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EFFECTS OF CAPTURE AND TRANSPORT ON BLOOD PARAMETERS IN FREE-RANGING MOUFLON (*OVIS AMMON*)

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Abstract: Twelve free-ranging mouflon (*Ovis ammon*), eight adults and four lambs, were captured in northeast Spain. The animals were transported for 9 hr from the capture site to the Wildlife Rescue Center at Vallcalent (Lleida), 300 km SW. Blood samples were taken at capture and after transport to study the effects on hematologic and biochemical parameters. The RBC, PCV, Hb, and alkaline phosphatase values were significantly higher in lambs than in adults, and total protein levels were significantly lower in lambs. Comparisons of blood parameters of adult animals at capture and after transport revealed significantly lower RBC, PCV, Hb, PLT, lymphocyte counts, glucose, cholesterol, creatinine, and alkaline phosphatase values and significantly higher neutrophil count, AST, ALT, CK, LDH, and total bilirubin levels after transport. The differences observed in the blood parameters of lambs before and after transport were not statistically significant.

Key words: Mouflon, *Ovis ammon*, capture, transport, stress, blood parameters.

INTRODUCTION

Capture and handling are some of the most stressful events that can happen to wild ungulates and are sometimes associated with considerable mortality.[27] It is necessary to understand physiological responses to capture and handling to reduce the frequency and severity of those adverse effects.

Blood parameters can be measured to assess animal homeostasis. In mouflon (*Ovis ammon*), there is little information available.[10,11] In closely related species such as bighorn sheep (*Ovis canadensis*) and other wild ungulates, there are more reports on the effects of capture and handling on these physiologic parameters.[1,5,8,9,13,15–18,22,25,29] However, in the few reports that evaluate the effects of capture and transport, chemical immobilization has been used.[5,19,24]

This paper evaluates the effects of physical capture and transport on blood parameters in 12 free-ranging mouflon.

MATERIALS AND METHODS

Blood was obtained from three female and one male 2-mo-old juveniles and seven female and one male 2–7-yr-old adult mouflon captured at the National Hunting Reserve of Freser-Setcases (NE Spain) in the spring of 1993. The population of mouflon in this area is about 250 but varies through the year, since individuals move to the northern slopes of the mountains in France during the summer.

An enclosure-trap of 15 × 15 m, baited with salt, was used for capture. Once the animals were inside, the door was closed remotely. Animals were then captured with a hand-net, physically restrained, and blindfolded, and 10-ml blood samples were obtained from the jugular vein.

The animals were transported in individual wooden boxes measuring 125 × 100 × 45 cm, with 5-cm diameter holes for ventilation, to the Wildlife Rescue Center at Vallcalent (Lleida), 300 km SW of the capture site. Transport lasted for 9 hr (from 0900 until 1800 hours). Temperature during transport was 27°C. Blood samples (10 ml) were again taken from the jugular vein, using manual restraint of the animals, after arrival at the Center.

All blood samples were obtained using disposable syringes and 20-Ga needles. A 2.5-ml portion of each blood sample was placed in a commercial tube with anticoagulant (ethylenediamine tetraacetic acid K_3). The remainder was placed in a tube without additive, left to clot at room temperature for 1 hr, and then centrifugated at 3,000 rpm for 10 min. Serum was removed and kept at 4°C until arrival at the laboratory.

Red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), and platelet count (PLT) were calculated with a semiautomatic hematology analyzer (Sysmex F-800, Toa Medical Electronics, Hamburg, Germany) adapted to work with animal blood by adjusting the two mobile volume discriminators, one low and one high. Packed cell volume was measured by the standard microhematocrit method with a hematocrit centrifuge (Micro-Haematocrit Centrifuge, Hawksley, Lancing, U.K.) at 11,000 rpm for 5 min to adjust the values obtained with the analyzer. Dif-

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Table 1. Hematology values of mouflon (*Ovis ammon*) at capture and after transport (mean ± SD).

