal. A GPS reading of the location was recorded, including the elevation, and notes taken on the weather, wind speed and habitat type. Before a shot was attempted, the pressure of the rifle was calibrated using data provided by the rifle manufacturer, according to the distance of the animal and the wind speed. The marksman approached the animal as close as possible and recalibrated the rifle, if necessary, before making an attempt to dart the animal. If the dart missed, another attempt was made if the animal was still within range, or else another target was sought. If a dart bounced off the body, the animal was observed for at least 20 minutes to determine whether it had received a sufficient dose of the drug to induce sedation. If no visible signs of sedation were detected, another target was sought. If darting was successful, animals were observed until they lay down with their head on the ground (taken as the induction time). They were then approached as rapidly as possible, and the handling and sampling procedure started.

Handling and sampling procedure

All animals were already in sternal recumbency when they were approached and do did not need to be moved. The head of the animal was immediately raised to an upright position and a blind-fold placed around the eyes. If necessary, an additional intra-muscular dose of anaesthesia was administered to complete sedation (see results section for details). The site where the dart had entered was inspected and an antiseptic applied to prevent infection. Blood samples were collected by jugular venipuncture into 3ml Vacutainers© (Becton, Dickinson and Company) and later used to determine levels of corticosteroids (analysed at the Endocrinology Laboratory at the Pontificia Universidad Católica de Chile – see Bonacic et al. 2003 for details). The following vital signs were monitored opportunistically throughout the handling process: cardiac rate, respiratory rate, rectal temperature and relative arterial oxygen saturation (using a blood oxymeter). Body condition was estimated and standard body measurements (mm) taken: total length; tail length; hind-foot length; ear length; head length (nose to condials); head circumference (around and behind the eyes) and girth. Age was estimated from tooth wear by experienced personnel. A faecal sample was taken from each individual by manually extracting faecal pellets from the anus. If any parasites were seen during the examination, a sample was collected and stored. A fleece sample was also collected from the flank of each animal. Finally, a length of plastic tape with the animal's identification code was loosely attached to the neck of the animal to aid future identification.

While sedated, physiological measures were recorded and samples taken.

Behaviour observations

When a potential target was spotted, group size and composition were estimated, including sex and age category (adult or cria) of all identifiable group members, if possible. Instantaneous focal sampling, in the case of solitary animals, or instantaneous scan sampling, in the case of a group of animals, was used to record behaviour every minute prior to darting (Martin & Bateson 1993) recording both the posture and activity of the animal. Vocalisations were recorded continuously as these

were considered events rather than states. The same person made all behaviour observations from a vantage point within 100m of the target. See Table 1 for a list of behaviours recorded prior to darting. Behaviour observations continued after the dart had been fired, using the same methods, although instantaneous scan sampling was used if the identity of the darted animal was not known immediately (see Table 2).

During handling of the sedated animal, changes in behaviour were noted on an *ad libitum* basis (Martin & Bateson 1993). These included head movements, ear twitches, vocalisation, palpatory reflexes and trembling (see Table 3). After the reversal drug had been administered, instantaneous focal sampling, with a sample interval of one minute, continued for at least 30 minutes or until it was clear that the animal could stand and walk with little trouble (see Table 2).

Table 1: Ethogram showing the categories of behaviour recorded prior to darting

Posture	Description
Stand	No change in location
Walk	Slow locomotion
Run	Fast locomotion, usually in response to approach by another animal or person
Lateral lie	Lying on the ground, with the side of the body in contact with the ground
Sternal recumbency	Lying on the ground, belly in contact with the ground, legs folded under the body
Roll	Rolling on the ground
Out of sight	Whole animal not visible

Activity	Description
Head on ground	Head in contact with the ground
Alert	Looking at an object or area with the head raised, ears up
Looking	All looking other than alert
Eating	Grazing, including chewing between bouts
Ruminate	Chewing movement with no sign of having eaten
Groom	Scratching with fore- and hind-feet and biting and chewing the fur
Vocalisation	The number of times the animal vocalised (event)
Out of sight	Animal visible but unable to accurately determine activity

Table 2: Ethogram showing additional behaviour categories recorded after darting, and after release, relating to sedation

Posture	Description
Stand unstable	Standing stationary, body swaying from side to side
Stand give way	Standing, hind-legs buckling
Walk unstable	Stumbling or swaying from side to side during slow locomotion
Run unstable	Stumbling during fast locomotion
Fall	Loss of balance, partially or completely

Activity	Description
Head low	Head far lower than normal when lying down or when standing
Head bob	Repetitive movement of the head up and down
Head sway	Repetitive movement of the head from side to side
Head supported	Head held in upright position due to animal's inability to support itself
Head movement	Side to side movement of the head, or vigorous shaking of the head

Table 3: Ethogram showing behaviour recorded during manipulation while sedated

Behaviour	Description
Struggle	Kicking, jumping, and/or attempting to stand
Head movement	Movement or shaking of head from side to side
Blink	Opening and shutting of the eyes
Mouth movement	Small movement of the mouth
Ear movement	Movement of ears back and forth
Tail movement	Movement of tail back and forth
Body movement	Shifting of body while in sternal recumbency
Grunt	Single, grunting vocalisation, often when a needle was inserted
Burp	Noisy expulsion of air

Results

Social grouping prior to darting

During five days of searching, a total of 13 guanacos and 13 vicuñas that were potential targets were spotted from the vehicle, not including other members of the group. Of these 13 guanacos, five were observed in a family group (mean group size: 7, range: 4 – 9), three were members of a bachelor herd (mean: 5, range 3 – 7) and five were solitary. Of the vicuñas spotted, three belonged to a family group (mean: 5,

range 3 – 6), five belonged to a bachelor herd (mean: 21, range 8 – 32) and seven were part of an unidentified group type (mean: 5, range 2 – 6).

Behaviour prior to darting

Few quantitative data were gathered prior to darting, primarily because darting occurred as soon as possible after a possible target was spotted. Due to the terrain, it was not possible to approach the herd undetected, and therefore most of the behaviour recorded before darting consisted of 'alert' behaviour. Guanacos and vicuñas were generally not fearful of humans, allowing the marksman to slowly approach within shooting distance. While this happened, most members of the herd displayed the 'alert' behaviour, following the course of the approaching marksman. Occasionally, animals walked, or sometimes, ran off when they were approached, particularly when they were alone (only guanacos were observed alone). All animals that were successfully darted were part of a group containing three or more animals, and on the whole, groups were easier to approach.

Darting success

Darting was not attempted for five guanacos and one vicuña that were spotted. This was because the wind speed was judged to be too high (one guanaco); the animal was, or moved out of, the required range for the rifle (three guanacos, one vicuña); or the animal was solitary (two guanacos). Animals in the last category were judged, with experience, to be too flighty and likely to run some distance after darting, making them difficult to find, as well as increasing the possibility of injury on the steep rocky habitat.

Darting attempts were made for seven guanacos (74% of potential targets) and 12 vicuñas (92% of potential targets). Animals that were hit successfully, and appeared to have been administered the drug, included three guanacos (43% success rate) and five vicuñas (42% success rate). Failure was mainly due to missed shots and darts bouncing off the body of the animal, probably due to the dart hitting a bone. Four shots were missed because the dart fell short of, or flew over, the animal, one because the aim was off and another due to an unknown cause. On average, animals were shot from 30m away (range 20-52m). Range was not found to significantly influence success (Kruskal-Wallis, $H = 2.01$, $df = 1$, $p = 0.157$).

Sedation and recovery

Guanacos

All three guanacos that were successfully darted were males in good condition, estimated to be between two and over four years of age (see Table 4). Drugs administered via the dart were sufficient to complete sedation in two animals (G1 and G2, see Table 4), but a further dose of ketamine was required after the capture of one male (G3) after a short struggle and physical restraint. In the two former animals, the first signs of sedation were observed four to five mins earlier than in the animal that required a further dose. However, induction time (sternal recumbency with the head lying on the ground) was observed at around the same time in all three animals, between 9 and 13 mins after darting (see Table 4). Sedation remained at a sufficient level throughout the procedure in G1 (up to 34 mins after darting), but G2 showed head movements and recovered the palpatory reflex after 1hr 5 mins, while G3 tried to stand after 54 mins (see Table 4). Animals could stand unaided within three minutes after injection of the reversal, albeit rather unsteadily, although G3 was standing within 1 min. Walking was observed at the same time, with the exception of G3, which was first observed to walk 7 mins after reversal. The first 20 mins after the reversal was injected were spent mainly walking unsteadily or standing, either looking around or in an alert posture (see Table 5). Sternal recumbency was also seen in two animals in the recovery period, but this was in the minutes immediately following injection (see Table 4 for details of recovery times).

Most animals were unsteady on their feet during the recovery period.

Vicuñas

Five vicuñas were successfully darted, but only two were sufficiently sedated to allow capture, both of which were males of two to three years of age (see Table 4). The other three were successfully darted, but sedation was only partial, with the first signs being observed between 5 and 17 mins after darting. Despite these signs of sedation, they were quick to stand up and run off when approached, albeit somewhat unsteadily. Subsequently, the dose was increased and complete sedation was later achieved in one animal (V1). This animal did need an additional dose of ketamine due to

incomplete sedation, but this induced a heavy level of sedation (discussed later in this section). An additional vicuña was darted (V2), but the dart punctured the rumen. The animal was captured after an extensive chase in order to administer treatment to the wound. Unfortunately, despite treatment and administration of a reversal, the animal never recovered and was found in a search for the carcass dead the next day. A necropsy revealed signs of capture myopathy, including internal bleeding, wasted muscles and dehydration. This animal will therefore not considered in the remainder of the results sections given the problems that were encountered.

The animal that was captured showed a deep level of sedation after the additional dose of ketamine, which lasted throughout the procedure. The animal stopped breathing at the same time the reversal was injected, 52 mins after darting (42 mins after the second dose of ketamine). Breathing resumed immediately after the nose was pricked with a needle. Oxygen was then administered twice. Subsequently, this animal took a long time to recover, supporting its own head and standing for the first time (very unsteadily) 35 mins after the reversal injection (see Table 4 and Table 5).

Sternal recumbency was the first sign of sedation in many animals

Animals were approached when in sternal recumbency with the head on the ground (taken as the induction time)

Behaviour during handling while sedated

Guanacos

In sedated animals, few changes in behaviour were observed during handling. Vocalisations were recorded in all three animals, which usually consisted of grunting when injected or a blood sample was taken, suggesting they could still detect painful stimuli. In one animal (G2), which had been sedated for 65 minutes, head movements were observed just prior to the palpebral reflex was recovered, closely followed by struggling, indicating recovery from sedation prior to reversal.

Table 4: Details of the darted animals, dosages given and behaviours observed

	Guanacos			Vicuñas				
	G1	**G2**	**G3**	**Va***	**Vb***	**Vc***	**V1**	**V2†**
Sex	Male	Male	Male	-	-	-	Male	Male
Estimated age (years)	2	>4	>4	-	-	-	3	2-3
Group size prior to darting	7	3	9	12	12	6	32	5
Group type prior to darting	Unknown	Bachelor	Family Gp	Unknown	Unknown	Unknown	Bachelor	Family Gp
Doses for sedation (mcg/kg): medetomidine	0.1	0.1	0.06	0.1	0.1	0.2	0.2	0.2
ketamine	2.0	2.0	1.9 + 0.8	2	2	1.5	2	2 + 1
Doses for reversal (mcg/kg): antipamezole	0.1	0.3	0.2	n/a	n/a	n/a	0.01	0.01
First signs of sedation observed (mins after darting)	5	4	9	11	5	17	4	3
Details of first signs of sedation	Sternal recumbency	Swaying	Sternal recumbency	Sternal recumbency	Back legs giving way	Sternal recumbency	Stumbling walk	Lying on side
Time to induction (mins after darting)	13	9	12	24	not seen	not seen	6	n/a
Captured and procedure started (mins after darting)	14	14	23	n/a	n/a	n/a	7	49
Reversal administered (mins after darting)	54	67	56	n/a	n/a	n/a	52	85
First signs of recovery observed (mins after reversal)	3	0	3	n/a	n/a	n/a	35	n/a
Details of first signs of recovery	Walking, unstable	Struggling prior to reversal	Walking unstable, head low	n/a	n/a	n/a	Stand unstable	Never recovered

*Sedation insufficient for capture.
†Dart punctured the rumen and animal later died from capture myopathy (see section 'Sedation and recovery: vicuñas').
‡Figure after a '+' sign indicate give additional doses that were administered after capture.

