DOI: 10.7589/2019-02-045

Journal of Wildlife Diseases, 56(2), 2020, pp. 294–305
© Wildlife Disease Association 2020

CHALLENGES TO ANIMAL WELFARE ASSOCIATED WITH CAPTURE AND LONG ROAD TRANSPORT IN BOMA-ADAPTED BLACK (*DICEROS BICORNIS*) AND SEMI-CAPTIVE WHITE (*CERATOTHERIUM SIMUM*) RHINOCEROSES

Friederike Pohlin,[1,2,8] Markus Hofmeyr,[1,3] Emma H. Hooijberg,[1,4] Dee Blackhurst,[5] Mmadi Reuben,[6] Dave Cooper,[7] and Leith C. R. Meyer[1,2]

[1] Centre for Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
[2] Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
[3] Great Plains Conservation and Rhinos Without Borders, PO Box 22127, Boseja, Maun, Botswana
[4] Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
[5] Division of Chemical Pathology, Faculty of Health Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa
[6] Department of Wildlife and National Parks, PO Box 131, Gaborone, Botswana
[7] Ezemvelo KZN Wildlife, PO Box 13053, Cascades 3202, South Africa
[8] Corresponding author (email: friederike.pohlin@gmail.com)

ABSTRACT: Capture and transport are part of translocation and expose animals to a variety of stressors that can lead to morbidity and mortality. We aimed to establish a better understanding of the physiologic responses to capture and transport in black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses in Southern Africa. Fourteen adult black rhinoceroses were transported 600 km by vehicle and 32 white rhinoceroses (24 adults and 8 juveniles) were transported 1,300 km by vehicle. The black rhinoceroses had been wild-caught and boma-adapted over 6 wk prior to the translocation and were only sedated to allow for loading into the transport crates. The white rhinoceroses originated from a game farm and were chemically immobilized from a helicopter and then loaded. Paired blood samples were collected from animals at loading (capture) and after transport and evaluated for changes in clinical chemistry analytes, acute phase reactants, and oxidative stress biomarkers. The Wilcoxon rank sum test was used to compare changes in measured analytes from capture and after transport. All rhinoceroses survived capture and transport. Rhinoceroses experienced total body water loss, mobilization of energy reserves, and muscular damage. Alterations in acute phase reactants suggested that animals mounted a stress response. Oxidative stress was observed in black rhinoceroses. We identified the following challenges to animal welfare during transport: hydration status, energy balance, skeletal muscle fatigue, and stress-induced immunomodulation. Measures to mitigate these challenges, such as administration of fluids, need to be included in the planning of future translocations.

Key words: Energy balance, fatigue, hydration, rhinoceros, stress, translocation, transport.

INTRODUCTION

The Southern-central black rhinoceros (*Diceros bicornis minor*) is listed as critically endangered, and the Southern white rhinoceros (*Ceratotherium simum simum*) as near threatened, by the International Union for Conservation of Nature (IUCN) *Red List of Threatened Species* (Emslie 2011, 2012). The main reasons for these assessments are the continued and increased poaching threat and the increasing illegal demand for rhinoceros horn associated with the increased involvement of organized international criminal syndicates in rhinoceros poaching (Emslie et al. 2016). Translocation for population reintroduction or reinforcement, or metapopulation management, represents an essential tool for the management of these species and is an integral part of national and international rhinoceros conservation plans (Knight 2017). Translocation involves capture, temporary captivity, transport, and release into a novel environment, exposing the animals to a variety of stressors such as prolonged periods of water- and food deprivation that can lead to

morbidity or mortality (Teixeira et al. 2007; Dickens et al. 2010). Rhinoceroses frequently traumatize themselves during transport (Morkel and Kennedy-Benson 2007) or become sick and die after they have been released (Emslie et al. 2009; Miller et al. 2016). The current mortality rate for rhinoceros translocations in South Africa and Namibia is estimated to be 5%. The prevalence of morbidity is likely underestimated (Miller et al. 2016).

The morbidity and mortality associated with rhinoceros translocation raises serious concerns for animal welfare (Teixeira et al. 2007; Swaisgood 2010; Harrington et al. 2013). The physiologic responses to translocation must be understood to reduce adverse events.

Handling and transport of farm animals influences the activity of certain enzymes and hormones, mobilization of energy and protein metabolism, concentration of acute phase reactants (APRs), and the balance between oxidants and antioxidants (Adenkola and Ayo 2010; Casella et al. 2012; Padalino et al. 2017). However, these effects have been poorly studied in rhinoceroses. Increases in creatine kinase and aspartate transaminase, changes in serum electrolyte concentrations, and a marked rise in blood cortisol and glucose concentrations have been found in captured and transported black rhinoceroses (Kock et al. 1990a), but similar investigations have not been carried out in white rhinoceroses. Furthermore, the effects of capture and transport on APRs and oxidative balance have not been evaluated in either black or white rhinoceroses. The APRs are a group of positive and negative immunomodulatory proteins and other analytes whose serum concentrations increase or decrease in response to stressors (Petersen et al. 2004; Cray et al. 2009). Concurrent stress-induced alterations in plasma oxidants and antioxidants might result in an imbalance in favor of oxidants which may induce oxidative stress, causing cellular damage and increased susceptibility to disease associated with translocation (Kock et al. 1992, 1999; Lykkesfeldt and Svendsen 2007).

Our aim was to better understand the physiologic responses to capture and transport by evaluating clinical chemistry analytes, APRs, and oxidative stress biomarkers during actual translocation operations of black and white rhinoceroses and to identify challenges associated with transport that should be addressed in order to improve animal welfare.

MATERIALS AND METHODS

Fourteen boma-adapted black rhinoceroses were transported just over 600 km from KwaZulu-Natal to the north of South Africa and 32 white rhinoceroses, originating from a game-farm, were transported just over 1,300 km from the Free State in South Africa to Botswana. Trucks and International Air Transport Association-approved rhinoceros crates were used in both translocations, which followed the practical guidelines for transport of live wild animals (CITES 2013) and rhinoceroses (Morkel and Kennedy-Benson 2007; Emslie et al. 2009). The translocations were planned and took place independently of this study and the University of Pretoria Animal Ethics Committee (V067-17) approved opportunistic sample collection from these animals.

Rhinoceros capture

All black rhinoceroses were adults (seven females and seven males) captured from the wild and confined, for adaptation purposes, in bomas for 6 wk prior to translocation. Temporary confinement facilitated veterinary examinations, disease screening and quarantine, and allowed for a quicker capture process on the day of transport.