Parameters	Lambs (n = 4)		Adults (n = 8)	
	At capture	After transport	At capture	After transport
Red blood cells ($\times 10^{12}$/L)	13.71 ± 0.46[a]	12.50 ± 0.43[a]	11.54 ± 1.08[b]	11.02 ± 1.04[c]
Packed cell volume (%)	42.25 ± 3.34[a]	38.13 ± 3.09[a]	35.19 ± 4.41[b]	33.31 ± 3.37[c]
Hemoglobin (g/dl)	15.35 ± 0.97[a]	14.25 ± 1.09[a]	13.39 ± 1.25[b]	12.75 ± 1.09[c]
MCV (fL)	30.80 ± 2.03	30.49 ± 2.17	30.47 ± 2.24	30.28 ± 1.73
MCH (pg)	11.20 ± 0.59	11.43 ± 0.81	11.63 ± 0.49	11.61 ± 0.48
MCHC (g/dl)	36.40 ± 1.12	37.40 ± 0.49	38.22 ± 1.69	38.34 ± 0.97
White blood cells ($\times 10^9$/L)	9.60 ± 2.15	10.50 ± 2.85	9.75 ± 4.25	8.79 ± 2.42
Differential leukocyte count				
Lymphocytes ($\times 10^9$/L)	7.24 ± 1.83[a,b]	4.19 ± 0.91[a,b]	6.42 ± 3.08[a]	3.32 ± 0.76[b]
Monocytes ($\times 10^9$/L)	0.17 ± 0.09	0.04 ± 0.04	0.38 ± 0.33	0.11 ± 0.11
Neutrophils ($\times 10^9$/L)	1.95 ± 0.39[a,b]	6.19 ± 2.17[a,b]	2.59 ± 1.47[a]	5.14 ± 1.62[b]
Neutrophil band ($\times 10^9$/L)	0.07 ± 0.07	0.09 ± 0.15	0.14 ± 0.24	0.22 ± 0.18
Eosinophils ($\times 10^9$/L)	0.16 ± 0.15	0 ± 0	0.22 ± 0.45	0 ± 0
Basophils ($\times 10^9$/L)	0.02 ± 0.03	0 ± 0	0 ± 0	0 ± 0
Platelet count ($\times 10^9$/L)	546.00 ± 108.60[a,b]	484.00 ± 73.19[a,b]	358.00 ± 123.53[a]	314.00 ± 115.62[b]

[a,b,c] Means with different superscripts are significantly different from each other ($P < 0.05$); means without superscripts are not significantly different from each other.

ferential leukocyte count was performed with blood smears stained with commercial Diff-Quick stain (Química Aplicada, S.A., Amposta, Spain). Cells were observed under ×1000 magnification, and a total of 100 leukocytes were classified.

Biochemical parameters were analyzed using a Kovas Bio autoanalyzer (Roche, Nutley, NJ 08876, USA). These included glucose, lactate, conjugated bilirubin, total bilirubin, blood urea nitrogen (BUN), creatinine, cholesterol, total lipid, phospholipids, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CK), lactic dehydrogenase (LDH), γ-glutamyltransferase (GGT), and alkaline phosphatase (AP). Total serum protein concentration (TP) was determined by the Biuret method, using a 4010 photometer (Boehringer Manheim, Hamburg, Germany).

Mean blood parameters between young and adult groups at capture and after transport were compared by nonparametric tests using a statistics software package (SPSS-PC, SPSS Inc., Chicago, Illinois, USA). A Mann-Whitney U test was used for the independent groups (lambs and adults), while a Wilcoxon test was used for the captured and transported groups.

RESULTS

Tables 1 and 2 show the hematologic and biochemical values for the young and adult groups of mouflon at capture and after transport. Values for RBC, PCV, Hb, and AP were significantly higher in the young group, while TP was significantly lower ($P < 0.05$) in young animals both before and after transport.

The differences observed between capture and transport were significant only in the adult group ($P < 0.05$). Values for RBC, PCV, Hb, lymphocyte, and PLT were significantly lower after transport than at capture, while the mature neutrophil count was significantly higher.

Values for ALT, AST, CK, LDH, and total bilirubin were significantly higher after transport in the adult group, while glucose, cholesterol, creatinine, and AP were lower ($P < 0.05$) after transport.

DISCUSSION

The hematologic results of our study concur with other reports on mouflon.[10,11] The PCV, however, tended to be higher in the previous reports. Hematologic results from our study are also similar to those reported for domestic sheep,[3] as well as bighorn sheep[22] and Dall's sheep.[4] Nevertheless, PCV and mature neutrophil counts were higher in these other species, while the lymphocyte count and MCHC were lower than in our mouflon.