Table 5: Details of behaviour during the 20 minutes following injection of the reversal agent Atipamezole

The data shown below refer to the behaviour of animals during the period immediately following injection of the reversal drug. All figures represent the proportion of scans, which occurred every minute, during which the behaviour was observed. The exception is vocalisations as these events were recorded continuously. Details of the behaviour of V2 are not given as this animal suffered from complications (see the following section).

	Guanacos			Vicuña
Posture	**G1**	**G2**	**G2**	**V1***
Stand	31.6	38.1	9.5	
Stand unstable		9.5	19.0	
Walk		14.3	4.8	
Walk unstable	63.2	28.6	52.4	
Sternal recumbency	5.3		14.3	100.0
Fall		9.5		
Activity				
Alert	57.9	19.0	23.8	
Look	36.8	33.3	38.1	9.5
Head on the ground	5.3		9.5	
Head low		9.5	23.8	
Head bob		9.5		
Head supported				90.5
Head movement			4.8	
Ruminate		14.3		
Vocalise (number of times)		1		3

*This animal appeared to have received too high a dose of sedative, following administration of an additional dose of ketamine after capture, and so took a longer time to recover than the others (see Table 4 for recovery times).

Vicuña

Again, very little changes in behaviour were noted in the one animal handled, with the exception of vocalisation during the initial struggle, prior to the administration of an additional dose of ketamine. During full sedation, burping was noted as a consequence of minor bloating.

Physiological measurements taken while sedated

Body functioning was monitored throughout the handling procedure while the animal was under sedation. Data on heart rate, respiratory rate, rectal temperature and blood oxygen content are given in Table 6. Heart rates remained relatively stable throughout the procedure, but showed some degree of slowing in one animal (G2). Similarly,

respiratory rates showed little change, except in one animal (G1) that showed an increase over the three measurements from 20.7 to 44 breaths per minute. There did not appear to be any associated change in behaviour at this time. A marked decrease in rectal temperature over the handling period was evident in all three animals from which data were collected (see Figure 1).

Table 6: Physiological measures during sedation

The data shown below represent the average figures for the four physiological measures taken while animals were sedated. Standard errors of the mean are given below each value, and ranges below that. Boxes marked with 'n/a' indicate that it was not possible to get a reading, or not enough readings were taken to provide a range. When there were insufficient data to give a range, the values themselves have been given.

	Guanacos			Vicuña
	G1	**G2**	**G3**	**V1**
Cardiac rate (beats per min)	34.2	25.0	53.0	39.7
	+/- 1.79	+/- 3.00	n/a	+/- 6.95
	32-36	22-28	46 & 60	27-44
Respiratory rate (breaths per	33.6	34.0	34.7	28.2
	+/- 11.84	+/- 2.83	n/a	+/- 4.75
	20.7-44		33 & 36	21-32
Rectal temperature (^{0}C)	37.7	n/a	37.5	37.7
	+/- 0.77		+/- 0.21	+/- 0.52
	36.7-38.3		37.3-37.6	36.9-38.4
Arterial oxygen saturation	81.2	n/a	72.7	73.0
	n/a		n/a	n/a
	n/a		n/a	n/a
Pulse (bpm)	35.8	n/a	60.2	40.6
	n/a		n/a	n/a
	n/a		n/a	n/a

Plasma cortisol

Results from plasma corticosteroid analyses revealed that all guanacos had similar concentrations of cortisol: 41.8 nmol/l (G1); 41.9 nmol/l (G2); 40.7 nmol/l (G3). The vicuña had a far higher concentration of 82.9 nmol/l. The results from the problem vicuña, which later died of capture myopathy, were also analysed and found to be extremely high at 116.4 nmol/l.

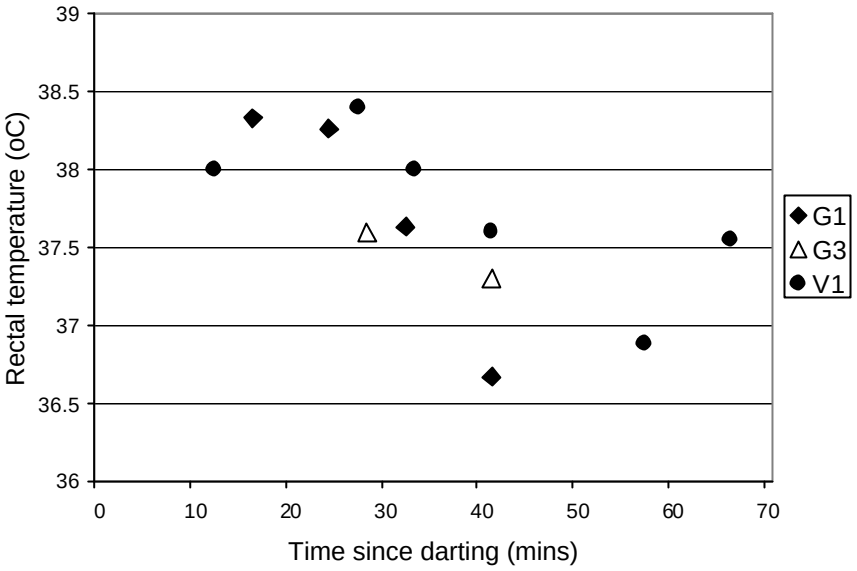


Figure 1 Rectal temperature of sedated animals

Body temperature decreased during sedation with medetomidine-ketamine in all three animals monitored: two guanacos (G1 and G2) and one vicuña (V1).

Body measurements

Body measurements were obtained from two guanacos and one vicuña. Data are summarised in Table 7.

Table 7: Physical measurements from sedated animals

Body measurement	G1	G2	V1
Body condition	Lower end of normal	Normal	Good
Total length (mm)	1890	2070	1650
Tail length (mm)	240	320	230
Hind-foot length (mm)	500	520	530
Ear length (mm)	130	140	110
Head length - nose to condials (mm)	390	380	280
Head circumference behind eyes (mm)	460	510	400
Head circumference around eyes (mm)	475	510	415
Girth (mm)	1090	1150	750

Discussion

This preliminary study shows that the drug combination medetomidine-ketamine, delivered via a projectile dart, can be an effective means of anaesthetising wild guanacos and vicuña. The first signs of sedation were rapid, occurring within three to

nine minutes in captured animals, and induction times ranged from 2 to 3 minutes. Sedation was complete for at least 30 minutes, and seemed to wear off in guanaco after about 50 minutes. Guanacos generally responded as predicted to the sedative, but due to the small sample size further work is required to calculate the optimal dosage. It was not possible to determine the optimal dosage for wild vicuña from this study, as a far higher dose than expected was required to ensure a sufficient level of sedation for capture. This may relate to the physiological adaptations of these animals to living at high altitudes.

Although data were limited, there was no obvious decrease in cardiac or respiratory rates during sedation, in contrast to studies of other species sedated with medetomidine-ketamine (e.g. Thurmon *et al.* 1994; Lock *et al.* 1998; Caulkett *et al.* 1999; Chittick *et al.* 2001; Rauser *et al.* 2002), elevated respiratory rates have also been reported in giraffes, but again this was not observed in this study (e.g. Bush *et al.* 2001). There was, however, a steady drop in rectal temperature in the three animals monitored here, which was also noted by Langan *et al.* (2000) in ostriches.

Serum cortisol levels were consistent across the three guanacos sampled, ranging between 40 to 42nmol/l. This is lower than the 55.18nmol/l baseline value reported in hand-captured young wild guanacos (Gustafson *et al.* (1998), but far higher than baseline values reported for captive guanacos (e.g. 16nmol/l: LeRoy 1999; 24.4nmol/l pre-shearing: Zapata *et al.* in prep.; 21.7nmol/l pre-transport: Zapata *et al.* submitted). Thus, chemical sedation by darting may yield more representative values for wild guanacos than capture by chasing, but these would appear to be far from baseline if we assume that captive animals are not likely to have dramatically lower baseline values than free-living animals. Only one data point was collected from vicuñas due to the difficulty in attaining a sufficient level of sedation. This revealed a cortisol level of 82.8nmol/l, which is far higher than baseline values from captive animals (35.5nmol/l), but far lower than peak values obtained during an ACTH challenge (116.4nmol/l) (Bonacic *et al.* 2003). Further work is obviously needed to increase the sample size before any conclusions can be drawn.

Several improvements were made to the darting protocol during fieldwork. First, it was noted that solitary animals (only solitary guanacos were observed) appeared far more flighty and nervous compared to those in a social group, and so these were avoided as targets. Second, no darting was attempted when animals were more than 40m away, as accuracy could not be guaranteed. Third, it was judged that the administration of additional drugs after capture to complete sedation should be avoided until at least 15 minutes after the initial darting. Otherwise, there is the risk of heavy sedation, as encountered in one of the study animals here. Fourth, carrying out more

than three chasing events during attempts to capture incompletely sedated animals should be avoided due to the significant risk of capture myopathy. Behaviour recordings proved to be useful for quantifying the effects of the drug, for instance providing data on the time to induction. Longer-term observations would have been particularly useful in determining the effects of sedation, capture and handling on the general behaviour of the animal, for instance in terms of social behaviour, feeding, spatial use, etc., as well to determine when sedation had worn off completely.

The data presented here is obviously limited by a small sample size of animals, and so more fieldwork is needed to gather more data. Although some insight was gained during this research, more work is required to determine the correct dosage required to achieve an optimal level of sedation in vicuñas. This would ideally be done in captive animals, using a similar darting procedure, before doing more work in the altiplano, although there would remain the possibility that required dosages may be affected by altitude. Addition work on captive vicuñas and guanacos would also allow the detailed clinical monitoring of a large group of animals, which could include measures of reflex activity, pain sensitivity and muscle relaxation, as well as accurate determination of actual doses administered, as it was not feasible to weigh guanacos in the field. A comparison could also be made between the sedative administered by darts, as in this study, and by hand injection. Ryeng *et al.* (2002) looked at this recently in reindeer using the same drug combination. They found a longer time to induction and mean induction time in darted animals, and although no difference was noted in most physiological measures, darted animals had significantly lower heart rates compared to hand-injected animals. A similar study of SACs would reveal how these different methods of administration affect sedation.

As a means of obtaining good baseline data from wild animals, this method of capture would appear to have some potential, given the values obtained for plasma cortisol concentrations. Further improvement of the dosage protocol, as outlined above, could improve this further by reducing time to induction and ensuring full sedation was achieved prior to animals being approached. More research in this area could also expand the range of blood parameters measured, for example including white blood cell counts, blood glucose concentration and levels of creatine kinase – all measures regularly used in studies looking at stress responses – as well as determining how the med-ket drug combination affects these parameters directly.

5. A pilot study on the response of farmed guanacos to a routine husbandry procedure: vaccination

Introduction

Farmed guanacos are subject to a range of husbandry procedures, some on a regular basis, such as body condition inspections, shearing, vaccinations and transportation (e.g. Bas & Gonzalez in press). These frequently involve handling of some sort, and the process as a whole consists of a variety of potential stressors, on top of the actual procedure being performed, including removal from the home enclosure, separation from the group, restraint and direct handling. Studies on a range of farmed species have shown handling to cause changes indicative of activation of the stress response (e.g. Carragher *et al.* 1997; Andrade *et al.* 2001), and similar results have been found in recent studies of farmed guanacos handled for the purposes of shearing and transportation (e.g. Zapata *et al.* in prep.; Zapata *et al.* submitted).