The translocation took place overnight in October 2017 in order to avoid extreme ambient temperatures. The rhinoceroses were sedated via darting within the boma with a combination of 0.6–0.8 mg etorphine (Captivon®, 9.8 mg/mL, Wildlife Pharmaceuticals, Karino, South Africa) and 60 mg azaperone (Azaperone tartrate, 50 mg/mL, Wildlife Pharmaceuticals) delivered remotely using 1.5-mL plastic darts (DAN-INJECT®, International S.A., Skukuza, South Africa) with 60-mm uncollared needles propelled by compressed air. Using a low-sedative dose of etorphine is a common capture method in boma-adapted rhinoceroses, which allows for conscious loading of the animals without the need for full immobilization. Once in the crate, a blood sample was collected from an auricular vein. To partially reverse some of the sedative effects of etorphine, 1.2 mg diprenorphine (Activon®, 12 mg/mL, Wildlife Pharmaceuticals) and 10 mg butorphanol

(Butorphanol tartrate, 50 mg/mL, Wildlife Pharmaceuticals) were administered intravenously. Additionally, 1 g carprofen (Rimadyl®, 50 mg/mL, Zoetis, Sandton, South Africa), a nonsteroidal anti-inflammatory drug, was given intramuscularly to all animals.

The white rhinoceroses comprised 24 adults (18 females, six males) and eight juveniles (five females, three males) and originated from a 340-ha private game farm where they received additional water and supplementary food prior to the translocation. Animals were captured in the early morning in September 2017 by darting from a helicopter with a combination of etorphine (3–5 mg/adult or 0.1–1.5 mg/juvenile), azaperone (20–40 mg/adult or 0–10 mg/juvenile), and 5,000 IU hyaluronidase (adult only; Hyalase®, Kyron Laboratories, Johannesburg, South Africa) delivered remotely using 2.0-mL darts (Pneu-dart, Inc.®, Williamsport, Pennsylvania, USA) with 63.5-mm barbed needles.

The animals became recumbent within 5 min of darting and a blood sample was collected immediately from an auricular vein. If an animal tremored severely (n=10), butorphanol, at 2–5 times the etorphine dose in milligrams, was administered intravenously in order to mitigate the hypoxemia associated with the muscle tremors (de Lange et al. 2017). Within 10 min of darting, diprenorphine (0.2–0.8 mg/adult or 0–0.1 mg/juvenile; M5050®, 12 mg/mL, Novartis, Midrand, South Africa) was administered intravenously to partially reverse the immobilizing effects of etorphine, which facilitated loading. Each rhinoceros, including the juveniles, was loaded into its own transport crate where adult animals received another 2.5–15 mg of intravenous diprenorphine to complete the etorphine reversal. Additionally, either 5 g vitamin C (n=4; ascorbic acid, 500 mg/5 mL, Fresenius Kabi, Bloemfontein, South Africa) or 500 mg vitamin E and 50 mg selenium (n=4; vitamin E acetate 17 mg/mL, sodium selenite 1.67 mg/mL, Kyron) were given intramuscularly to some adult rhinoceroses to support the animals' antioxidant defenses (Lykkesfeldt and Svendsen 2007).

Rhinoceros transport

The tranquilizer zuclopenthixol acetate (Clopixol-Acuphase®, 50 mg/mL, H. Lundbeck Pty. Ltd., Randburg, South Africa) was administered intramuscularly via hand-injection (150–220 mg/black rhinoceros or 100–250/adult white rhinoceros or 10–50 mg/juvenile white rhinoceros) just after loading into the crates. Transport commenced once all rhinoceroses had been captured and loaded, which took a mean of 3.25 h (SD 0.75) in both translocations.

During transport, the vehicles stopped at 2–4-h intervals to allow for additional intramuscular administration of azaperone and midazolam (Dazonil®, 50 mg/mL, Wildlife Pharmaceuticals) to restless animals. Eight black rhinoceroses received at least one top-up dose of 40–100 mg azaperone, of which two animals required additional one to three top-up doses of azaperone and midazolam (15–30 mg). All white rhinoceroses received at least one top-up dose of azaperone (80–120 mg/adult or 10–80 mg/juvenile), which was combined with 2.5–15 mg midazolam in the juveniles. Sixteen adult and six juvenile white rhinoceroses required up to three additional top-up doses of azaperone, alone or in combination with midazolam (10–20 mg/adult), during transport.

Water was not provided to the animals, as past experience has shown that rhinoceroses do not drink during transport and affixed water containers are known to cause injury (CITES 2013). At the heat of the day, however, white rhinoceroses were doused with water during stops and small amounts of alfalfa hay were offered to some of the animals.

At the release site, all adult rhinoceroses were reimmobilized with etorphine (3.5–4 mg/black rhinoceros or 3.5–6 mg/white rhinoceros) and azaperone (40 mg/black rhinoceros or 20–40 mg/white rhinoceros) via pole syringe or hand injection into the nuchal hump while standing in the transport crate. Juvenile white rhinoceroses received 0.5–2.5 mg etorphine and 5 mg midazolam intramuscularly via pole syringe. Before animals became immobile, they were released from their crates and manually restrained with ropes until they became recumbent. Subsequently, blood samples were collected from the immobilized animals from cephalic or auricular veins. Naltrexone (Trexonil®, 50 mg/mL, Wildlife Pharmaceuticals), at 20 times the etorphine dose in milligrams, was administered intravenously to reverse the immobilization and release the rhinoceroses into the private game reserve (black rhinoceroses) or national park (white rhinoceroses).

Blood sample analysis

Blood from rhinoceroses was collected directly into serum and potassium oxalate/sodium fluoride (NaF) tubes (BD Vacutainer, Becton and Dickinson, Plymouth, UK), stored in a cooler box with ice packs, and centrifuged within 24 h. Serum and plasma were aliquoted and stored at –80 C until analysis. Samples from the white rhinoceroses were stored at –20 C for 1 mo prior to being stored at –80 C.

Samples were analyzed in the clinical pathology laboratory of the Onderstepoort Veterinary

Academic Hospital (Pretoria, South Africa) and the University of Cape Town Division of Chemical Pathology laboratory (Cape Town, South Africa).

