

## The effect of transport on cortisol, glucose, heart rate, leukocytes and body weight in captive-reared guanacos (*Lama guanicoe*)

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### Abstract

Current procedures for ranching and sustainable use of guanacos necessitate their transport. Transportation is a risky process for animals, and is a particular concern for wild-caught or semi-domesticated species such as the guanaco — a wild South American camelid species increasingly being established on farms in Chile and Argentina. This study investigated the effect of transport on the physiological and behavioural responses of eight castrated adult male guanacos, transported on a single 2 h journey at a stocking density of 113.5 kg m<sup>-2</sup> (0.76 m<sup>2</sup> per animal). Plasma cortisol and blood glucose concentration, total and differential white blood cell (WBC) counts, heart rate, and body weight were measured one week before, immediately before, immediately after, 2 h after and one week after transport. Behavioural responses were recorded during handling prior to loading. Immediately after transport we found significant increases in plasma cortisol concentrations and neutrophil:lymphocyte (N:L) ratio, the latter peaking 2 h after transport. Heart rate increased significantly only during loading, while body weight remained constant throughout. Behavioural responses related to handling (jumping, vocalising, kicking, spitting and urinating) were not associated with the physiological response. All variables returned to pre-transport values within one week. Transport of guanacos under these conditions produced physiological changes similar to those associated with a mild and transient stress response in other species and which, we judge, fall comfortably within acceptable limits for their welfare.

**Keywords:** animal welfare, cortisol, guanaco, stress, transport

### Introduction

Transportation is a potentially aversive and stressful process for most farm species, with obvious relevance to welfare (for a review see Grandin 1993). For domestic stock, potentially problematic aspects of transportation include removal from the home environment, loading and unloading, confinement, fasting, water deprivation, social mixing, forced physical exercise, handling, noise and vibration (Warriss 1990; Grigor *et al* 1998). These, and other factors, are likely to be even more relevant to the transportation of wild species, a practice increasingly being carried out within conservation and management programmes (ie for the restoration, sustainable use, and study of wild animals). For this reason, and others, there is growing interest in evaluating the stress experienced by such species. Indeed, Macdonald (2000) suggests that animal welfare science is an important, but hitherto under-emphasised, aspect of conservation biology. Recent approaches to evaluating the stress experienced by wild mammals in association with transport include measures of blood biochemistry and

hormones in vicuña, *Vicugna vicugna* (Bonacic & Macdonald 2003; Bonacic *et al* 2003), and of leucocyte responses in Eurasian badgers, *Meles meles* (McLaren *et al* 2003; Montes *et al* 2004). It is important to consider that stress responses may be species-specific (Morton *et al* 1995); Bonacic *et al* (2003) have highlighted this for South American camelids.

In this context, the guanaco (*Lama guanicoe*), a South American wild camelid, is particularly relevant because it is increasingly the subject of ranching initiatives in Chile and Argentina. This involves not only the transportation of wild-caught guanacos from their site of capture to a farm, but also subsequent journeys as other farms are stocked (Bas & González 2000). Since this is a new initiative in wildlife use, little is known about the guanaco's response to the stressors involved. However, studies investigating the stress response of domestic camelids to transportation (Anderson *et al* 1999a,b) found that short-duration transportation produced an increase in serum cortisol concentrations, but heart rate did not change significantly and behavioural

characteristics were not associated with changes in serum cortisol concentration.

Since stress involves changes in neuroendocrine and autonomic nervous systems, and in behaviour, there is a case for measuring several dimensions of the stress response, including physiological, haematological and behavioural variables (Mason & Mendl 1993; Fraser & Broom, 1997 p 436; Dawkins 1998). Furthermore, it is sensible for these measures to include some that reflect a short-term response, such as heart rate, plasma cortisol and blood glucose concentration, and others that reflect a medium-term effect, such as decreased body weight, impaired growth and immune response (Fraser & Broom 1997, p 436).

Here, we report on a preliminary study of the short- and medium-term stress responses of captive guanacos to transportation. Plasma cortisol and blood glucose concentration, heart rate and white blood cell (WBC) changes were measured as indicators of a short-term stress response, and body weight as an indicator of a medium-term stress response to the transportation process.