The aim of this study was to collect data from guanacos subject to a routine management procedure, namely vaccination. This was primarily to gain experience in running experiments with farmed guanacos, and to provide a run through of the data collection procedure. The secondary aim was to determine whether any correlations existed between the behavioural, clinical and physiological measures taken during handling. The measures collected during this study were based on previous studies of camelids (e.g. Bonacic *et al.* 2003; Zapata *et al.* in prep.; Zapata *et al.* submitted), as well as similar studies on other farmed species (Grandin 1997).

Methods

Study animals and study site

A total of 46 (19 males, 27 females) guanacos, housed in two farms on the outskirts of Santiago, Chile, were used in this study. Animals ranged from 5 months to 5 years of age (see Table 8 for body weights). Young animals had been born on the farms, while the oldest individuals had been captured from the wild. The first farm, Pirque (PI), which is an experimental farm managed by the Catholic University, held 27 of the study animals: 8 males (seven castrates around 18 months old and one 5-year old breeding male) and 19 females (two around 18 months old and 17 5 to 6-year olds). Enclosures consisted of flat, short grassland areas with some tree cover, bound by open-wire fences. Animals were fed a mixture of hay and pellet food throughout the year, and had *ad libitum* access to water in one area of the enclosure. The second farm, El Tahluen (TA), which although privately owned has the herd managed by the Catholic University,

held 19 of the study animals: 10 males (9 were 5 to 6 months old, and one breeding male was 6 years old) and 9 females (5 to 6 years old). Enclosures in this farm were much larger and naturalistic. Extensive vegetation provided cover, as well as browse and grazing material, which was supplemented with a small amount of alfalfa hay. Water was available *ad libitum* in one area of the enclosure, as in the other farm.

Animals held at El Tahluen (TA) had large enclosures containing extensive natural vegetation.

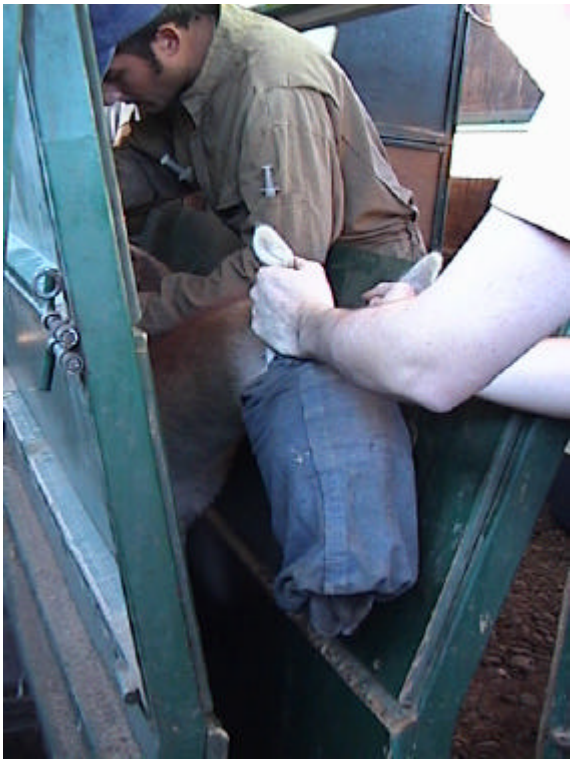
Table 8 Body weights of guanacos

Average body weights are given for each age class at each farm. Standard errors of the mean are given below averages. Where there were insufficient data to calculate standard errors, n/a is noted, or the actual figures given. Dashes denote age classes that were not present on the farm.

Sex and age class		Body weight (kg)	
		Pirque (PI)	El Tahluen (TA)
Males:	5-6 months old	-	98.8 ±1.6
	18 months	66.4 ±2.4	-
	5 years	108 n/a	-
	3 years	-	97 n/a
Females:	18 months	81.5 (80 & 83)	-
	5-6 years	96.3 ±4.9	93 ±2.3

Handling

At both farms, the animals are subject to routine husbandry practices, including vaccinations, on a yearly basis. Both sites had specially designed handling areas consisting of a central enclosure containing a weighing platform leading into a restraint chute consisting of a drop-floor crush. Two to three small pens were connected to this area which were used to hold animals prior to handling. For the handling procedure, all guanacos were herded from their home pen into a holding pen. Individual animals were then separated from the group in the holding pen, and herded through the corridors into a chute containing the electronic weighing platform. Once an accurate measurement had been obtained, a sliding door was opened to allow the animal to enter the restraint chute, and the floor dropped to restrain the animal. If the animal refused to enter they were manually pushed from behind. A blindfold was immediately placed over the head of the animal and the head held in an upright position by the ears throughout the procedure. Once the procedure was finished, the sides of the restraint chute were released and the animal moved into a second holding pen. When all animals in the group had passed through the system, the whole herd was released back into their home pen. This basic procedure process occurred during all handling procedures, such as clinical examination, vaccination and shearing, and so all animals had some experience of this procedure prior to the experiment.



A drop-floor restraint chute was used to contain animals for handling. A blindfold was applied to reduce stress.

Pilot observations

Prior to this study, observations were made at TA during routine shearing. This allowed familiarisation with the handling procedures typically used on these farms (see section 2), as well as allowing dummy behaviour observations to be conducted.

Vaccinations

All mature animals were vaccinated twice a year (March and September) with Clostridium A, B, C, D (2 ml), an enterotoxemy vaccine (2 ml) and ivermectine (1 ml per 50 kg. Calves were vaccinated immediately after birth again two months later.

Physiological measurements

Heart and respiratory rates were measured twice during the handling procedure in each animal: just after entering the restraint chute and just before release. These were averaged for analyses. Heart rates were measured with the use of a stethoscope, and respiratory rates taken by observing physical signs of breathing. The total number of beats or breaths were counted over the same 15 second period and multiplied by four to give rates per minute. Rectal temperatures were then measured immediately afterwards using a digital thermometer.

Vaccinations were administered to each animal whilst restrained in the chute

Samples

Blood samples were obtained by jugular venipuncture into 3ml Vacutainers© (Becton, Dickson and Company) containing no additives, after vaccinations had been given. Samples were allowed to cool to room temperature before being placed into a cool box where they were then stored until they could be centrifuged. Within four hours of collection, all blood samples were centrifuged in a Gemmy Industrial Corporation centrifuge at speed 4 for 10 minutes. Serum was then pipetted into labelled eppendorfs and frozen at –20^{o}C. All samples were then sent for analysis to the Endocrinology Laboratory at the Pontificia Universidad Católica de Chile.

In addition to blood samples, saliva and urine was collected from animals on an opportunistic basis during the sampling procedure. The purpose of collecting these samples was primarily to facilitate the later validation of corticosteroid analyses from these mediums (in a separate study by Jessica Gimpel), rather than to provide

accurate data for this study. Saliva samples were collected from all animals that produced a sufficient volume by running the top of a glass test tube around the mouth. Samples were taken prior to vaccination and blood samples, with the collection time limited to a period of three minutes or less. Whenever possible, urine samples were also collected in 5ml glass test tubes. All samples were stored in a cool box and then frozen at –20^{o}C for later use.

Behaviour observations

The behaviour of guanacos was scored while entering the restraint chute and during restraint. A total of eight behaviours were recorded (see Table 9), based on previous studies and observations of the same animals during handling.

Table 9: Ethogram showing the behaviours scored in guanacos immediately before and during handling in the restraint chute.

Behaviour	Description
Reluctance to enter the restraint chute (1-2)	The willingness of the animal to move from the weighing platform through to the restraint chute was classified into two categories: 1 = little or no coercion required; 2 = a moderate to a significant amount of pushing required.
Temperament (1-5)	Temperament was scored according to a modified version of the scale used by Grandin (1997) for cattle. On a scale of one to five, animals were scored as follows: 1 = calm, no movement; 2 = restless shifting; 3 = squirming and occasional shaking of the restraint chute; 4 = continuous, vigorous movement and shaking of device; 5 = rearing, twisting or violent struggling.
Vocalisation (1-2)	The degree to which animals vocalised was recorded: 1 = no vocalisation, or only when moved or initially restrained; 2 = frequent to continuous vocalisation during handling.
Urination (Y/N)	It was noted whether or not an animal urinated.
Defecation (Y/N)	It was noted whether or not an animal defecated.
Salivation (1-3)	The degree to which an animal salivated was scored on a scale from one to three: 1 = no salivation; 2 = some saliva seen around the mouth, no dribbling; 3 = a lot/stream of salivation.
Regurgitation (no. times)	The number of times an animal regurgitated was noted, as identified by the sound of regurgitation, chewing and/or grass visible in the mouth.
Spitting (no. times)	The number of times an animal spat was noted.

Timing of events

The timing of all procedures was recorded. This included the: start of the initial herding process from the home pen into the holding area; entry into the holding pen as a group;

isolation from the group and entrance into the corridor leading to the restraint chute; entry into the chute containing the weighing platform; entry into the restraint chute; administration of the first vaccination; time of blood sampling; the time any saliva or urine was collected, and release.

Statistical analyses

Two-sample t-tests were used to compare results for animals held at the two farms. General Linear Models were used to compare the different physiological measures with each other, including farm as a factor in the model. Data were log-transformed to meet the assumptions of this parametric model. Chi-squared tests were used to compare behavioural measures collected during handling. When expected counts were less than five, Fisher's Exact tests were used to calculate the probability values (Rees 1995). Kruskal Wallace tests were used to compare behavioural and physiological measures, as data could not be transformed to meet the assumptions of parametric statistics. Levels of significance were selected using Bonferonni corrections to control for the number of tests performed. Standard errors of the mean are given after averages.

Results

Physiological measures and serum cortisol levels

During handling in the restraint chute, average heart rate was 90 beats per minute (+/- 2.51); respiratory rate 71 breaths per minute (+/- 2.94), and core body temperature 38.5^{o}C (+/- 0.06). The average serum cortisol concentration was 32.9 nmol/l (+/- 1.94). Animals held at PI had significantly higher heart rates (two sample t-test: $t = 6.93$, $df = 39$, $p < 0.001$) and respiratory rates ($t = 3.14$, $df = 38$, $p = 0.003$), but lower rectal temperatures ($t = -2.59$, $df = 23$, $p < 0.05$) than animals at TA. No differences between farms were found in cortisol levels ($t = 0.36$, $df = 37$, $p > 0.05$). See Table 10 for a breakdown of the data by farm. None of the physiological measures were found to significantly correlate with each other.

Behaviour measures

Most guanacos needed little or no coercion to enter the restraint chute (37 out of 46). Temperament scores were almost entirely confined to the first three points of the scale, with only one exception (score 5). Thus, scores were collapsed into a three-point scale, with the score of 5 being assigned to the third category. Twenty-one animals were in the first category, 9 in the second and 17 in the third. Out of the 46 animals studied, 18

displayed frequent vocalisations during the handling period, 12 showed excessive salivation, 18 regurgitated at least once, and 10 spat at least once during the procedure. Only two animals were observed to urinate during the handling process, and none defecated, so these data were not used in analyses. None of the behaviour measures were found to significantly correlate.

Table 10: Summary of values of physiological measures during handling

Average values are given for the three physiological variables measured, plus serum cortisol concentration, with standard errors of the mean given below. Animals at PI had significantly higher heart and respiratory rates, but significantly lower core body temperatures during handling.