Serum clinical chemistry analysis was done using a Cobas Integra 400 Plus automated biochemistry analyzer (Roche Diagnostics Ltd., Rotkreuz, Switzerland) using commercially available kits. Measured analytes included: total serum protein, albumin, globulin, sodium, chloride, urea, creatinine, potassium, magnesium, phosphorus, total calcium, total bilirubin, cholesterol, triglycerides, alkaline phosphatase, γ-glutamyl transferase, glutamate dehydrogenase, aspartate aminotransferase (AST), and creatine kinase (CK). Plasma glucose and lactate concentrations were measured by spectrophotometric methods with the same analyzer. Serum beta hydroxybutyrate (BHB) and nonesterified fatty acid (NEFA) concentrations were measured by kinetic enzymatic and colorimetric methods, respectively, using BHB and NEFA kits (Randox Laboratories, Crumlin, Antrim, UK). Serum cortisol concentration was assessed by a chemiluminescent immunoassay using the Immulite/Immulite 1000 Cortisol® following manufacturer's instructions (Siemens Healthcare, Erlangen, Germany).

Serum haptoglobin was determined by the hemoglobin-binding method using a commercial kit (PHASE Haptoglobin Assay, Tridelta Development Limited, Kildare, Ireland) with the Cobas Integra 400 Plus analyzer. Concentrations of serum amyloid A (SAA) were determined by a solid-phase sandwich enzyme-linked immunoassay using a commercial kit (PHASE SAA Assay, Tridelta Development Limited) previously validated for use in black and white rhinoceroses (Schook et al. 2015; Hooijberg et al. 2018). Serum iron was measured with the Cobas Integra 400 Plus biochemistry analyzer.

The lipid peroxidation products, conjugated dienes, and thiobarbituric acid reactive substances (TBARS) were measured by spectrophotometric methods (Nduhirabandi et al. 2011). The antioxidant capacity of plasma was assessed by the oxygen radical absorbance capacity (ORAC) method (Cao et al. 1993; Huang et al. 2002).

Statistical analysis

Statistical tests were performed using R 3.3.1 for Windows (The R Foundation, Vienna, Austria). Descriptive tables and scatter plots were generated. Mean and SDs were calculated for each analyte and presented for descriptive purposes. Due to the small sample size, nonparametric analyses were used to compare concentrations of measured analytes between capture and after transport by using the Wilcoxon rank sum test with data divided by species and age. A P-value <0.05 was considered significant.

RESULTS

All rhinoceroses survived capture and transport. The mean (SD, range) overall time animals spent in the transport crates was 19.7 (2.3, 16.3–23.0) h for black rhinoceroses and 34.3 (3.2, 30.5–40.3) h for white rhinoceroses. Environmental temperatures ranged from 7.9 C and 13.7 C during the night to 28.2 C and 40.3 C during the day in the black and white rhinoceros translocations, respectively.

Capture and transport influenced many clinical chemistry analyte concentrations. Means (SD) for measured analytes at capture and after transport and the corresponding P-values for the Wilcoxon rank sum test are shown in Table 1 for the different species and age groups.

Briefly, total serum protein and sodium concentrations increased from capture to after transport in black (P=0.004 and P<0.001, respectively) and adult white rhinoceroses (P=0.043 and P<0.001, respectively). Albumin concentrations increased in black rhinoceroses (P=0.004). Chloride concentrations increased and potassium concentrations decreased in all animals (P=0.020 and P<0.001 in black, P<0.001 and P<0.011 in adult white, and P=0.010 and P=0.007 in juvenile white rhinoceroses, respectively). Magnesium and phosphorus concentrations decreased in adult rhinoceroses (P=0.037 and P<0.001 in black and P<0.001 and P<0.001 in white rhinoceroses, respectively). Total calcium concentrations increased in black rhinoceroses (P=0.022) but decreased in juvenile white rhinoceroses (P<0.001). Only in white rhinoceroses did creatinine and urea concentrations increase (P<0.001 and P<0.001 in adults, P=0.027 and P=0.002 in juveniles, respectively). Total bilirubin concentrations rose in all animals (P=0.014 in black, P<0.001 in adult white, and P=0.001 in juvenile white rhinoceroses). Nonesterified fatty acids and BHB concentrations increased in adult (P<0.001 and P=0.044, respectively) and juvenile (P=0.001 and

TABLE 1. Mean (SD) concentrations or activities of blood clinical chemistry analytes of black rhinoceroses (*Diceros bicornis*) and adult and juvenile white rhinoceroses (*Ceratotherium simum*) at capture and after transport. A significant difference (shown in bold) between capture and after-transport samples represented the effects of transportation.