## Materials and methods

### Animals

Eight castrated male guanacos, ranging from four to five years of age and with body weights ranging between 90 and 110 kg, were transported between two farms in the vicinity of Santiago, Chile. They were caught when they were approximately one month old, and bottle-fed until they reached a body weight of 35 kg (at around four months of age).

All guanacos were clinically examined before transport and found to be in good health. They belonged to the same social group and were placed alone in a new paddock, both before and after transportation. These animals were subject to common guanaco farming practices prior to the study, including monthly weighing, prophylactic treatment for endo- and ecto-parasites twice a year, and shearing annually. The animals had been castrated at two years of age. On both farms, guanacos grazed and browsed natural vegetation typical of the Mediterranean climate at that location; they were also provided with an alfalfa hay supplement.

### Transportation protocol

The journey of 102 km (90 km of paved road, and the remainder un-paved) was undertaken in February 2001, the austral summer. The average speed was 50 km h<sup>-1</sup> and the journey was made early in the afternoon. Transportation consisted of three stages: loading, journey, and unloading. Animals were loaded individually into a 2.1 × 3 m stock trailer by pushing them up a ramp (20° inclination). Loading the whole group took 17 min and the journey lasted 1 h 51 min (from 1356h to 1547h). Unloading the group took approximately 3 min. Alfalfa hay was used as bedding material during the journey, but no water was provided.

### Sampling protocol

Body weight (kg) ( $\pm$  0.01 kg, Micro-power®), heart rate (beats min<sup>-1</sup>), blood glucose concentration (mmol l<sup>-1</sup>), plasma cortisol concentration (nmol l<sup>-1</sup>), total WBC counts (10<sup>9</sup> l<sup>-1</sup>),

and differential WBC counts (10<sup>9</sup> l<sup>-1</sup>) were measured. The ratio of neutrophils to lymphocytes (N:L) was then calculated. The following behaviours were recorded for each animal as they were individually loaded: jumping, vocalising (a scream), spitting, kicking and urinating. Individuals were classified into one of three categories on the basis of these responses: mild, if they showed none or one of those behavioural responses; moderate, if they showed two or three of those behavioural responses; or severe, if they showed four or five of those behavioural responses.

The animals were manually restrained and blood samples were obtained by jugular venepuncture (Vacutainer®, Becton Dickinson, USA) using ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. Blood samples were taken one week prior to transport, immediately before transport, immediately post-transport, 2 h post-transport, and one week after transport. Two tubes were obtained for each animal (6 ml): one for haematological studies, and one for plasma cortisol analysis. The process took approximately 1 min per animal and the samples for the WBC count were placed on ice prior to processing. The samples for cortisol determination were immediately centrifuged at 5000 rpm and the plasma obtained was separated into 1.5 ml microtubes, which were kept on ice during the fieldwork. Body weight was measured one week prior to transport, immediately post-transport, and one week after transport. Heart rate was measured using a cardiac monitor (Polar X-Trainer® Polar Electro, Finland), which was fitted with its belt around the animal's chest 1 h prior to loading and removed approximately 1 h after unloading. The cardiac monitor was programmed to record heart rate every 60 s, and the data were downloaded using the Polar interface®.

### Blood analysis

Blood glucose, plasma cortisol and WBC counts were measured from samples taken one week prior to transport, immediately before transport, immediately post-transport, 2 h post-transport and one week after transport. Blood glucose concentration was measured in fresh blood using a portable glucometer (Glucomer 4, Bayer®, USA) and test strips. Plasma cortisol concentration was obtained from centrifuged blood samples (stored at -70°C until assay) using the radioimmunoassay technique (RIA) with reagents supplied by the World Health Organisation (WHO) and according to the recommended procedures (Hall 1978). The mean inter-assay and intra-assay coefficients of variation were 11.56% and 7.5% respectively. WBC counts were conducted by the same person, and in a Neubauer counting chamber using the haemocytometer technique; differential WBC counts were performed according to Schalm *et al* (1975), and the N:L ratio was calculated. Manual counts were performed instead of automated cell counts because in Chile few veterinary clinical laboratories use the latter technique.