Measurement	Farm PI	Farm TA
Heart rate (beats per minute)	101 +/- 2.41	77 +/- 2.45
Respiratory rate (breaths per	79 +/- 4.20	62 +/- 3.04
Rectal temperature (^{o}C)	38.4 +/- 0.32	38.7 +/- 0.09
Serum cortisol concentration (nmol/l)	33.5 +/- 2.55	32.0 +/- 3.04

Relationships between behaviour and physiological measures

Animals that required coercion to enter the restraint chute had significantly higher heart rates ($H = 8.89$, $df = 1$, $p = 0.003$), respiratory rates ($H = 11.44$, $df = 1$, $p = 0.001$) and serum cortisol concentrations ($H = 4.16$, $df = 1$, $p < 0.05$) than animals that entered of their own accord. Animals that vocalised during the handling process had a tendency to have lower respiratory rate ($H = 6.36$, $df = 1$, $p = 0.012$) but higher cortisol levels ($H = 3.85$, $df = 1$, $p = 0.05$).

Effects of the handling procedure

A closer look was taken at the handling procedure to determine whether this could explain some of the differences found between animals held in the two different farms. This revealed that PI animals had significantly shorter herding times ($t = -4.12$, $df = 22$, $p = 0.0001$) and shorter waiting times, spent in the holding pen, prior to being handling ($t = -3.86$, $df = 30$, $p = 0.001$) compared to those at TA. The order in which animals were taken from the holding pen to be vaccinated had a significant effect on heart

rates, which were higher in animals that were sampled later ($F_{1,38}$ = 14.38, p = 0.001, see Figure 2).

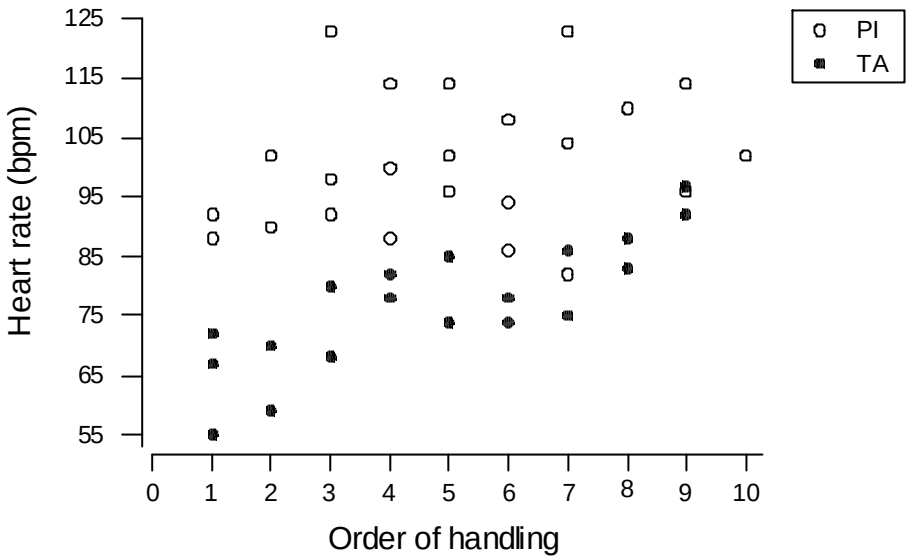


Figure 2 Heart rate recorded during handling by order of handling

A significant positive correlation was found between the order in which animals were handled and heart rate. Overall, animals held at PI were found to have significantly higher heart rates than those TA animals.

Discussion

None of the physiological measures looked at here, namely heart rate, respiratory rate and rectal temperature, were found to correlate with each other, or with levels of corticosteroid in the blood. Similarly, measures of behaviour during handling were not related to each other.

There were some significant relationships between measures of behaviour and physiology. Lower breathing rates were found in animals that vocalised during the procedure. This is likely to have been caused by the breath-holding that was commonly observed in vocalising animals, thus reducing estimates of breathing rates. Measures of vocalisation should therefore be considered when evaluating data on respiratory rates. Animals that required more coercion to enter the restraint chute displayed higher respiratory rates and heart rates during handling. This may relate to the physical effort required to resist entry, but the same animals also showed higher concentrations of cortisol levels in the blood, suggesting that these animals experienced higher levels of stress when they passed through the handling procedure.

No relationship was found between behaviour and heart rate, which was also reported by Zapata *et al.* (submitted). In another study, on shearing, Zapata *et al.* (in prep.) also found a significant relationship between the frequency of vocalisations of differing intensity and cortisol levels, and the frequency of urination. In this study, no record was made of the duration of vocalisations and so a similar comparison cannot be made here. In this study, urination was recorded so infrequently that analysis was not possible.

Significant differences were found between the measures recorded from animals held at the two farms. Animals held at PI had significantly higher respiratory rates and heart rates during handling compared to animals at TA farm, possibly suggesting a higher reactivity to the handling procedure. However, animals at PI also had lower core body temperatures than those in TA and no difference was found in the levels of cortisol in the blood, or in the behaviours recorded during handling. The difference may instead be due to the handling procedure: animals at PI were held in the holding pens for a shorter time before handling, compared to TA, and possibly had less time to rest following the herding process. There may also have been differences in the activity levels of animals while they were in the holding pens, but no observations were conducted at this time to confirm whether or not this was the case.

Overall, this study constituted an effective learning experience for conducting experiments on the guanaco farms, and collecting data. Few correlations were found either between or within behavioural and physiological measures taken during handling, with the exception of the need to coerce animals into the restraint chute. Significant differences were found between animals held at the two farms, which although may have been due to slightly different handling practices, would be interesting to investigate further, given the very different size and content of the enclosures.

6. A study on the effect of analgesia on the behaviour and physiology of farmed guanacos following surgical castration

Introduction

As a general herd practice, male guanacos other than those specifically kept for breeding purposes are routinely castrated once they reach puberty. The primary reasons for this are to control reproduction and reduce aggression between males (Fowler 1989). Studies on a range of species have provided considerable evidence that surgical castration induces pain, both acute and chronic (e.g. horses: Merl *et al.* 2000; calves: Earley & Crowe 2002; lambs: Stafford *et al.* 2002), indicated by changes in physiology (e.g. an increase in cortisol concentration and heart rate) and behaviour (e.g. the appearance of abnormal postures). The only study to have investigated this in guanacos suggests a similar response. Zapata *et al.* (2001) found significant increases in plasma cortisol and an increase in lying behaviour in castrated four-month-old guanacos compared to controls that were only handled. It has now become relatively common in a range of species to administer analgesic drugs prior to (perioperative) or after castration (e.g. Kay 2001; Price & Nolan 2001; Stafford *et al.* 2002) to reduce pain and distress caused by castration. In this study, the use of analgesia during castration was investigated in farmed guanacos, as suggested by Zapata *et al.* (2001).

The analgesic selected was phenylbutazone, which is a non-steroidal anti-inflammatory drug (NSAID). This class of drug acts centrally to induce analgesia and peripherally to reduce inflammation and fever (Wolfensohn & Lloyd 1996). Although the effect of phenylbutazone on guanacos has not been assessed, the pharmacokinetics have been studied in llamas (Navarre *et al.* 2001). The aim of this study was to determine what effect the administration of phenylbutazone had on post-operative behavioural and physiological measures in castrated male guanacos.

Methods

Study animals

Twelve male guanacos were used in this study. Ten animals were one year old at the time of the study (average body weight 53.4 kg, range 43.5 - 66) and two animals were two years of age (body weights 63.7 & 67 kg). Study animals were equally divided between two farms located near Santiago, Chile: Pirque Experimental Research Farm (PI) and El Tahluen (TA). TA held both two-year olds. All study animals were born in captivity to wild-caught parents. See the methodology section in the previous chapter

for details of housing and husbandry ('Study animals and study site' on p23). All study animals were healthy and passed a physical examination prior to the experiment.

Treatments

The experimental protocol involved the surgical castration of all males in the study. Immediately prior to castration, the animals received one of two treatments. Group 1 males received an intravenous dose of the analgesic phenylbutazone, at a dose of 5mg/kg as recommended for llamas (Navarre *et al.* 2001). Group 2 males received an intravenous dose of the same volume of saline solution. Each treatment group contained six animals (three from each farm), which were randomly selected. The two two-year-old males were divided between the treatment groups. The order in which animals were treated was also randomised, while ensuring that the first animal was in Group 2, the second in Group 1, the third in Group 2, and so on. The same order was used for all handling procedures, thus negating the need to control for order during analysis.

Sampling and handling protocol

The experiment took place over three consecutive days within each farm during March, with one week between farms (TA followed by PI). Animals were handled and sampled on the first day (baseline); handled, sampled and castrated the following day (castration); and handled and sampled on the third day (post-castration). See Figure 3 for a summary of the whole procedure.

On the first day, animals were herded from their home enclosure into a holding pen. They were then led one by one in the pre-determined order into a weighing chute, their body weight recorded and then a slide-door opened to allow entry into the restraint chute, which is a drop-floor crush similar to that used with deer (see 'Handling' section on p25 for more details). Immediately after restraint, a blindfold was attached around the head, and held upright throughout the procedure. Various physiological measurements were taken, blood samples collected and a dose of antibiotic (20mg/kg of amoxycillin) given immediately afterwards. Heart rate monitors (Polar Xtrainer Plus™) were attached around the girth of each animal, just behind the foreleg, and a gel applied to aid conductance. These monitors were set to record heart rate every minute. A colour collar was then attached to the neck of the animal to aid identification during subsequent observations. The animal was then released into a holding pen. This was repeated for all six animals in the same order on each day.

On the second day, the procedure started at approximately the same time and followed a similar sampling protocol. Immediately after the blood samples were taken, animals were given either analgesia (Group 1) or saline (Group 2) intravenously. Instead of being released into a holding pen, however, animals were manually restrained as they were leaving the restraint chute. They were then tied with ropes in preparation for castration and carried to a nearby area where the operation was to take place, with the blindfold still attached. Local anaesthesia (3 ml lidocaine 2%) was then injected in a radial pattern around the testes, and the animal left for five to seven minutes to allow the anaesthetic to take effect. Surgical castration proceeded as recommended by Fowler (1989). In short, this involves making two incisions, excising the testicles and transecting the cord. No sutures were used, to facilitate drainage of the wound. An antiseptic spray with fly repellent was applied at the end of the operation. Physiological measures (heart rate, respiratory rate and rectal temperature) and blood samples were taken again before the animal was released into a holding pen. This was repeated for each animal in the predetermined order. One hour after the end of the surgery, each animal was led back into the restraint chute where physiological measures and the last blood sample of the day were taken. A sampling time of one hour post-castration was selected as a study on vicuñas (Bonacic *et al.* 2003) showed that the ACTH-induced rise in cortisol levels peaks between one and two hours after injection (90-120 minutes). This suggests that in order to get the maximal stress response, samples should thus be taken within one to two hours after the stressful event. Animals were then released back into an enclosure.

On the last day of the experiment, day three, the same procedure as employed on day one was carried out, but heart rate monitors were removed prior to release. Again, handling started at around the same time of day. Animals were released into a holding pen and then into the home enclosure as a group.

Physiological measures

During each handling event in the restraint chute, the following physiological measurements were recorded: heart rate, respiratory rate and rectal temperature, in that order. See the section ‘Physiological measurements’ on page 26 for details of how each was measured. One measurement was taken at each phase of the handling process. Baseline values were calculated by averaging the two values taken prior to castration on days one and two.

Heart rates were also recorded using polar heart rate monitors, thus providing data for periods when animals were not being handled. These data were used to derive

minimum heart rates during three time periods: 1) pre-castration (baseline) - from the time monitors were fitted on day one to one hour before handling on day two; 2) immediately post-castration – from the time animals were released after castration on day two to the time the last blood sample was taken one hour later; 3) post-castration - from the time animals had been released into the home pen on day two to one hour before handling on day three. Resting heart rates were calculated by taking an average over the lowest 50 measurements, for the pre- and post-castration time periods, and over the lowest 10 measurements for the period immediately after castration (due to the shorter time period).

Blood sampling and analysis

Blood sampling was kept to a minimum due to the stress caused by the handling procedure. This is not only detrimental to the animal but may also confound results: previous studies have found that with subsequent handling, particularly within the same day, guanacos become more difficult to handle, and cortisol levels increase accordingly (Zapata *et al.* in prep., Jose Luis Riveros pers. comm).