Clinical chemistry analyte	Black rhinoceroses (n=14)			Adult white rhinoceroses (n=24)			Juvenile white rhinoceroses (n=8)		
	At capture	After transport	*P*	At capture	After transport	*P*	At capture	After transport	*P*
Albumin (g/L)	32 (1)	34 (2)	**0.004**	31 (3)	32 (2)	0.085	31 (2)	32 (2)	0.798
Alkaline phosphatase (U/L)	82 (41)	92 (28)	0.239	82 (29)	81 (26)	0.959	175 (34)	141 (22)	0.093
Aspartate transaminase (U/L)	78 (32)	206 (130)	**<0.001**	62 (13)	313 (313)	**<0.001**	64 (13)	375 (384)	**0.002**
Beta hydroxybutyrate (mmol/L)	0.20 (0.04)	0.23 (0.10)	0.942	0.24 (0.07)	0.30 (0.11)	**0.044**	0.16 (0.06)	0.39 (0.15)	**0.004**
Chloride (mmol/L)	94 (2)	96 (3)	**0.020**	89 (2)	95 (2)	**<0.001**	90 (1)	94 (3)	**0.010**
Cholesterol (mmol/L)	1.34 (0.23)	1.09 (0.35)	**0.044**	2.13 (1.42)	1.83 (0.57)	**0.011**	4.54 (1.59)	3.98 (1.80)	0.442
Cortisol (nmol/L)	49 (25)	44 (16)	0.627	76 (25)	78 (26)	0.853	52 (16)	49 (33)	0.798
Creatine kinase (U/L)	499 (300)	7,919 (7,514)	**<0.001**	199 (71)	9,097 (6,554)	**<0.001**	3,234 (64)	6,062 (5,453)	**<0.001**
Creatinine (µmol/L)	93 (15)	89 (14)	0.382	141 (21)	177 (45)	**<0.001**	127 (19)	153 (20)	**0.027**
Globulin (g/L)	52 (5)	55 (4)	0.159	55 (4)	56 (4)	0.212	43 (7)	43 (7)	1.000
Glucose (mmol/L)	4.97 (0.69)	5.49 (0.49)	0.080	7.56 (1.39)	8.15 (1.06)	0.490	7.68 (1.26)	7.58 (0.77)	0.959
Glutamate dehydrogenase (U/L)	3 (1)	3 (1)	0.225	4 (2)	3 (1)	**0.016**	3 (2)	2 (1)	0.082
γ glutamyl transferase (U/L)	14 (3)	17 (4)	0.055	15 (5)	14 (3)	0.773	10 (4)	9 (3)	0.599
Lactate (mmol/L)	2.44 (1.77)	7.91 (3.23)	**<0.001**	17.03 (6.58)	6.77 (2.74)	**<0.001**	19.79 (7.58)	2.95 (0.81)	**<0.001**
Magnesium (mmol/L)	0.89 (0.12)	0.8 (0.08)	**0.037**	1.12 (0.11)	0.80 (0.07)	**<0.001**	1.14 (0.14)	1.02 (0.17)	0.092
Non-esterified fatty acids (mmol/L)	0.39 (0.31)	0.47 (0.28)	0.481	0.10 (0.10)	0.42 (0.23)	**<0.001**	0.06 (0.03)	0.75 (0.43)	**0.001**
Potassium (mmol/L)	6.8 (1.4)	5.4 (0.4)	**<0.001**	4.9 (0.6)	4.6 (1.4)	**0.011**	4.72 (0.55)	3.81 (0.61)	**0.007**
Phosphorus (mmol/L)	1.56 (0.26)	1.03 (0.30)	**<0.001**	1.48 (0.23)	0.67 (0.37)	**<0.001**	2.02 (0.24)	1.87 (0.38)	0.234
Sodium (mmol/L)	129 (2)	136 (3)	**<0.001**	132 (2)	137 (2)	**<0.001**	133 (3)	135 (3)	0.195
Total calcium (mmol/L)	2.99 (0.08)	3.07 (0.10)	**0.022**	2.95 (0.18)	2.85 (0.22)	0.061	3.13 (0.13)	2.81 (0.11)	**<0.001**
Total bilirubin (µmol/L)	3.2 (1.2)	4.7 (1.8)	**0.014**	2.0 (0.4)	4.5 (1.8)	**<0.001**	2.1 (0.5)	6.7 (2.6)	**0.001**
Total serum protein (g/L)	84 (5)	90 (4)	**0.004**	85 (5)	88 (5)	**0.043**	74 (6)	75 (5)	0.959
Triglycerides (mmol/L)	0.29 (0.12)	0.60 (0.33)	**0.001**	0.51 (0.14)	0.40 (0.13)	**0.022**	0.60 (0.12)	1.16 (0.68)	0.066
Urea (mmol/L)	5.0 (0.8)	5.7 (0.8)	0.058	3.6 (0.6)	4.9 (1.2)	**<0.001**	2.5 (0.9)	4.6 (1.2)	**0.002**

TABLE 2. Mean (SD) concentrations or activities of acute phase reactant and oxidative stress biomarkers of black rhinoceroses (*Diceros bicornis*) and adult and juvenile white rhinoceroses (*Ceratotherium simum*) at capture and after transport. Significant differences (shown in bold) between capture and after-transport samples represented the effects of transportation.

Biomarkers	Black rhinoceroses ($n=14$)			Adult white rhinoceroses ($n=24$)			Juvenile white rhinoceroses ($n=8$)		
	At capture	After transport	*P*	At capture	After transport	*P*	At capture	After transport	*P*
Acute phase reactant									
Haptoglobin (g/L)	4.0 (1.0)	3.5 (1.9)	0.209	2.2 (0.8)	2.3 (1.0)	0.676	1.6 (0.6)	1.3 (1.0)	0.336
Serum amyloid A (mg/L)	24 (53)	104 (82)	**0.002**	<7 (0)	10 (7)	**0.013**	<7 (0)	14 (17)	0.310
Iron (µmol/L)	25.8 (4.3)	19.5 (6.7)	**0.006**	23.6 (4.2)	19.7 (5.3)	**0.006**	26.3 (3.3)	16.4 (5.4)	**0.005**
Oxidative stress biomarker[a]									
ORAC (µmol/L trolox equivalents)	764 (272)	974 (389)	0.183	781 (255)	660 (194)	0.113	1,136 (493)	919 (432)	0.505
CD (µmol/L)	41.5 (8.0)	53.7 (7.5)	**0.001**	56.0 (10.1)	53.4 (8.6)	0.726	58.0 (10.2)	53.4 (10.4)	0.958
TBARS (µmol/L MDA equivalents)	0.39 (0.15)	0.29 (0.07)	0.135	0.32 (0.14)	0.34 (0.18)	0.570	0.24 (0.08)	0.26 (0.22)	0.527

[a] ORAC = oxygen radical absorbance capacity; CD = conjugated dienes; TBARS = thiobarbituric acid reactive substances; MDA = *malondialdehyde*.

$P=0.004$, respectively) white rhinoceroses. Cholesterol declined in black ($P=0.044$) and adult white rhinoceroses ($P=0.011$), whereas triglyceride concentrations increased in black ($P=0.001$), but decreased in adult white rhinoceroses ($P=0.022$). Capture and transport did not appear to change the liver enzymes alkaline phosphatase and γ glutamyl transferase ($P>0.05$); however, glutamate dehydrogenase concentrations were lower following transport in white rhinoceroses ($P=0.016$). Serum CK and AST were markedly elevated after transport compared to capture in all adult ($P<0.001$, both analytes) and juvenile ($P<0.010$ and $P<0.003$, respectively) rhinoceroses. After transport, lactate concentrations were higher in black ($P<0.001$), but lower in white ($P<0.001$), rhinoceroses. No changes in cortisol and glucose concentrations were detected in any rhinoceros group ($P>0.05$).

Mean (SD) of APR concentrations at capture and after transport and the corresponding *P*-values for the Wilcoxon rank sum test are shown in Table 2 for the different species and age groups. There were no changes in haptoglobin concentrations from capture to after transport in the groups ($P>0.05$). Serum amyloid A concentrations in white rhinoceros capture samples were below detection range (<7.0 mg/L), but increased to detectable concentrations in adult white rhinoceroses after transport ($P=0.013$) and were also higher in black rhinoceroses after transport ($P=0.002$). Iron decreased from capture to after transport in all animals ($P=0.006$ in black, $P=0.006$ in adult white, and $P=0.005$ in juvenile white rhinoceroses).