### Statistical analysis

The Kolmogorov-Smirnov test was used to test for normality of variables. The ratios of neutrophils to lymphocytes were transformed using their arcsine-square root.

The mean heart rate was calculated during pre-loading, loading, the journey, and 45 min post-journey. Heart rate,

**Table 1** Mean  $\pm$  SD glucose, cortisol and body weight at different sampling times before and after transport (n = 8).

	Glucose (mmol l <sup>-1</sup> )	Cortisol (nmol l <sup>-1</sup> )	Weight (kg)
Baseline	6.5 $\pm$ 0.25	21.7 $\pm$ 2.76	96.1 $\pm$ 7.24
Post-transport	7.8 $\pm$ 1.56	37.3 $\pm$ 10.25*	94.5 $\pm$ 5.88
2 h post-transport	7.4 $\pm$ 1.31	25.4 $\pm$ 6.56	–
1 week post-transport	5.9 $\pm$ 0.71	22.4 $\pm$ 8.75	95.2 $\pm$ 6.69

\* Significant increase from baseline ( $P < 0.05$ ).

**Table 2** Mean  $\pm$  SD heart rate at different sampling times before, during and after transport (n = 7<sup>†</sup>).

	Pre-loading (baseline)	Loading	Journey	Post-journey
Heart rate (bpm)	67.8 $\pm$ 3.01	144.6 $\pm$ 23.33*	76.1 $\pm$ 17.76 <sup>‡</sup>	60.7 $\pm$ 2.51 <sup>‡</sup>
n	7	7	6 <sup>‡</sup>	4 <sup>‡</sup>
Mean duration (min)	51.1	1.2	111	45

\* Significant increase from baseline ( $P < 0.05$ ).

<sup>†</sup> Heart rates were recorded from only seven of the eight guanacos, because one cardiac monitor failed to work throughout the study.

<sup>‡</sup> Two and four cardiac monitors did not record heart rate, during the journey and post journey, respectively. Both of these time samples were excluded from statistical analysis.

body weight, plasma cortisol concentration, blood glucose concentration, total WBC count and the N:L ratio were analysed by ANOVA with repeated measures. A contrast with the first level was performed to allow comparison of the subsequent values with the baseline values. Baseline blood parameters were obtained from the mean of the first two blood-sampling occasions (one week and immediately before transport), thus producing a more robust baseline value. These were previously tested using the paired *t*-test. Chi-square tests were performed to evaluate the association between behavioural responses and the cortisol and glucose concentration, heart rate and N:L values. Probability values of  $< 0.05$  were considered significant.

## Results

There was a significant increase in cortisol concentration ( $\times 1.7$ ) after transport, compared with baseline values ( $F_{1,7} = 16.3$ ;  $P = 0.005$ ; see Table 1). Plasma cortisol concentrations peaked immediately after transport and returned to baseline values 2 h post-transport, whereas blood glucose and body weight were statistically similar at all sampling times.

Heart rate was significantly higher than baseline during the loading procedure ( $F_{1,6} = 9.13$ ;  $P = 0.029$ ) (see Table 2). However, during the journey, four of the eight animals displaced their heart rate sensors. As a result, post-journey measures of this variable were excluded from the ANOVA.

Total WBC counts and neutrophils peaked 2 h after transport ( $F_{1,7} = 6.2$ ;  $P = 0.041$  and  $F_{1,7} = 23.1$ ;  $P = 0.002$ , respectively) and remained elevated for up to one week after transport ( $F_{1,7} = 5.5$ ;  $P = 0.05$  and  $F_{1,7} = 8.4$ ;  $P = 0.02$ , respectively; see Table 3). The number of lymphocytes decreased significantly following transport ( $F_{1,7} = 14.3$ ;  $P = 0.007$ ) and remained low for up to 2 h ( $F_{1,7} = 15.0$ ;  $P = 0.006$ ). Consequently, the N:L ratio changed: it was significantly higher than baseline immediately after

transport ( $F_{1,7} = 8.4$ ;  $P = 0.023$ ), peaked 2 h post-transport ( $F_{1,7} = 156.1$ ;  $P < 0.001$ ), and then returned to baseline at the last sampling.