During each sampling period, blood was collected by jugular venipuncture into two 3ml Vacutainers© (Becton, Dickinson and Company), one containing heparin to slow clotting, and another with no additives. Heparinised tubes were gently rocked to aid mixing, left with the other sample to cool down to room temperature, and then both stored in a cool box. Within four hours of collection, all blood samples were centrifuged in a Gemmy Industrial Corporation centrifuge at speed 4 for 10 minutes. Serum (samples with no additive) or plasma (from heparinised tubes) were then pipetted into labelled epindorfs and frozen at -20^{o}C. All samples were then sent for analysis within two weeks of collection to the Endocrinology Laboratory at the Pontificia Universidad Católica de Chile.

Plasma cortisol concentrations were determined using radioimmunoassay techniques (see Bonacic et al, 2003 for details). Baseline cortisol concentrations were calculated by taking an average over values from the day prior to castration and from the handling event immediately before surgery. Changes in cortisol concentration were expressed as percentage change from the baseline level for each individual.

Serum was collected with the aim of quantifying levels of a second component in the blood: haptoglobin. Haptoglobin is an acute-phase protein which, when present in high concentrations, is indicative of inflammation, infection or trauma (Skinner 2001), and is increasingly being used in studies of animal welfare (e.g. Price & Nolan 2001; Earley & Crowe 2002). Samples are currently with the Endocrinology Laboratory, but as yet have not been analysed.

Behaviour

An observer, blind to the treatment administered, recorded the behaviour of the guanacos in two ways. The first was via direct observations to record specific behavioural events of focal animals during the handling process in the restraint chute. The behaviours recorded were as follows: a) reluctance to enter the crush (1 = little or no coercion required; 2 = a moderate to a significant amount of pushing required); b) struggling, i.e. vigorous movement of the body and legs (number of events were also recorded, where one event represented a single movement or a set of continuous movements); c) vocalisation (1 = none, or only when moved or initially restrained; 2 = frequent to continuous vocalisation during handling); d) urination (yes/no); e) spitting (number of events). These measures were recorded throughout the handling process in the restraint chute and, on the day of castration, during surgery.

The four different behaviours recorded during handling in the restraint chute (struggling, vocalisation, urination and spitting) were combined into a single composite score for each individual. First of all, to give added weight to individuals that displayed high frequencies of struggling and spitting, these behaviours were given a score of zero to three, based on their observed frequencies (0 = none; 1 = one to nine times; 2 = more than nine times). Vocalisation and urination were given a score of one if they were observed during the handling period, and zero if they were not. All these scores were then summed to give one value for each animal for each handling period, ranging from 0 to 6, with higher values indicating higher reactivity to handling. The reluctance to enter the restraint chute was considered separately. A similar process was carried out to derive a behaviour score for when the animal was undergoing surgery.

The second form of behaviour recording was by videotape. Animals were recording on a Sony digital camera in the holding pen immediately after handling (days one and three) and castration (day two), and also in the home enclosure after castration (day two) to allow comparison between freely moving animals in the different treatment groups. Recordings were limited to 10 and 20 minutes (see sampling protocol) due to the constraints of daylight after processing all study animals. Continuous focal sampling (Martin & Bateson 1993) was used to score the posture and activity of each animal (see Tables 11 to 13). Studies of castration in other species provided information about what type of abnormal postures and behaviours may have been expected in castrated animals (e.g. Molony *et al.* 1995), and these were refined following observation of the video-recordings.

Table 11: General activities recorded

Activity	Description
Groom	Biting and chewing the fur, or scratching body with hind-legs or against inanimate objects.
Groom area around scrotum	Biting and licking the area around the scrotum
Eat	Includes grazing, browsing and chewing between feeding bouts
Alert	Head raised, ears erect with directed gaze
Other	All other activities
Out of sight	Not visible

Table 12: Postures recorded

Posture	Description
Stand	Standing stationary in normal posture
Unsteady standing*	Standing: swaying, stumbling etc.
Walk	Walking in the normal way
Unsteady walking*	Walking unsteadily: swaying, stumbling etc.
Run	Running in the normal way
Unsteady running*	Running unsteadily: swaying, stumbling etc.
Kneel	Resting on knees of forelegs whilst standing on hind-legs (seen in animals looking out through the bottom of walls in the holding pen)
Sternal recumbency	Lying with chest on the ground, all four legs tucked underneath
Sternal recumbency with fore-legs stretched out	Lying with chest on the ground, one or two fore-legs fully or partially stretched out
Lateral lie	Lying with one shoulder and one hindquarter on the ground, legs
Roll	Rolling in the dirt
Total changes in posture	Sum of the number of times an animal partially or fully stands up or
Sum of inactivity	Sum of all standing and lying categories
Sum of abnormal postures*	Sum of all lying, standing and moving postures considered to be

*Postures considered abnormal and possible indicators of pain and discomfort

Table 13: Specific activities recorded that were considered to be potential responses to castration

Activity	Description
Tail posture	Position of the tail in relation to the body was scored according to the following: low = flat or nearly flat to the body; medium = the base of the tail perpendicular to the body; high = the tail is raised higher than the body.
Foot stamping/kicking	The number of times an animal stamps foot while standing (one action consists of the leg being lifted and forcefully placed on the ground) or kicked outwards while standing or lying (one action consists of the foreleg or hind-leg being stretched out and back).
Hind-leg scratch	Lifting of one hind-leg and movement against the back of the other leg. One event is classed as one action, or a set of continuous actions.
Tail movement	The number of times the tail moves. One action describes the tail moving side to side, or else a series of simultaneous movements side to side (irritation by flies may interfere with this measure)
Head turning	The number of head turns towards the hind quarters (from the midpoint of the body to the tail), with the head behind the shoulders

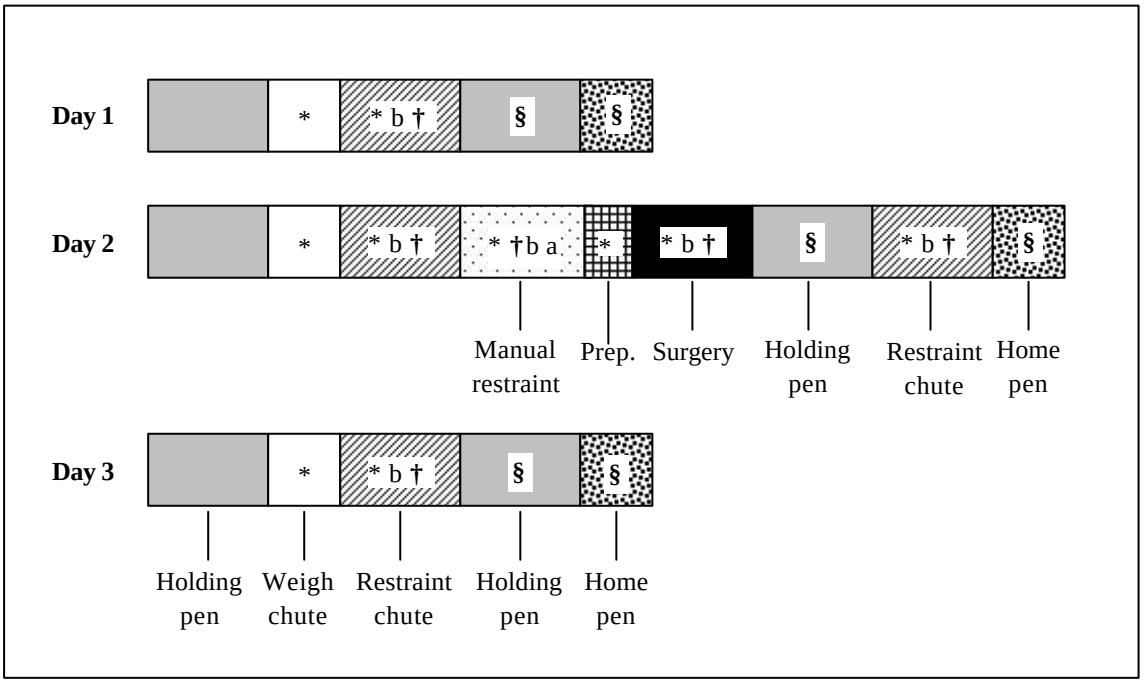


Figure 3 Summary of the handling and sampling procedure

Symbols denote the following procedures: *behaviour scored during handling; † = physiological measures taken (heart rate, respiratory rate, rectal temperature); § = behaviour videotaped; a = analgesia (Group 1) or saline (Group 2) administered intravenously; b = blood samples taken.

Statistical analyses

General Linear Models (GLMs) were used to investigate the effects of treatment on heart rate, respiratory rate and rectal temperature during handling; minimum heart rate during periods when animals were not being handled; and behaviour following handling. Farm and sampling period were included as factors in the model, and if results were significant, post-hoc Tukey tests were used to investigate where significant differences were located. Data were log-transformed to meet the assumptions of this test. Paired t-tests were used to compare serum cortisol concentrations after castration with baseline values. Behavioural reactivity scores (0 - 6) during handling events were subject to ordinal logistical regression analyses. Treatment, sampling period and farm were entered as factors in the model. The standard error of the mean is given after average values. All analyses were conducted in Minitab version 13.1.

Results

The collection of physiological measures and blood samples one hour after castration was only achieved for animals held at PI (mean 1hr 8min, range 1hr 4 min - 1hr 12 min). Animals at TA were sampled an average of 4hrs 13min after surgery had ended (range 2hrs 30min – 6hrs 5min) due to logistical problems. Sampling on the following day occurred, on average, 15hrs 52min after castration (range 14hrs 6min – 18hrs 12 min). Sampling periods thus consisted of baseline (an average over day one and day two, pre-castration), immediately post-castration, 1 to 6 hours post-castration, and 14 to 18 hours post-castration.

Problems were encountered while trying to capture one animal on day two, due to a lengthy chase period. Data on this animal, which received analgesia after castration (Group 1), were therefore not included in the analyses.

Minimum heart rate

Problems were encountered when attempting to fit heart rate monitors on the first day (TA only), so these pre-castration data were missing for animals held at TA. Baseline resting heart rates for PI animals averaged 49.7bpm (+/-3.04). In the periods following castration, there was no significant difference between treatment groups ($F_{1,15} = 0.20$, $p > 0.05$). A significant drop in minimum heart rate was found between the period immediately following castration, comparing animals in the holding pen (mean

75.1±5.24 bpm) to the same animals in the home pen on the following day (mean 51.6±2.10 bpm).

Physiological reaction to handling

Analysis of heart rate data, taken manually during handling, revealed an interaction between treatment and sampling time, although this did not quite reach significance ($F_{1,18}$ = 3.06, p = 0.072). Post-hoc tests showed that heart rates of analgesia-treated animals (Group 2) were significantly higher in the handling period immediately after castration compared to the two other post-castration periods (one to several hours after: t = 3.705, p < 0.05; one day after: t = -4.116, p < 0.01), and these were higher, but not significantly so, compared to control animals (t = -3.136, p = 0.054).

Rectal temperature was found to increase significantly from the period just after surgery to one hour after (t = 2.69, p < 0.05), and from this point, decrease the following day (t = -3.652, p < 0.005). But, treatment group had no effect on rectal temperatures ($F_{1,18}$ = 0.23, p > 0.05) or respiratory rates during handling after castration ($F_{1,18}$ = 0.01, p > 0.05).

Behaviour

During handling

Behaviour reactivity scores varied from 1 to 6 during the period prior to castration, and from 0 to 4 in the periods after. Median scores: were 3 on day one; 5 on day 2 immediately before castration; 1 in the hours after castration and 2 on day three. Animals held at PI had significantly higher scores both before castration (Z = -3.39, p = 0.001) and after (Z = -2.55, p = 0.01). There was very little variation in behaviour scores during surgery, with two animals attaining a score of 3 (one from each treatment group) and the remainder a score of 2. There was also a trend for higher scores in control animals after castration, compared to those treated with analgesia (Z = -1.71, p = 0.087).