Mean (SD) of oxidative stress biomarker concentrations at capture and after transport and the corresponding *P*-values for the Wilcoxon rank sum test are shown in Table 2 for the different species and age groups. Namely, CD concentrations were higher after transport compared to capture in black rhinoceroses only ($P=0.001$). No significant changes in TBARS and ORAC were detected ($P>0.05$).

DISCUSSION

Capture and transport of black and white rhinoceroses induced changes in serum electrolyte, enzyme and metabolite concentrations, APRs, and, in the black rhinoceroses, plasma oxidants. Based on these changes, we identified the following challenges to animal welfare during transport: hydration status, energy balance, skeletal muscle fatigue, and stress-induced immunomodulation. Most clinical chemistry analytes remained within published reference intervals for black and white rhinoceroses measured at capture (Kock et al. 1990b; Mathebula et al. 2012; Hooijberg et al. 2017). However, because these reference intervals were established using different laboratory methods, and the value in comparing once-off measurements to population-based reference intervals can be limited (Perrin et al. 2018), the changes of variables within individuals were used to identify these challenges.

The increase in total serum protein and albumin concentrations from capture to after transport point to a decrease in plasma volume, most likely because rhinoceroses did not drink (Gupta et al. 1999). The parallel increase in the concentrations of sodium and chloride also indicates a relative body water loss and dehydration. Despite this increase, both analytes remained within normal limits for the species (Kock et al. 1990b), suggesting that rhinoceroses are fairly tolerant to the effects of water deprivation. Depending on the season and location, rhinoceroses sometimes only drink every second day (Mukinya 1977; Morkel and Kennedy-Benson 2007).

However, the white rhinoceroses responded to the water deprivation with an additional increase in serum urea and creatinine concentrations, indicating a decreased renal glomerular filtration rate (DiBartola 2012). Similar changes in serum electrolytes and urea and creatinine concentrations have been correlated with journey duration and ambient temperatures in transported domestic animals (Minka and Ayo 2009; Nielsen et al. 2011). The white rhinoceroses were transported over a longer time-period than the black rhinoceroses and were exposed to higher ambient temperatures; therefore, they likely experienced a greater degree of dehydration. Even though the animals appeared to clinically cope with the prolonged time of water deprivation, total body water loss could pose as an additional stressor. Rhinoceroses, especially those caught from the wild, seldom drink water during transport, therefore future actions should include research on effective methods of fluid administration and planning and scheduling appropriate administration of fluids during long transports.

Transportation induced an increase in total bilirubin concentrations and a decrease in serum potassium concentrations in all animals. Magnesium and phosphorus concentrations also decreased in the adults and calcium concentrations in the juveniles. These changes are known to occur after a period of fasting (Gupta et al. 1999; Muñoz et al. 2010) and a lack of dietary intake of these electrolytes (Fisher et al. 1999; Muñoz et al. 2010; DiBartola 2012). Food deprivation leads to protein catabolism and mobilization of lipid stores from the adipose tissue for energy (Brinkmann et al. 2013). Mobilization of lipid stores results in elevated plasma NEFA concentrations, which were observed in the white rhinoceroses in our study and also have been reported in transported domestic animals (Brown et al. 1999; Knowles et al. 1999; Saeb et al. 2010). Typically, only a part of NEFA is utilized as an energy source and the remainder is converted into triglycerides or metabolized to ketone bodies such as BHB (Brinkmann et al. 2013). Accordingly, we found an increase in triglyceride concentrations in transported black rhinoceroses and an increase in BHB concentrations in transported white rhinoceroses indicating a negative energy balance. Cholesterol concentrations, however, decreased in adult rhinoceroses, possibly in response to a release of inflammatory cytokines and acute phase reaction (Feingold and Grunfeld 2010). The observed negative energy balance during the long transports was likely a stressor and should be mitigated. Whether feeding can correct this imbalance, especially in animals unaccus-

tomed to eating unnatural food, or if metabolic supplements or parenteral feeding could be used successfully, requires further investigation; this investigation should focus on nutritional planning (i.e., type and amount of food) and consider the gastrointestinal side effects of the tranquilizing drugs.

Animals likely become tired if they remain standing during a long journey. Plasma indicators of muscle exertion (AST and CK) increased from capture to after transport in all rhinoceroses. Additionally, calcium concentrations increased in the black rhinoceroses, most likely due to increased muscular activity (Ayo et al. 2009). Elevated muscle enzymes have occurred in a variety of transported animals (Kock et al. 1990a; Montané et al. 2002). When an animal is immobilized by physical or chemical restraint, or standing in a transport crate, its muscles are in an increased state of contraction. Consequent compression of vessels may lead to poor muscular perfusion resulting in tissue hypoxia and ultimately muscle cell damage (Spraker 1993). Repetitive intramuscular administration of the tranquilizing drugs throughout transport may have also caused additional muscle cell injury and release of CK into the blood stream (Lefebvre et al. 1996). Serum lactate concentrations increased from capture to after transport in the black rhinoceroses but decreased in the white rhinoceroses. Lactate is produced in skeletal muscle and other tissues as a direct result of increased metabolic rate and glycolytic carbon flow. Following intense exercise, muscular hypoperfusion, or hypoxia, lactate production will increase, creating energy anaerobically and allowing for the continuation of exercise (Phypers and Pierce 2006). Normal resting lactate concentrations in rhinoceroses are not available in the literature; however, mean values for ground-immobilized white rhinoceroses (4.6 mmol/L; Cole et al. 2017) indicate that the lactate concentrations in our helicopter-captured white rhinoceroses were profoundly elevated. This finding is not unexpected, as the helicopter-captured rhinoceroses likely experienced higher muscular activity prior to immobilization compared to the ground-immobilized rhinoceroses in the study of Cole et al. (2017) or our boma-confined black rhinoceroses. Additionally, the white rhinoceroses in our study were fully immobilized and likely endured greater hypoxia (Hattingh et al. 1994; Buss et al. 2015) than did the black rhinoceroses, which were only sedated.

These results suggest that differences in capture techniques had substantial implications for animal welfare during transport. Temporary confinement of rhinoceroses in bomas allowed for a smoother capture before transportation, without the need for full immobilization, thereby mitigating hyperlactataemia and other capture-associated pathophysiology at the beginning of the transport. However, temporary captivity itself may adversely affect animal welfare (Miller et al. 2016) and its value, as a component of the translocation process, remains to be investigated in rhinoceroses.