Based on behavioural observations, the response to handling was categorised as mild in two animals (25.0%), moderate in three animals (37.5%) and severe in three animals (37.5%). However, there was no association between the categorised behavioural response to handling and concentrations of cortisol or glucose, heart rate, or the N:L ratio ( $P > 0.05$ ).

## Discussion

Both journey duration and stocking density have important implications for the welfare of animals during transportation (Tarrant *et al* 1992; Warriss *et al* 1995; Waas *et al* 1997; Grigor *et al* 1998; Knowles 1999; Weeks 2000). In this study, both journey duration (less than 2 h) and stocking density (113.5 kg m<sup>-2</sup>; 0.76 m<sup>2</sup> per animal) were within the ranges recommended for red deer (MAFF 1989; New Zealand Animal Welfare Advisory Committee 1994) — a comparably sized wild species also adopted as a farm animal.

In the sampled guanacos, transportation had a significant effect on heart rate, plasma cortisol concentration, total WBC count, and neutrophils, and, as a consequence, the N:L ratio. However, there was no significant effect on blood glucose concentration or body weight. The increase in plasma cortisol concentration found in the guanacos was similar to that reported by Anderson *et al* (1999a) for alpacas (*Lama pacos*), in which serum cortisol was significantly higher after 30 min of transportation compared with baseline levels and with levels after a 4 h recovery period. Nevertheless, that report showed that in alpacas the cortisol concentration peak was higher (2.17  $\times$  baseline) than in guanacos (1.72  $\times$  baseline). In red deer, plasma cortisol concentrations have been shown to increase after 3 h travelling and to return to pre-journey values at the end of a 2.75 h

**Table 3 Mean  $\pm$  SD total and differential white blood cell counts of guanacos before and after transport (n = 8).**

	Leukocytes (10 <sup>9</sup> l <sup>-1</sup> )	Neutrophils (10 <sup>9</sup> l <sup>-1</sup> )	Lymphocytes (10 <sup>9</sup> l <sup>-1</sup> )	Monocytes (10 <sup>9</sup> l <sup>-1</sup> )	Eosinophils (10 <sup>9</sup> l <sup>-1</sup> )	N:L ratio <sup>†</sup>
Baseline	8.6 $\pm$ 1.56	5.7 $\pm$ 1.27	2.2 $\pm$ 3.66	0.1 $\pm$ 0.05	0.5 $\pm$ 0.10	0.17 $\pm$ 0.02
Post-transport	5.8 $\pm$ 0.80	4.4 $\pm$ 0.71	1.0 $\pm$ 0.12**	0.2 $\pm$ 0.04	0.3 $\pm$ 0.08	0.21 $\pm$ 0.01*
2 h post-transport	11.1 $\pm$ 1.58*	9.3 $\pm$ 1.37**	1.2 $\pm$ 0.22**	0.1 $\pm$ 0.03	0.3 $\pm$ 0.11	0.28 $\pm$ 0.02**
1 week post-transport	11.3 $\pm$ 1.49*	8.4 $\pm$ 1.22*	2.3 $\pm$ 0.51	0.2 $\pm$ 0.03	0.4 $\pm$ 0.14	0.20 $\pm$ 0.02

\*Significant change from baseline at the  $P < 0.05$  level.

\*\*Significant change from baseline at the  $P < 0.01$  level.

<sup>†</sup> Values are arcsine-square root transformed.

recovery period (Grigor *et al* 1998). Similar results have been reported for goats (Kannan *et al* 2000), in which cortisol concentrations peaked immediately post-transport and then decreased dramatically.

The cortisol response observed in the present study in guanacos immediately after transport suggests a moderate stress response, compared with the maximal cortisol response of a 4.6-fold increase obtained after injection with exogenous adrenocorticotrophic hormone (ACTH) in captive guanacos (Le Roy 1999). However, we do not know what cortisol levels were reached during transportation in the present study. Waas *et al* (1999) measured the real-time physiological response of red deer to translocation using remote blood samplers, and found a 3-fold increase in cortisol concentration during the course of translocation, suggesting that the stress response of the red deer was progressive. Similarly, Stull and Rodiek (2000) reported a continuing increase in plasma cortisol concentration in horses throughout the duration of transit.