After handling

In terms of general activity, no difference was found between the two treatment groups in total levels of inactivity ($F_{1,23}$ = 0.31, p > 0.05), the frequency of alert behaviour ($F_{1,23}$ = 0.05, p > 0.05), the frequency of tail movements ($F_{1,23}$ = 0.01, p > 0.05) or the time spent eating ($F_{1,23}$ = 2.65, p < 0.05). The latter was, however, affected by sampling period ($F_{2,23}$ = 3.59, p < 0.05), with lower levels found after castration (baseline cf. immediately after castration: t = -2.22, p = 0.089; baseline cf. day after castration: t = -

2.41, p = 0.061). Insufficient data negated analyses involving the frequency of grooming behaviour; grooming the area around the scrotum; head-turns; scratching the hind-legs; foot stamping and kicking; changing postures and abnormal postures. Abnormal postures were only observed in four animals throughout the whole observation period. Two of these were during the period immediately after castration (one in each treatment group) and one the following day (control group), but one occurred during the baseline period, presumably as a direct result of handling.

Tail position was the only behaviour found to be affected by treatment, with animals in the control group tending to be observed more often with the tail flat to the body ($F_{1,23}$ = 3.90, p = 0.061) than animals treated with analgesic. There was also a trend for this tail position to be seen more frequently immediately after castration compared to baseline conditions (t = 2.28, p = 0.079).

Serum cortisol concentration

Blood samples were taken, on average, four minutes after animals were restrained in the chute (range 2 - 11 min). During baseline conditions, average serum cortisol concentration was 42.0mnol/l (range 23.5 – 55.4). Looking at PI animals only (sampled one hour after castration), relative changes in cortisol concentration, expressed as percentage change from baseline for each individual, was significantly affected by treatment ($F_{1,12}$ = 6.60, p < 0.05): animals treated with analgesia showed a lesser increase in cortisol following surgery. A similar result was found when data from both farms were combined, and the time since surgery entered as a covariate in the model: ($F_{1,29}$ = 4.37, p < 0.05) (see Figure 4). In the control group only, serum cortisol was significantly higher immediately after surgery (t = -4.35, p < 0.01), and in the following sampling period, one to six hours after (t = -3.15, p < 0.05), compared to baseline levels. No significant change in cortisol levels was evident in animals treated with analgesia. In the control group, levels had returned to baseline by the day after castration. See Table 14 for data showing the changes in cortisol concentration in absolute terms.

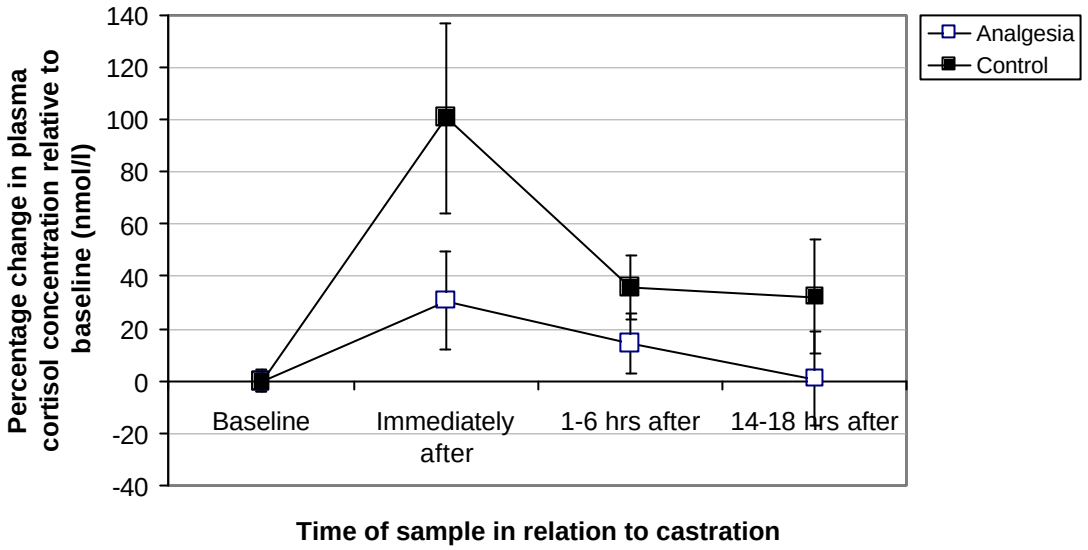


Figure 4 Relative changes in serum cortisol concentration in castrated guanacos treated with pre-operative analgesia compared to controls treated with saline

Treatment with analgesia prior to castration prevented the significant increase in cortisol level observed following surgery in control animals. In the control group, cortisol concentrations were significantly higher immediately after surgery, marginally so during sampling between one to six hours after, but not significantly different by the following day.

Table 14: Serum corticosteroid levels

Sampling period	Serum cortisol concentration (nmol/l)	
	Control group (n = 6)	Analgesia group (n = 5)
Baseline	40.8 (+/-4.27)	43.4 (+/-3.59)
Immediately after castration	75.7 (+/-6.15)	55.5 (+/-7.63)
1-6 hrs after castration	55.0 (+/-7.54)	48.3 (+/-2.10)
14-18 hrs after castration	49.9 (+/-4.16)	43.9 (+/-8.45)

Discussion

Treatment with analgesia had little effect on the behaviour observed in animals after castration. The exception was position of the tail: control animals held their tails flat to their body more frequently than analgesia-treated animals, and this occurred significantly more often after surgery compared to baseline periods. This could indicate that control animals experienced more pain at the site of surgery, with the function being to protectively cover the area from potential knocks. On the other hand, the position of the tail could represent a more general signal, lowering when the animal experiences pain from all manner of sources. Recording tail position should therefore

be recording in future studies investigating the effect of potentially painful procedures on guanacos to determine if it has wide applicability as a pain indicator. Animals that did not receive analgesia also had marginally higher reactivity scores during handling after castration. This could also indicate that analgesia-treated animals experienced lower levels of pain and discomfort, causing them to struggle less while being handling (e.g. Pollard *et al.* 1992).

Other measures of body posture and activity were found to be unaffected by treatment or were observed so rarely that analysis was impossible (e.g. abnormal postures). This could be interpreted as an indication that surgical castration causes little pain and discomfort in guanacos. Alternatively, this procedure could cause significant amounts of pain but this was not reflected in the behavioural data collected here for one of two reasons. The first is that the observation period used was simply not long enough. More time spent observing the animals in their home pen may have revealed at least higher levels of behaviours indicative of pain, but this was not possible in this study due to the lack of daylight after the animals had been processed. This could be overcome by conducting more replicates over different days with fewer animals. Another possibility is that guanacos have evolved to show little outward signs of pain and distress, having responded to pressure from predators (Bateson 1991; Clubb & Mason in press), and so a lack of obvious behaviour signs would not necessarily mean that the animal is not experiencing pain. Considering that Zapata *et al.* (2001) report a significant increase in lying behaviour in castrated four-month old guanacos, then perhaps it is more likely that too short an observation period is responsible for the lack of significant behaviour results found here.

Castration itself appeared to have a significant affect on the physiology of all animals. The sampling periods immediately after castration was characterised by high minimum/resting heart rates; high heart rates during handling (for analgesia treated animals only); high rectal temperatures and high levels of serum corticosteroids. A possible contributory factor, unrelated to castration, is that animals were held in small holding pens at this time for later sampling, where they were seen to be quite agitated and active. This could have been overcome by adding an additional control group of animals that experienced the same handling procedure but which were not castrated.

Treatment with analgesia was found to have a significant effect on cortisol levels following surgery. Control animals experienced a significant rise in blood cortisol levels in the period immediately following castration, averaging 1.9 times higher than baseline. This was notably absent in animals treated with the analgesia phenylbutazone, showing only a small rise to 1.3 times above baseline. A similar increase has been reported in castrated four-month old guanacos, peaking four hours

after surgery at 1.8 times baseline (Zapata *et al.* 2001). In the Zapata *et al.* study, cortisol levels remained high for 24 hours, returning to normal within a week, yet here, cortisol levels in control animals were no different to baseline within 15 to 18 hours after surgery. Another interesting contrast between these studies is the baseline levels of cortisol, being far higher in this study compared to Zapata *et al.* (42.0 cf. 28.3nmol/l), as well as compared to other studies on the same farms (e.g. Zapata *et al.* in prep,; Zapata *et al.* submitted). Even animals transported for over an hour had lower levels than the baseline levels found here (37.3nmol/l: Zapata *et al.* submitted).

Overall, it would seem that castration does induce significant activation of the stress response, which may be indicative of pain, as concluded in a previous study of young farmed guanacos (Zapata *et al.* 2001). This would, however, appear to be relatively short-lived (less than one day). The use of intravenous phenylbutazone as a pre-operative analgesic largely prevents this stress response, suggesting that it worked effectively. Although minimal changes in behaviour were observed, it is suggested that this is largely due to the restricted time period during which study animals were observed, and more extensive work could be more revealing. The use of analgesics during routine castration is thus recommended as a means of improving the welfare of guanacos, which may otherwise be compromised in the short-term.

7. Transportation: effects on behaviour, physiology and stress hormone output of farmed guanacos

Introduction

A study of the transport of tied and unrestrained animals was planned, but due to logistical problems with the transport vehicle and enclosure fences, this was unfortunately not possible during fieldtrips. A protocol for the study had been prepared and so is attached to the end of this chapter. A pilot study of two guanacos that were transported while restrained did take place and so the results of this are presented below.

South American camelids are sometimes transported while tied up with ropes, which has unknown effects on their welfare.

Pilot work

During the routine management of farmed guanacos held in two research farms near Santiago (see 'Study animals and study site' p23), the opportunity arose to study the transportation of two female guanacos over a distance of 100 km from Pirque (PI) to El Tahluen (TA). A larger study of transportation was being planned (see the previous and following sections), and so this procedure was used to carry out some pilot work. The findings from this work are presented briefly here.

Methods

Study animals and study site

Two female guanacos held at TA were used in this study (F1: 63kg; F2: 59kg, both approximately 18 months old). Refer to p23 of this report for details of the management and housing at this farm.

Handling and sampling

Prior to the transportation period, the study animals were separated from the group by moving the whole herd through a restraint chute (see p23 for details of the handling procedure). Study animals were caught while leaving the chute and manually restrained. A coloured collar was attached around each animal's neck to aid identification during subsequent behavioural observations. A heart rate monitor (Polar Xtrainer Plus^{TM}) was then attached around the girth of the animal, just behind the left foreleg, and a gel applied to aid conductance. The first animal was then released into a holding pen containing the rest of the herd and the procedure repeated with the other female. The whole herd was then released into a small outdoor enclosure where behaviour observations were made. One and a half hours after release, the animals were herded into the holding pen again. The two study animals were captured one at a time, immediately blindfolded and then tied in a sternal recumbent position with ropes. Blood samples were then taken and a sedative administered intravenously (2mg xylazine and 1mg ketamine). Each animal was then loaded into the back of a pickup truck ready for transport. A second blood sample was taken on arrival at PI.

Transportation and unloading

Transportation from TA to PI took a total of 2hrs 54mins with no stops, 2hrs 23mins of which took place on motorways and main roads. Animals were unloaded from the pickup truck, one by one, untied and released as a pair into a small enclosure for a period of one hour, before being moved into a different enclosure containing seven other guanacos (4 females and 3 bachelor males).