Elevations in plasma cortisol and glucose concentrations have been documented in transported domestic (Fazio and Ferlazzo 2003; Fisher et al. 2009; Minka and Ayo 2009) and wild animal species (Zapata et al. 2004; Saeb et al. 2010), including black rhinoceroses (Kock et al. 1990a), indicating a stress response to transport. Interestingly, we did not find any significant changes in serum cortisol and glucose concentrations in our rhinoceroses. Previous studies in farm animals have shown that blood cortisol concentrations peak within the first 3 h of transport and then return to baseline concentrations within 9 h (Knowles et al. 1995; Warriss et al. 1995). If the timing of cortisol release is similar in rhinoceroses, we may have missed sampling at peak plasma concentrations. Alternatively, it could be that cortisol concentrations were elevated at both sample points or that, due to the sedative drugs, rhinoceroses were not stressed. In order to identify a stress response during long transports, future studies need to collect serial blood samples at shorter time intervals or include other analytes that indicate a stress response and whose concentrations change more slowly.

There appears to be a link between stress response and an increase in APR concentra-

tions and oxidative stress biomarkers during transport (Wernicki et al. 2006; Giannetto et al. 2011; El-Deeb and El-Bahr 2012). By inducing proinflammatory cytokines in immunity-related cells, the activation of the hypothalamic-pituitary-adrenal axis promotes the initiation of an acute phase response (Murata 2007). In white rhinoceroses, haptoglobin and SAA are positive APRs, which increase during the acute phase response, and albumin and iron are negative APRs which decrease during an acute phase response (Hooijberg et al. 2018). Serum amyloid A has also been shown to be a positive acute phase protein in black rhinoceroses (Schook et al. 2015). We observed an increase in SAA concentrations and a decrease in iron concentrations from capture to after transport, indicating immunomodulation in response to stress. An acute phase response is often accompanied by alterations in plasma oxidants and antioxidants that may lead to an excessive production of free radicals, resulting in oxidative stress (Cray et al. 2009). Oxidative stress has been implicated in numerous disease processes so that oxidative parameters have been proposed as biomarkers to identify animals at risk of disease (Lykkesfeldt and Svendsen 2007). Of all the oxidative stress biomarkers that we measured, transport elevated the concentration of conjugated dienes only in the black rhinoceroses, indicating an increased production of free radicals in these animals (Adenkola and Ayo 2010). Unlike the white rhinoceroses, we gave the black rhinoceroses a nonsteroidal anti-inflammatory drug, which might have contributed to the oxidative damage (Asensio et al. 2007). However, the administration of the potent antioxidants vitamin C or E to some of the white rhinoceroses could have prevented an increase in lipid peroxidation products (Lykkesfeldt and Svendsen 2007); therefore, the role of oxidative stress in translocation requires further research.

Our primary aim was to determine the physiologic responses to capture and transport of African rhinoceroses. Because the translocations took place for conservation purposes, independently of this study, it was not possible to standardize interventions or change how the animals were managed. Therefore, a number of confounding variables, such as the varying use of sedatives and other drugs, immobilization techniques, or the different times spent in the transport crate could have influenced the results in some animals. Interspecies and interage group differences were only noted within and between time points, but were not statistically compared because this was beyond the scope of this study. These comparisons should be directly investigated in the future. Nevertheless, we believe this study highlighted some important effects of capture and transport which likely influenced the welfare of translocated rhinoceroses in a real-world setting. These effects likely resulted from challenges such as the lack of water, food, and rest, and stress-induced immunomodulation, which could be minimized by implementing simple measures such as providing water and food or administering fluids or metabolic supplements. However, some measures, such as feeding or providing water, might be difficult to implement or might cause side-effects such as colic, and therefore need to be systematically investigated. Practical guidelines developed for the nonair transport of live wild animals (CITES 2013) and for rhinoceroses (Morkel and Kennedy-Benson 2007; Emslie et al. 2009) mention measures that are currently undertaken to guarantee animal welfare during transport. These measures mainly focus on management considerations around boma housing and transport and the transport crate design. For rhinoceroses, currently there are no recommended limits for transport duration, or water and food deprivation times; it is important that these factors should be established in future studies.

ACKNOWLEDGMENTS

We thank Rhinos Without Borders (Great Plains Conservation & Beyond), the World Wildlife Fund, and Ezemvelo KwaZulu-Natal Wildlife for allowing access to rhinoceros transports. We thank Johannes Eksteen and Thomas Lengate (Kroonstad Dierehospitaal), Comfort Nkgowe, Les Charlisle, Grant Tracy, Rob Jackson,

Jacques Flamand, Ursina Rusch, Rowan Leeming, Jacques O'Dell, and Janine Meuffels for helping with sample collection and storage. We thank the Department of Paraclinical Sciences of the University of Pretoria, National Research Foundation, and the Wildlife Group of the South African Veterinary Association for funding of these projects. We thank DEFFink and the students of the University of Veterinary Medicine Vienna for raising awareness and funds. Many thanks to Radoslaw Karpienko for helping with statistical analysis of data.

LITERATURE CITED

Adenkola AY, Ayo JO. 2010. Physiological and behavioural responses of livestock to road transportation stress: A review. *Afr J Biotechnol* 9:4845–4856.

Asensio C, Levoin N, Guillaume C, Guerquin MJ, Rouguieg K, Chrétien F, Chapleur Y, Netter P, Minn A, Lapicque F. 2007. Irreversible inhibition of glucose-6-phosphate dehydrogenase by the coenzyme A conjugate of ketoprofen: A key to oxidative stress induced by non-steroidal anti-inflammatory drugs? *Biochem Pharmacol* 73:405–416.

Ayo JO, Minka NS, Sackey AKB, Adelaiye AB. 2009. Responses of serum electrolytes of goats to twelve hours of road transportation during the hot-dry season in Nigeria, and the effect of pretreatment with ascorbic acid. *Onderstepoort J Vet Res* 76:409–418.

Brinkmann L, Gerken M, Riek A. 2013. Effect of long-term feed restriction on the health status and welfare of a robust horse breed, the Shetland pony (*Equus ferus caballus*). *Res Vet Sci* 94:826–831.