In the current study, glucose concentrations were within the normal range reported for guanacos (Zapata *et al* 2003). Glucose concentration increased after transport; however, this increase was not significant and may be explained by wide inter-subject variability. There was a tendency for changes in glucose concentration to parallel those in cortisol concentration, increasing immediately post-transport and decreasing to baseline values 2 h after transport. Exposure to stress or aversive conditions are thought to activate adrenalin, which leads to an elevation of plasma glucose, preparing the animal to respond to the perceived challenge (Wingfield *et al* 1997). Similar results have been found in horses (Stull & Rodiek 2000), in which glucose levels, together with cortisol levels and the N:L ratio, increased with journey duration and then returned to baseline values during the post-transport period. In goats, increased concentrations of glucose were found up to 3 h post-transport (Kannan *et al* 2000), whereas in red deer, using remote blood samplers, glucose levels remained stable (Waas *et al* 1999).

The guanacos displayed an increase in total WBC counts and neutrophils, and a decrease in the number of lymphocytes after transport. Total and differential WBC counts, however, were within the normal range for guanacos

(Hawkey & Gulland 1988; González *et al* 1998; Bas & González 2000; Zapata *et al* 2003). Nevertheless, the N:L ratio, which summarises the change in the number of neutrophils and lymphocytes, was significantly higher than baseline immediately post-transport. It peaked 2 h post-transport (2.6-fold increase) and then decreased to pre-transport levels. Wild mammals such as mouflons (*Ovis ammon*) and rhinoceros (*Diceros bicornis michaeli*) have shown similar changes in WBC count following transportation (Marco *et al* 1997; Kock *et al* 1999). In goats, after 2.5 h of transportation, the N:L ratio rose above pre-loading values for up to 18 h post-transportation (Kannan *et al* 2000). The observed changes in the N:L ratio could be explained in terms of changes in cell trafficking and reservoir site. These changes might indicate that transport affected the immune system; however, one week after transport, the baseline ratio had been re-established.

Studies on the effect of loading for transportation in cattle and sheep have shown that heart rate increases with loading and initial transportation, but decreases with time (Sartorelli *et al* 1992; Warriss *et al* 1995; Grigor *et al* 1998). Waas *et al* (1999) found increased heart rates during 'transitional events' of red deer transport, such as round-up, penning, and loading onto a truck. In fact, they reported that heart rate increased sharply during these events, but recovered rapidly when deer were undisturbed. In the present study the increase in heart rate may be interpreted as a stress response to loading.

Body weight may fall as part of a chronic stress response or as a result of food and water deprivation (Grigor *et al* 1998; Knowles 1999). In small wild mammals, at least, changes in body weight can occur rapidly (McLaren *et al* 2004). Grigor *et al* (1998) reported that in red deer, in conditions with access to feed and water, weight loss increased from 0.8% to 1.7% as journey time increased, but returned to pre-transport levels within 3 h of transport. In the present experiment, in which only alfalfa hay was available during transport, body weight did not change over the course of the study. Water deprivation did not affect body weight. The absence of any detectable effect of transport on body weight in the present study could be explained by considering the short duration of the journey (cf Warriss 1990; Grigor *et al* 1998).



## Conclusions

Transportation of adult male guanacos under the conditions described produced some physiological changes, without body weight loss. All variables returned to pre-transport values within one week after transport. This suggests that the duration of transport and the stocking density in this study produced only a moderate and transient stress response.

## Animal welfare implications

It is a matter of judgement as to whether the observed effects were acceptable, and our judgement is that the welfare of the guanacos was not problematic under the conditions we describe. Of course, it is obvious that different circumstances — for example, different pre-transport preparation, longer or rougher journeys, greater stocking densities or more extreme temperatures — might lead to different responses and an opposite judgement. We are also mindful that, although our study involved eight individuals, their transport together in the same trailer reduced the independence within our sample. Nonetheless, while we consider our results as only a first step, we consider that step important in the context of the increasing trade in guanacos, and the absence, hitherto, of any data on their welfare within the farming process. Hopefully, our findings, and those that build on them, will help those formulating guidelines for the transportation and husbandry of farmed guanacos.

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