Behaviour observations

Instantaneous scan sampling (Martin & Bateson 1993) was used to record the posture and activity of study animals, using a 30s sample interval (see Table 15). Behaviour was recorded in this way during three periods: the day prior to transportation in the home enclosure (09:00-17:00 over two days); immediately before transportation in a small enclosure (11:10-12:10); and immediately after transportation (15:20-16:20). The

first period consisted of observations of the whole herd (n = 12) whilst in the home enclosure, and do not refer specifically to the study animals (individuals were not marked). These were made for interest only, to get some impression of the activity budget of captive animals in a naturalistic enclosure (see page 23 for details of the farm), and to determine whether it was possible to get good quality baseline data for animals held in this farm (TA).

Table 15: Ethogram showing the postures and activities recorded when guanacos were in outdoor enclosures

Posture	Description
Stand	Standing stationary
Walk	Slow locomotion
Run	Fast locomotion, usually in response to approach by another animal or person
Sternal recumbency	Lying on the ground, belly in contact with the ground, legs folded under the body
Lateral lie	Lying on the ground, with the side of the body in contact with the ground
Out of sight	Not visible

Activity	Description
Roll	Rolling on the ground
Alert	Looking at an object or area with the head raised, ears up
Look	All looking other than alert
Graze	Feeding on material on the ground, including chewing between bouts
Browse	Feeding on material above the ground, including chewing between bouts
Ruminate	Chewing movement with no sign of having eaten
Groom	Scratching with fore- and hind-feet and biting and chewing the fur
Other	All other activities
Out of sight	Unable to see activity

Behaviour during handling was also recorded, as described in other sections of this report: a) reluctance to enter the crush (1 = little or no coercion required; 2 = a moderate to a significant amount of pushing required); b) number of struggles, i.e. vigorous movement of the body and legs (one event represented a single movement or a set of continuous movements); c) vocalisation (1 = none, or only when moved or initially restrained; 2 = frequent to continuous vocalisation during handling); d) urination (yes/no); e) spitting (number of events).

Results

Behaviour of the herd in the home pen

Observations of the herd in their home pen were extremely difficult. The enclosure was extensive and contained a large amount of natural vegetation cover, mainly in the form of *Acacia* trees. Animals were therefore difficult to find, and once found difficult to follow. Over the two-day period, a total of 15 separate observation events took place, with behaviour being recorded for up to three animals within each of these. Animals were followed for a period of 25 minutes on average (range 3min - 1hr 19min). The averaged figures over all these scans are given in Table 16 as a proportion of scans in which each animal was visible.

Table 16: Frequency of postures and activities of a group of guanacos in an outdoor naturalistic enclosure (TA)

Posture	Frequency (% visible scans)
Stand	83.3
Walk	11.6
Run	0.2
Sternal recumbency	4.4
Lateral lie	0.6
Activity	**Frequency (% visible scans)**
Roll	0.2
Alert	21.5
Look	9.6
Graze	2.7
Browse	53.6
Ruminate	8.0
Groom	0.9
Other	3.4

Behaviour of study animals before and after transport

Observations of the two female guanacos before transport revealed that most time was spent standing and browsing (F1) or standing and looking (F2), see Figure 5 and Figure 6. After transportation F1 spent far more time alert (4 times more) and in a sternal recumbent position. No browsing was recorded after transport due to lack of browse at PI, although in FI, eating in total decreased by a factor of seven. The other female (F2) showed lower frequencies of walking, and spent more time standing after transportation. This animal also showed a large increase in the time spent alert (3

times higher), as well as a reduction in browsing (although eating in total decreased by just a factor of 2, cf. F1).

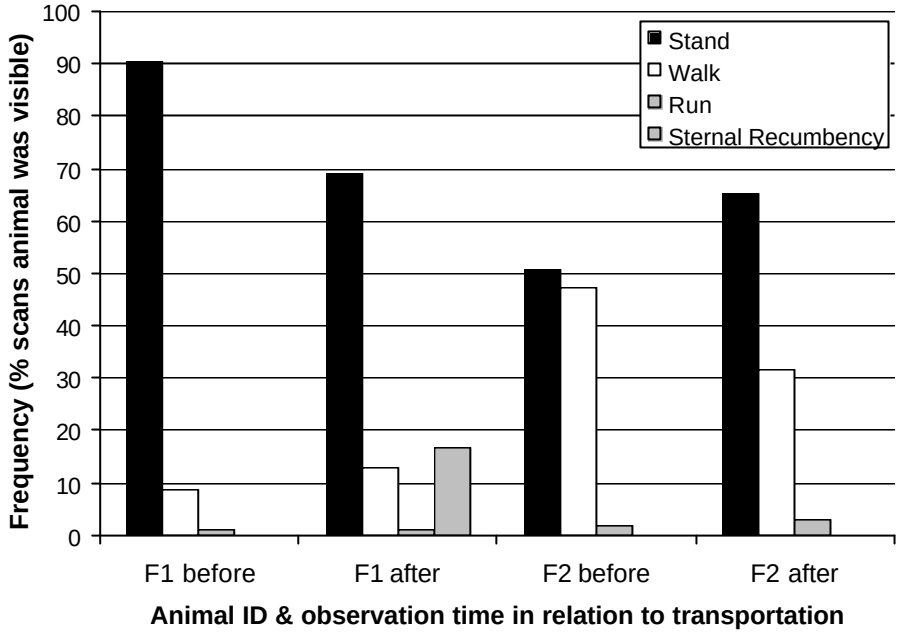


Figure 5 Postures before and after transportation

The postures of two female guanacos recorded before and after transportation (while tied and sedated) are shown here. Observations took place when the pair was alone in outdoor enclosures at TA farm (before) and PI farm (after).

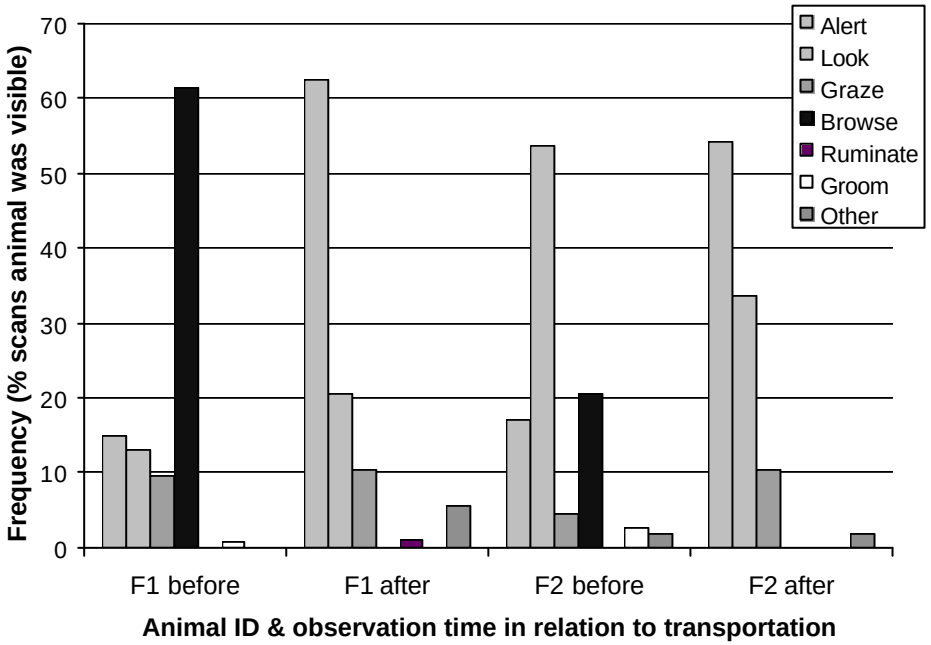


Figure 6 Activities before and after transportation

The activity of two female guanacos recorded before and after transportation (while tied and sedated) is shown here. Observations took place when the pair was alone in outdoor enclosures at TA farm (before) and PI farm (after).

Discussion

This pilot study yielded valuable data and experience, which are useful in the design and implementation of a larger scale study. Behaviour observations in the home pen at TA were found to be extremely difficult. This could be overcome by moving the animals to be transported into a smaller enclosure prior to the experiment to aid observation. Behaviour observations immediately following transport showed that movement decreased, with more time spent lying down and standing stationary compared to before transport. This is likely due to the sedative rather than transport *per se* and so this should be considered in future experimental design. It would be interesting to see whether sedating the animals has a beneficial effect, possibly reducing the stress of the procedure, and hence whether it is necessary.

Suggested protocol for the study of the effects of tied and unrestrained transport on the behaviour, physiology and stress hormone output of farmed guanacos

Introduction

As mentioned at the beginning of this section, a transport study was planned to take place. Unfortunately, it was not possible to conduct this study during fieldtrips to Chile, and therefore the protocol for the study is given here for information. The study aimed to transport a group of guanacos between two farms near Santiago (the same route taken in the pilot study but in the opposite direction). The aim was to compare the behaviour, physiology and stress hormone output of guanacos that were transported while restrained (tied with ropes) with a group of unrestrained guanacos. The protocol given below is specific to this planned project, but could easily be adapted for other situations.

Methods

Animals

A total of 16 male and female guanacos will be used in this study. Animals consist of six one-year-old males, two one-year-old females and seven two-year-old males. All animals are currently held at Pirque experimental farm (PI). Study animals are the F1 generation of parents caught from the wild and transported to captivity in 1996.

Treatment groups

There will be two treatment groups:

1) Restrained during transport: this group will be tied with ropes in the traditional way (in a sternal recumbent position) prior to transportation as in the pilot study. Individuals will be loaded one by one by carrying them into the truck where they will remain restrained throughout the transportation. This group will consist of three one-year-old males, four two-year-old males and one female (one-year-old).
2) Unrestrained during transport: this group will be allowed to move freely while being transported, and will consist of three one-year-old males, three two-year-old males and one female (one-year-old).

Animals will be randomly allocated to each treatment group, ensuring an even distribution of ages and sexes between groups (due to the uneven sample size, an

extra animal will be assigned to Group 1). The order of sampling will be such that that first animal will belong to Group 1, the second to Group 2, the third to Group 1 and so on. The order of individuals within groups will be randomly allocated, ensuring that animals of different ages are evenly distributed, and the two females are sampled at a similar time (see table below).

Handling

At least two weeks prior to the study, both groups (currently held separately) will be mixed within a single enclosure. The experiment will take place over a period of fifteen days in total. Animals will be sampled and marked one week prior to transport (day 1 - baseline), sampled following transportation on day 8, sampled the following day (day 9) and then again one week later (day 15). Each handling episode should occur at approximately the same time of day within each period.

On the first experimental day, all animals will be moved into the restraint chute. Physiological measures and blood samples will be collected while restrained, and behaviour recorded. A heart rate monitor will be attached around the girth of the animal and conductance gel added, and a colour collar will be attached around the neck for identification purposes. The animal will then be released into the home pen and the procedure repeated for all animals.

One week later (day 8), animals will be taken through the restraint chute, and upon release will either enter a holding pen (Group 2), or manually restrained and tied with ropes (Group 1). Restrained animals will be carried into the back of the truck and left there with someone to monitor their behaviour. Once all of Group 1 have been loaded, Group 2 animals will then be released from the holding pen and loaded into the back of the truck. Transportation will then take begin.

On arrival, Group 2 animals will be unloaded into a holding pen. Group 1 will then be unloaded one by one. They will be untied and released into the same holding pen as Group 2 animals. Animals will be left to settle in the holding pen for a period of at least 10 minutes. Sampling will then take place as before and the animals released into an enclosure. This will continue until all the animals have been sampled.

The day after transport (day 9) and one week later (day 15), the same procedure as employed on day one will be carried out.

Physiological measurements

Heart rate, respiratory rate and rectal temperature will be recorded during each handling event in the restraint chute. Heart rate monitors will record heart rates during transportation.

Samples

Blood samples will be obtained by jugular venipuncture into Vacutainer® tubes. Blood will be analysed for the following components: cortisol; haptoglobin levels (see 'Blood sampling and analysis' p35); glucose levels; packed cell volume and creatine kinase.