No other reports of mouflon blood chemistry values have been found. Although our results were similar to those reported in domestic sheep, several differences are probably due to capture stress, including the higher initial levels of glucose, lactate, LDH, and CK in our mouflon. However, BUN, GGT, and AP were higher and ALT was lower than in domestic sheep.[3] Values for bighorn sheep and Dall's sheep showed differences in BUN and cho-

Table 2. Blood chemistry values of mouflon (*Ovis ammon*) at capture and after transport (mean ± SD).

Parameters	Lambs (n = 4)		Adults (n = 8)	
	At capture	After transport	At capture	After transport
Glucose (mg/dl)	160.43 ± 64.08[a,b]	77.28 ± 6.60[a,b]	191.18 ± 69.97[a]	88.66 ± 19.88[b]
Lactate (mg/dl)	49.19 ± 28.74	37.12 ± 6.94	55.77 ± 31.44	43.60 ± 9.10
Conjugated bilirubin (mg/dl)	0 ± 0	0.045 ± 0.03	0 ± 0	0.08 ± 0.08
Total bilirubin (mg/dl)	0.27 ± 0.19[a,b]	0.65 ± 0.47[a,b]	0.22 ± 0.08[a]	0.67 ± 0.28[b]
BUN (mg/dl)	54.23 ± 8.32	70.45 ± 12.16	87.57 ± 35.34	92.73 ± 44.60
Creatinine (mg/dl)	1.38 ± 0.15[a,b]	1.00 ± 0.10[a,b]	1.62 ± 0.24[a]	1.39 ± 0.24[b]
Cholesterol (mg/dl)	126.39 ± 18.66[a,b]	122.94 ± 17.60[a,b]	108.80 ± 11.81[a]	94.15 ± 10.56[b]
Total lipid (mg/dl)	552.2 ± 184.28	418.25 ± 133.71	612.80 ± 153.96	485.33 ± 101.86
Phospholipids (mg/dl)	71.25 ± 40.80	82.87 ± 5.73	95.44 ± 18.187	82.15 ± 19.35
Triglycerides (mg/dl)	44.90 ± 10.11	24.71 ± 5.86	45.93 ± 27.89	25.70 ± 10.90
TP (g/dl)	4.95 ± 0.38[a]	5.45 ± 0.50[a]	6.40 ± 0.26[b]	5.94 ± 0.90[b]
ALT (U/L)	17.80 ± 4.83[a,b]	21.12 ± 7.30[a,b]	19.19 ± 7.17[a]	32.52 ± 10.41[b]
AST (U/L)	210.19 ± 21.84[a,b]	660.15 ± 615.93[a,b]	264.38 ± 66.40[a]	591.04 ± 253.62[b]
LDH (U/L)	1845.91 ± 391.82[a,b]	2544.04 ± 436.62[a,b]	1649.42 ± 418.34[a]	3226.59 ± 1804.29[b]
CK (U/L)	771.77 ± 650.49[a,b]	2598.52 ± 3785.62[a,b]	433.58 ± 274.04[a]	3105.04 ± 2512.10[b]
GGT (U/L)	73.25 ± 11.42	86.77 ± 12.51	78.52 ± 34.56	66.49 ± 18.81
AP (U/L)	1078.31 ± 155.29[a]	983.69 ± 90.89[a]	602.83 ± 273.77[b]	432.02 ± 203.97[c]

[a,b,c] Means with different superscripts are significantly different from each other ($P < 0.05$); means without superscripts are not significantly different from each other.

lesterol, with higher and lower levels in mouflon, respectively.[4,16]

In the present study, RBC, PCV, and Hb were significantly higher in lambs than in adults. Similar results have been obtained in other wild[23,25] and domestic ungulates.[12] In mouflon, however, these parameters have been found to be lower in young animals aged from 3 days to 3 months than in adults.[11]

Higher levels of TP in adults have been described in many species. There is a general increase in TP with age, although in very old animals, it decreases again.[14] Alkaline phosphatase is generally higher in young animals, mainly because of bone growth. However, in ruminants, a wide range of normal values may complicate the interpretation of AP levels.[14]