Brown SN, Knowles TG, Edwards JE, Warriss PD. 1999. Behavioural and physiological responses of pigs to being transported for up to 24 hours followed by six hours recovery in lairage. *Vet Rec* 145:421–426.

Buss P, Olea-Popelka F, Meyer L, Hofmeyr J, Mathebula N, Kruger M, Brüns A, Martin L, Miller M. 2015. Evaluation of cardiorespiratory, blood gas, and lactate values during extended immobilization of white rhinoceros (*Ceratotherium simum*). *J Zoo Wildl Med* 46:224–233.

Cao G, Alessio HM, Cutler RG. 1993. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Bio Med* 14:303–311.

Casella S, Fazio F, Giannetto C, Giudice E, Piccione G. 2012. Influence of transportation on serum concentrations of acute phase proteins in horse. *Res Vet Sci* 93:914–917.

CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). 2013. *Guidelines for the non-air transport of live wild animals and plants*. https://cites.org/eng/resources/transport/index.php. Accessed June 2019.

Cole GC, Tordiffe ASW, Steenkamp G. 2017. Assessment of a portable lactate meter for field use in the white rhinoceros (*Ceratotherium simum*). *Onderstepoort J Vet Res* 84:1–10.

Cray C, Zaias J, Altman N. 2009. Acute phase response in animals: A review. *Comp Med* 59:517–526.

de Lange SS, Fuller A, Haw A, Hofmeyr M, Buss P, Miller M, Meyer LCR. 2017. Tremors in white rhinoceroses (*Ceratotherium simum*) during etorphine–azaperone immobilisation. *J S Afr Vet Assoc* 88:a1466.

DiBartola SP. 2012. *Fluid, electrolyte, and acid-base disorders in small animal practice*. 4th Ed. Elsevier Saunders, St. Louis, Missouri, 744 pp.

Dickens MJ, Delehanty DJ, Romero LM. 2010. Stress: An inevitable component of animal translocation. *Biol Conserv* 143:1329–1341.

El-Deeb WM, El-Bahr SM. 2012. Acute-phase proteins and oxidative stress biomarkers in water buffalo calves subjected to transportation stress. *Comp Clin Path* 23:577–582.

Emslie RH. 2011. *Ceratotherium simum* ssp. *simum*. In: *The IUCN red list of threatened species* 2011: e.T39317A10197219. http://dx.doi.org/10.2305/IUCN.UK.2011-2.RLTS.T39317A10197219.en. Accessed June 2019.

Emslie RH. 2012. *Diceros bicornis* ssp. *minor*. In: *The IUCN red list of threatened species* 2012: e.T39321A16981557. http://dx.doi.org/10.2305/IUCN.UK.2012.RLTS.T39321A16981557.en. Accessed June 2019.

Emslie RH, Amin R, Kock R. 2009. *Guidelines for the in situ re-introduction and translocation of African and Asian rhinoceros*. International Union for Conservation of Nature, Gland, Switzerland, 115 pp.

Emslie RH, Milliken T, Talukdar B, Ellis S, Adcock K, Knight MH. 2016. African and Asian rhinoceroses—Status, conservation and trade. *A report from the International Union for Conservation of Nature Species Survival Commission (IUCN SSC) African and Asian Rhino Specialist Groups and TRAFFIC to the CITES Secretariat pursuant to Resolution Conf. 9.14 (Rev. CoP15), No. CoP17 Doc. 68*. Convention on International Trade in Endangered Species of Wild Fauna and Flora Secretariat, Johannesburg, South Africa, 18 pp.

Fazio E, Ferlazzo A. 2003. Evaluation of stress during transport. *Vet Res Commun* 27:519–524.

Feingold K, Grunfeld C. 2010. The acute phase response inhibits reverse cholesterol transport. *J Lipid Res* 51: 743–754.

Fisher AD, Colditz IG, Lee C, Ferguson DM. 2009. The influence of land transport on animal welfare in extensive farming systems. *J Vet Behav Clin Appl Res* 4:157–162.

Fisher AD, Pearce PV, Matthews LR. 1999. The effects of long haul transport on pregnant, non-lactating dairy cows. *N Z Vet J* 47:161–166.

Giannetto C, Fazio F, Casella S, Marafioti S, Giudice E, Piccione G. 2011. Acute phase protein response during road transportation and lairage at a slaugh-

terhouse in feedlot beef cattle. *J Vet Med Sci* 73: 1531–1534.

Gupta AK, Mamta YP, Yadav MP. 1999. Effect of feed deprivation on biochemical indices in equids. *J Equine Sci* 10:33–38.

Harrington LA, Moehrenschlager A, Gelling M, Atkinson RPD, Hughes J, Macdonald DW. 2013. Conflicting and complementary ethics of animal welfare considerations in reintroductions. *Conserv Biol* 27:486–500.

Hattingh J, Knox CM, Raath JP. 1994. Arterial blood pressure and blood gas composition of white rhinoceroses under etorphine anaesthesia. *S Afr J Wildl Res* 24:12–14.

Hooijberg EH, Cray C, Buss P, Steenkamp G, Goddard A, Miller M. 2018. The acute phase response in healthy and injured white rhinoceros. In: *20th European Society of Veterinary Clinical Pathology—European College of Veterinary Clinical Pathology meeting*, Athens, Greece, 17–20 October, p. 92.

Hooijberg EH, Steenkamp G, Buss P, Goddard A. 2017. Method comparison and generation of plasma biochemistry RIs for the white rhinoceros on a point-of-care and wet chemistry analyzer. *Vet Clin Pathol* 46:287–298.

Huang D, Ou B, Hampsch-Woodill M, Flanagan JA, Prior RL. 2002. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J Agric Food Chem* 50:4437–4444.

Knight M. 2017. African Rhino Specialist Group report. *Pachyderm* 58:17–35. (http://www.pachydermjournal.org/index.php/pachy/article/view/488/347. Accessed February 2019.)

Knowles TG, Brown SN, Warriss PD, Phillips AJ, Dolan SK, Hunt P, Ford JE, Edwards JE, Watkins PE. 1995. Effects on sheep of transport by road for up to 24 hours. *Vet Rec* 136:431–438.

Knowles TG, Warriss PD, Brown SN, Edwards JE. 1999. Effects on cattle of transportation by road for up to 31 hours. *Vet Rec* 145:575–582.

Kock MD, du Toit R, Kock N, Morton D, Foggin C, Paul B. 1990a. Effects of capture and translocation on biological parameters in free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe. *J Zoo Wildl Med* 21:414–424.