Behaviour observations

General behaviour will be recorded in the week before transport (baseline) and in the week after (post-transport). Instantaneous scan sampling, with a interval of one minute, will be used to record the posture and activity of all study animals during alternate hours between 09:00 and 17:00. See the pilot study in this section for details of behaviours that will be recorded. In addition to these, behaviours that may occur due to restraint will be recorded, such as limping and stumbling (see Table 12, p37).

The reactivity of animals to handling in the restraint chute will be scored on day one (baseline), day 8 (after transport), day 9 (day after transport) and day 15 (one week after transport). The following behaviours will be recorded: a) reluctance to enter the crush; b) struggling; c) vocalisation (type and duration); d) urination, and e) spitting (see 'Behaviour' section on p36 for more details).

During transport, the behaviour of restrained animals will be recorded by direct observation, noting key behaviours such as struggling, spitting and vocalising. If possible (depending on the design of the truck), behaviour of the unrestrained animals will be recorded throughout the transport. This will include a) rapid foot adjustments (movement of the feet to maintain balance); b) falls (losing balance), and c) impacts with the side of the truck or another animal. Orientation of animals in relation to the direction of travel will also be recorded.

Timings

The time of key features of the transportation will be recorded by someone sitting in the front of the truck. This will include sudden braking, cornering, acceleration and speed bumps. The time of the journey will be recorded, as well as the distance travelled, taken from the odometer of the truck.

Transportation

The transport will take place between Pirque (PI) experimental farm and El Talhuen (TA). All animals will be transported at the same time in the same truck.

Restrained animals (Group 1) will be carried onto the truck immediately after they have been tied up and placed side by side in direct contact with one another (as is the common method of tied transport), surrounded by hay bales to minimise movement. From the time the first animal is placed in the truck, someone will be with them constantly to monitor their condition. Animals will not be blindfolded during restraint, as this is not normal practice for transporting restrained animals. It is not judged to be a welfare problem as they will not be being handled or exposed to people during this time.

Unrestrained animals (Group 2) will be enclosed in a section of the truck. Following the MAFF standards for red deer (Grigor *et al.* 1997), an area measuring c. 6.3m^2 for the seven animals in Group 2 will be cordoned off, thus allowing 0.9m^2 per guanaco. Unrestrained animals will be loaded directly onto the truck by driving them from the holding pen into the back. The truck will be in a hole deep enough to ensure that the floor is level with the ground, thus negating the need for a ramp (this reduced loading times considerably during transport of a group from TA, Jose-Luis Riveros, pers. comm.). The truck has an open top and so to facilitate loading of unrestrained animals, a covering will be applied *after* they have been loaded, ensuring the inside of the truck is adequately illuminated. Previous experience has also shown that loading is facilitated by having a restrained guanaco in the back of the truck (Jose-Luis Riveros, pers. comm.). Thus, the last restrained guanaco will firstly be loaded into the truck and once all the unrestrained guanacos are in place, it will be removed and placed with the others for the transport. Alfalfa hay will be used for bedding material for the journey but no water will be available.

Table 17 Summary of data to be collected

Day 1 **1wk before transport (baseline)**	**Day 8** **Day of transport**		**Day 9** **1 day after transport**	**Day 15** **1 wk after transport**
During handling:	*During transport*	*During handling after transport:*	*During handling:*	*During handling:*
Blood sample		Blood sample	Blood sample	Blood sample
Heart rate		Heart rate	Heart rate	Heart rate
Respiratory rate		Respiratory rate	Respiratory rate	Respiratory rate
Rectal temp.		Rectal temp.	Rectal temp.	Rectal temp.
Behaviour		Behaviour	Behaviour	Behaviour
When in enclosure:		*When in enclosure:*	*When in enclosure:*	*When in enclosure:*
General behaviour	Behaviour	General behaviour	General behaviour	General behaviour
Heart rate	Heart rate	Heart rate	Heart rate	Heart rate
	Orientation			

8. Digital catalogue of guanaco behaviour

During studies of wild and captive guanacos and vicuñas presented in this report, digital footage of behaviour was collected. These have been catalogued according to behaviours that would be recorded during behaviours studies. The aim is to collect a complete ethogram for guanacos and to place these on a website accessible to all people wishing to conduct behavioural studies on camelids. Such a website would act as an effective learning tool, as well as allow standardisation of scoring systems and methodology. The video clips that have been assembled so far are listed below (Table 18), with further behaviours to be added given in

Table 19.

Table 18. Digital film clips of guanaco behaviours

Posture	Description
Stand	Standing stationary, no change in location
Walk	Slow locomotion
Run	Fast locomotion, usually in response to approach by another animal or person
Alert	Looking at an object or area with the head raised and ears erect
Graze	Mouth at ground level, biting or chewing vegetation. This includes bites and chewing between bouts. Feeding material can consist of natural vegetation or food that has been provided (e.g. hay)
Browse	Feeding on material above ground level, including bites and chewing between bouts. This can be combined with 'Graze' to give an overall score for feeding.
Drink	Mouth level or submersed in water
Limp	Walking or running, with one leg either not touching the ground, or spending less time on the ground than the others
Head-nod	Movement of the head up and down. Often seen in animals that have been separated from the group, directed at the enclosure boundary
Head-shake	Vigorous movement of the head from side to side
Alarm call	Loud bleating vocalisation. Often seen when the animal is standing alert, with the tail raised.
Startle	Rapid movement of the head and/or body in response to a stimulus, sometimes involving movement away from the source. This can be a useful measure for future welfare studies, as research on other species has shown that startle responses are increased during states of fear and anxiety, and when anticipating an aversive experience[1]

[1] **Schmid, A., Koch, M. & Schnitzler, H.-U. (1995).** "Conditioned pleasure attenuates the startle response in rats." Neurobiology of Learning and Memory **64**(1): 1-3.
Lang, P. J., BRadley, M. M. & Curthbert, B. N. (1998). "Emotion, motivation and anxiety: brain mechanisms and psychopathology." Biological Psychiatry **44**: 1248-1263.

Table 19. Behaviours that will be added to the digital catalogue

Behaviour category	Description
Roll	Rolling from side to side in the dirt
Sternal recumbency	Lying on the ground, belly in contact with the ground, legs folded under the body
Lateral lie	Lying on the ground, with the side of the body in contact with the ground
Unsteady standing	Standing and stumbling, swaying, falling. This may be seen in animals subject to handling, surgery or after transportation
Unsteady walking	Walking unsteadily: swaying, stumbling etc. This may be seen in animals subject to handling, surgery or after transportation
Unsteady running	Running unsteadily: swaying, stumbling etc. This may be seen in animals subject to handling, surgery or after transportation
Look	All looking around other than 'Alert'
Ruminate	Chewing movement with no sign of having eaten
Groom	Scratching with fore- and hind-feet and biting and chewing the fur
Nose	Touching, or near touching of the ground with the nose. Often occurs before urination or defecation.
Threat	Erect body posture with ears laid back and tail held high
Urinate	
Defecate	
Foot stamp	Rapid lifting and dropping of the foot
Hind-leg scratch	Lifting of one hind-leg and movement against the back of the other leg
Tail movement	Movement of the tail back and forth
Head-twist	Animal leans head back and twists it around, sometimes to the point where the head almost touches the back. Possibly a stereotypic behaviour.
Fence-line pacing	Repetitive walking back and forth along the line of the fence. Possibly a stereotypic behaviour.
Tail posture	
Low	Tail is flat or nearly flat to the body
Medium	The base of the tail is perpendicular to the animal's back
High	The tail is raised higher than the level of the animal's back
Standing on end	The tail is upright, at right angles to the animal's back

9. Suggested future research projects

Automatic Blood Sampling Equipment (ABSE)

Once the ABSE has been used successfully for collecting blood from guanacos, it could be used for a wide-range of applications. First, baseline data on a range of blood variables could be collected from farmed guanacos (as collected by Zapata *et al.* 2003, in restrained animals). Second, an ACTH challenge could be conducted on captive animals without the confound of handling (cf. LeRoy 1999; Bonacic et al. 2003). Third, during studies on the effect of different procedures on guanacos (e.g. capture, shearing, transport), the ABSE could be used to collect samples in the days after treatment, thus negating the need for repeated handling which may interfere with some measures (e.g. Zapata *et al.* in prep.). Furthermore, the ABSE could be used to collect blood samples during transport, not just before and after (as suggested by Zapata et al. submitted). With further refinement, it would also be possible to carry out similar procedures on farmed vicuñas, and potentially on guanacos and vicuñas living freely in the wild.

Dosage protocol for sedation with medetomidine-ketamine drug combination

Further work is required, particularly on vicuñas, to determine the optimal dosage required to achieve complete sedation using the med-ket drug combination administered via projectile dart (see Section 4 for more details).

Comparison of animals transported while tied with ropes or while restrained

See section 7 for a full protocol for this study.

The use of sedatives during the transportation of restrained animals

In a pilot study described in section 7, restrained guanacos were sedated throughout the transportation period. It is unknown whether or not, and to what extent, sedation reduces the stress induced by this procedure and hence whether it is a necessary precaution. A study could be conducted to compare the behaviour and physiology of restrained guanacos transported while sedated with a control group of unsedated animals. See section 7 for details of measures that could be taken.

The use of blindfolds during the transportation of restrained animals

When SACs are transported restrained with ropes, traditionally no blindfolds are used. Blindfolding animals during handling has been shown to reduce handling times and stress responses of guanacos and vicuñas, but it is not know whether the application of a blindfold to restrained animals during transportation would be beneficial, or possibly detrimental to their welfare. A study could be conducted to compare the behaviour and physiology of two groups of animals with and without blindfolds. Measures that could be taken include behaviour during handling, behaviour whilst restrained, heart rates, rectal temperature, serum cortisol concentrations, blood glucose levels, creatine kinase levels and haptoglobin levels. Factors that would have to be considered include the duration of the transportation (restrained animals should not be transported over long distances due to welfare concerns) and the view animals are exposed to during transportation.

The aversiveness to the restraint device and handling area with and without conditioning and training (based on suggestions by Jessica Gimpel)

Studies on both vicuñas and guanacos have shown that handling induces a significant stress response. Both species are now commonly being held in farms where handling can be relatively frequent. Minimising the stress experienced by animals during the handling process is therefore vital to maximise their welfare. This topic has already been highlighted by Jessica Gimpel. Ways of doing this include habituation to the handling area and apparatus, during periods when nothing aversive happens to the animal. This could take the form of running animals through the device at regular intervals without restraint or handling. This experience could be immediately followed by a highly valued food reward. Experiments could reveal what frequency of exposure would be necessary to reduce the aversiveness to the handling area and the restraint device. Aversiveness could be measured in a number of ways. The time taken to enter a corridor leading to the handling area could be measured (see Rushen 1996, for a review). The 'startle' response to a loud auditory stimulus could also be measured, as this can increase when an aversive event is anticipated (e.g. Davis 1980; Schmid *et al.* 1995; Lang *et al.* 1998). These could be measured without the need for handling. Reaction to handling could be monitored intermittently at stages in the experiment to determine whether habituation and training has any affect.

Stereotypic behaviour in SACs held in varying types of captive conditions.

Casual observations of farmed guanacos revealed two potential behaviours that would fit the definition of stereotypies, being repetitive, invariant and with no obvious function (Mason 1991). These are repetitive head-twisting with the head held back, and pacing along the fence line. It is possible that similar behaviours may be seen in captive vicuñas, but this is unknown at present. Stereotypies are often linked to poor welfare due to sub optimal housing conditions, and thus the study of these behaviours, in terms of their prevalence, frequency and rigidity, could be a useful measure of the suitability of captive conditions. Anecdotally, head-twisting is observed only in animals held in small enclosures, but not in those held in large, naturalistic enclosures. Ideally, such a study would encompass as wide a range of conditions as possible, to maximise variation, covering a number of different farms around South America. Factors known to affect stereotypy levels in other species, which should be considered here, include source of the animal (captive-born vs. wild-caught), age, enclosure size, feeding regime, enclosure complexity and group size.

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