Stress was defined as a nonspecific reaction, with three phases: alarm, resistance, and exhaustion.[26] It consists of interacting behavioral, autonomic, and neuroendocrine components. However, recent research shows that stress responses are extremely complex, depending on the species, the individual, and the precipitating factors involved, so they are accepted as specific reactions.[7] Blood parameters (hematologic and biochemical), together with other physiological parameters, are sensitive indicators of alterations in animal homeostasis during capture and stress episodes in wild ungulates.[16]

Initially, stress activates the sympathetic nervous system of wild ungulates and domestic animals, stimulating the adrenal medulla, with release of catecholamines. These hormones cause splenic contraction, releasing erythrocytes to the peripheral circulation. This permits more uptake of oxygen, mainly for muscle use, and permits a quick fight-or-flight response.[1,2,8,12,25]

In our study, the higher values of RBC, PCV, and Hb at capture in adult animals may be due to catecholamine action and may reflect a high degree of excitement in this critical phase.

Total and differential leukocyte count also respond to a variety of stimuli, including capture and transport. Catecholamines released during the alarm phase may be responsible for the initial neutrophilia and lymphocytosis. Corticosteroids released during the resistance phase contribute further to neutrophilia, but they may cause a decrease in the lymphocyte count. In domestic animals, the neutrophilia and lymphopenia peaks appear after 4–8 hr of exposure to stress.[3,12]

Splenic contraction may affect the platelet count in the same way as erythrocyte count, as the spleen is also a platelet reservoir. Thus, splenic contraction following catecholamine release at capture could produce the high RBC levels seen at capture.[2,3,12]

In many wild ungulates, hyperglycemia due to catecholamine and glucocoricoid action following excitement in capture operations has been reported.[5,8,9,16,17,20,25,29]

Cholesterol level is influenced mainly by diet. However, some authors have associated an increase in cholesterol values with the alarm phase in short-term stress episodes in bighorn sheep.[5] In domestic animals, decreased cholesterol values after transport have been reported, as part of the cholesterol is diverted to corticosteroid synthesis.[21]

Creatinine level can be used to assess renal function. However, in some ungulates, an increased creatinine concentration due to muscular activity and a decrease in renal excretion because of vasospasm in the kidney produced by catecholamines after capture has been described.[6] In horses with myoglobinuria, there is an increase in the creatinine concentration. The same feature has been described after prolonged and intense exercise in humans, due to an increase in the production of creatinine in muscle with normal kidney excretion.[14]

The higher total bilirubin levels after transport might be related to increased hemoglobin from hemolysis after capture, but we could not confirm this. In one case of capture myopathy in an Arabian oryx (*Oryx leucorynx*),[28] the total bilirubin value was very high because of an increase in free bilirubin.

Muscle enzyme levels accurately reflect alterations due to capture and handling of wild ungulates. However, hemolysis can also increase these levels. After vigorous exercise, high blood levels can result from increased muscle cell permeability. In cases of muscular damage, blood levels can increase substantially.[3]

Elevated levels of CK, AST, and LDH have been described in many stressed wild ungulates.[1,16,19,20,24,25] However, some authors find that CK and AST levels are the most sensitive indicators of muscular disorders.[1,3] In some stress-related events such as capture myopathy, CK activity can increase dramatically. In one case of capture myopathy in an Arabian oryx, CK activities of 1,461,000 U/L were observed.[28] In our study, values higher than 9,000 U/L in one animal occurred after transport. The same animal also presented the highest levels of the other enzymes that can be involved in muscular damage: LDH, AST, and ALT. In ruminants, ALT has poor diagnostic value, as it is also found in small concentrations in the liver.[3,28]

In ruminants, AP can be found mainly in the liver, bone, gut, kidney, placenta, and leukocytes, and its range is wider than in other species, so it has poor diagnostic value.[3]

The results of this study indicate variation in blood composition of mouflon with age and confirm previous reports of changes in hematological and serum biochemical values with capture and transport of animals. The variations of blood parameters in the young group of mouflon were not significant, perhaps because of the small size of the group. Few data on blood parameters of mouflon are available in the literature, and further studies are needed to provide more accurate reference values for this species.

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