Kock MD, du Toit R, Morton D, Kock N, Paul B, Url S. 1990b. Baseline biological data collected from chemically immobilized free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe. *J Zoo Wildl Med* 21:283–291.

Kock N, Foggin C, Kock MD, Kock R. 1992. Hemosiderosis in the black rhinoceros (*Diceros bicornis*): A comparison of free-ranging and recently captured with translocated and captive animals. *J Zoo Wildl Med* 23:230–234.

Kock RA, Mihok SR, Wambua J, Mwanzia J, Saigawa K. 1999. Effects of translocation on hematologic parameters of free-ranging black rhinoceros (*Diceros bicornis michaeli*) in Kenya. *J Zoo Wildl Med* 30: 389–396.

Lefebvre HP, Laroute V, Braun JP, Lassourd V, Toutain PL. 1996. Non-invasive and quantitative evaluation of post-injection muscle damage by pharmacokinetic analysis of creatine kinase release. *Vet Res* 27:343–361.

Lykkesfeldt J, Svendsen O. 2007. Oxidants and antioxidants in disease: Oxidative stress in farm animals. *Vet J* 173:502–511.

Mathebula N, Miller M, Buss P, Joubert J, Martin L, Kruger M, Hofmeyr M, Olea-Popelka F. 2012. Biochemical values in free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa. *J Zoo Wildl Med* 43:530–538.

Miller M, Kruger MM, Kruger MM, Olea-Popelka F, Buss P. 2016. A scoring system to improve decision making and outcomes in the adaptation of recently captured white rhinoceroses (*Ceratotherium simum*) to captivity. *J Wildl Dis* 52:S78–S85.

Minka NS, Ayo JO. 2009. Physiological responses of food animals to road transportation stress. *Afr J Biotechnol* 8:7415–7427.

Montané J, Marco I, López-Olvera J, Manteca X, Lavín S. 2002. Transport stress in roe deer (*Capreolus capreolus*): Effect of a short-acting antipsychotic. *Anim Welf* 11:405–417.

Morkel P, Kennedy-Benson A. 2007. Translocating black rhino: Current techniques for capture, transport, boma care, release and post-release monitoring. In: *Report to African Rhino Specialist Group*. International Union for Conservation of Nature, Species Survival Commission, African Rhino Specialist Group, Tsavo, Kenya, pp. 1–85. (https://rhinos.org/research-publications/iucn-african-rhino-specialist-group/. Accessed February 2019.)

Mukinya JG. 1977. Feeding and drinking habits of the black rhinoceros in Masai Mara Game Reserve. *E Afr Wildl J* 15:125–138.

Muñoz A, Riber C, Trigo P, Castejón F. 2010. Hematology and clinical pathology data in chronically starved horses. *J Equine Vet Sci* 30:581–589.

Murata H. 2007. Stress and acute phase protein response: An inconspicuous but essential linkage. *Vet J* 173: 473–474.

Nduhirabandi F, Du Toit EF, Blackhurst D, Marais D, Lochner A. 2011. Chronic melatonin consumption prevents obesity-related metabolic abnormalities and protects the heart against myocardial ischemia and reperfusion injury in a prediabetic model of diet-induced obesity. *J Pineal Res* 50:171–182.

Nielsen BL, Dybkjær L, Herskin MS. 2011. Road transport of farm animals: Effects of journey duration on animal welfare. *Animal* 5:415–427.

Padalino B, Raidal SL, Carter N, Celi P, Muscatello G, Jeffcott L, de Silva K. 2017. Immunological, clinical, haematological and oxidative responses to long distance transportation in horses. *Res Vet Sci* 115: 78–87.

Perrin KL, Kristensen AT, Bertelsen MF, Gray C, Kjelgaard-Hansen M. 2018. How insensitive are population-based reference intervals for monitoring hematologic changes in Asian elephants (*Elephas maximus*). In: *Joint European Association of Zoo and Wildlife Veterinarians/American Association of Zoo Veterinarians/Leibniz-Institut für Zoo und Wildtierforschung Conference*, Prague, Czech Republic, p. 113.

Petersen HH, Nielsen JP, Heegaard PMH. 2004. Application of acute phase protein measurements in veterinary clinical chemistry. *Vet Res* 35:163–187.

Phypers B, Pierce JMT. 2006. Lactate physiology in health and disease. *Continuing Education Anaesthesia, Critical Care Pain* 6:128–132.

Saeb M, Baghshani H, Nazifi S, Saeb S. 2010. Physiological response of dromedary camels to road transportation in relation to circulating levels of cortisol, thyroid hormones and some serum biochemical parameters. *Trop Anim Health Prod* 42:55–63.

Schook MW, Wildt DE, Raghanti MA, Wolfe BA, Dennis PM. 2015. Increased inflammation and decreased insulin sensitivity indicate metabolic disturbances in zoo-managed compared to free-ranging black rhinoceros (*Diceros bicornis*). *Gen Comp Endocrinol* 217–218:10–19.

Spraker TR. 1993. Stress and capture myopathy in artiodactylids. In: *Zoo and wild animal medicine: Current therapy*, 3rd Ed., Fowler ME, editor. W. B. Saunders Co., Philadelphia, Pennsylvania, pp. 481–488.

Swaisgood RR. 2010. The conservation-welfare nexus in reintroduction programs: A role for sensory ecology. *Anim Welfare* 19:1–48.

Teixeira CP, De Azevedo CS, Mendl M, Cipreste CF, Young RJ. 2007. Revisiting translocation and reintroduction programmes: The importance of considering stress. *Anim Behav* 73:1–13.

Warriss PD, Brown SN, Knowles TG, Kestin SC, Edwards JE, Dolan SK, Phillips AJ. 1995. Effects on cattle of transport by road for up to 15 hours. *Vet Rec* 136:319–323.

Wernicki A, Urban-Chmiel R, Kankofer M, Mikucki P, Puchalski A, Tokarzewski S. 2006. Evaluation of plasma cortisol and TBARS levels in calves after short-term transportation. *Rev Med Vet* 157:30–34.

Zapata B, Gimpel J, Bonacic C, González BA, Riveros JL, Ramírez AM, Bas F, Macdonald DW. 2004. The effect of transport on cortisol, glucose, heart rate, leukocytes and body weight in captive-reared guanacos (*Lama guanicoe*). *Anim Welfare* 13:439–444.

Submitted for publication 18 February 2019.
Accepted 11 July 2019.