

HEMATOLOGIC AND BIOCHEMICAL VALUES FOR SPANISH IBEX (*CAPRA PYRENAICA*) CAPTURED VIA DRIVE-NET AND BOX-TRAP

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ABSTRACT: Between October 2002 and September 2004, 70 free-ranging Spanish ibex (*Capra pyrenaica*) were captured in Catalonia, northeastern Spain, using two different physical methods, drive-net ($n=26$) and box-trap ($n=44$). Blood samples were taken to determine 20 hematologic and 23 biochemical variables. Values obtained fell within already published reference intervals, with the following exceptions: higher values for red blood cells (RBC), packed cell volume (PCV), white blood cells (WBC), eosinophil count, triglyceride concentration, creatine kinase (CK; in box-trap), chloride, sodium, α -1, α -2, and gamma electrophoretic fractions of serum proteins; and lower values for hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), urea concentration, CK (in drive-net), albumin, and albumin:globulins ratio (A:G). Published values for aspartate aminotransferase (AST) concentration are both higher and lower than observed in this study. In our study, monocyte and eosinophil counts, as well as triglyceride and potassium concentrations, were lower in animals captured via box-trap than those captured via drive-net. Conversely, MCHC, neutrophil count, total bilirubin concentration, urea, and AST were higher in animals captured via box-trap. Hematologic and biochemical values obtained from Spanish ibexes show that the drive-net is a newer, less-stressful method of capture than the box-trap.

Key words: Biochemical values, box-trap, *Capra pyrenaica*, drive-net, hematologic values, Spanish ibex.

INTRODUCTION

Reference intervals for hematologic and biochemical parameters are necessary for assessment of the physiologic status of captured wild animals, as well as for assessment of their health and nutritional status (DelGiudice et al., 1992; Montané et al., 2002; López-Olvera et al., 2006). However, determining normal physiologic data in wild animals is made more difficult by the confounding effect of capture and handling stress (Gibert, 1993). Therefore, the effect of capture method and handling on the physiologic values of wild animals requires study, and separate reference intervals need to be established for each method (Kock et al., 1987b; Peinado et al., 1993; Marco et al., 1997; López-Olvera et al., 2006).

The Spanish ibex (*Capra pyrenaica*) is a medium-sized mountain ungulate, endemic to the Iberian Peninsula, that shows marked sexual dimorphism and is catalogued as a rare species (Fandos, 1991; Blanco and González, 1992). The entire

Spanish ibex population has been estimated at nearly 50,000 individuals (Escós and Alados, 1997; Granados et al., 2002).

Spanish ibex have been captured via corral-trap (Fernández-Arias et al., 1993; Pérez et al., 1999, 2003), box-trap, and chemical immobilization (Peinado et al., 1993). Drive-nets have been used successfully to capture other species of wild ungulates (Jones, 1984; Montané et al., 2003; López-Olvera et al., 2006), but to our knowledge have not been used for Spanish ibex. Animal safety is important when assessing a new method of capture. Capture stress and capture myopathy can be serious, capture-related risks that can be assessed through physiologic (clinical, hematologic, and biochemical) parameters (Williams and Thorne, 1996).

Hematologic and biochemical data for Spanish ibex, sampled under either physical or chemical restraint, have been described previously by other authors. However, some studies have used samples from dead animals (Pérez et al., 2006)

while others have collated data from physically captured and anaesthetized ibexes (Pérez et al., 1999). Other studies have provided reference intervals for Spanish ibexes captured via corral-traps (Pérez et al., 2003) or box-traps (Peinado et al., 1993), although in the latter study, the sample size ($n=14$ animals) was rather small. To our knowledge, this is the first time blood values have been determined for Spanish ibexes captured via drive-nets. The goals of the present study are to assess drive-net capture of Spanish ibex and to compare hematologic and biochemical parameters obtained from those animals with values obtained from animals captured with box-traps.

MATERIALS AND METHODS

Seventy free-ranging Spanish ibex (41 adult [≥ 2 -yr-old] males and 29 adult females) were captured in the National Game Reserve of Ports de Tortosa i Beseit ($40^{\circ}50'N$, $0^{\circ}30'E$), in Catalonia in north-eastern Spain. Twenty-six Spanish ibex—10 males (mean body weight 40.3 kg), and 16 females (mean body weight 31.3 kg)—were captured with 10×10 -cm mesh drive-nets (Ziboni Ornitecnica, Bergamo, Italy). Twelve operations, in 18 days, were carried out between October 2002 and September 2004; 10 ibexes were captured in spring and 16 in the fall. A line of beaters (mean of six in each battue) drove animals towards the net, where a group of researchers and volunteers lay concealed in order to assist animals as soon as they became entangled. A mean of 36 workers were needed for each capture. Forty-four Spanish ibexes—(31 males [mean body weight 49.4 kg] and 13 females [mean body weight 28.1 kg])—were caught in box-traps; traps were baited with salt and vegetables during a 96 day period. On entering a trap, the animal triggered an internal mechanism whereby two sliding doors closed the cage. Box-traps were visited daily by rangers, and the research team called in when an animal was discovered. A mean of 7.5 workers were needed for these captures. This latter method of capture was used concurrently for the same period of study as the drive-net; 25 ibex were caught in spring and 19 in the fall.

Once an ibex was caught in either the drive-net or box-trap, it was manually restrained. Each animal was then blindfolded, had their

legs restrained, was placed in a 4×4 -cm mesh transport sack net (Ziboni Ornitecnica, Bergamo, Italy), and weighed.

Blood samples were collected from the jugular vein with disposable 10 ml syringes fitted with 21 gauge 1" needles. For hematologic analyses, 2 ml of each sample were placed in a commercially available tube containing tripotassium ethylenediaminetetraacetic acid (EDTA K_3) as an anticoagulant. The remainder was placed in a serum collection tube with polystyrene granules and allowed to clot in a portable icebox. Once the clot had formed, samples were centrifuged at $1200 \times G$ for 15 min, and serum samples obtained were frozen at $-20^{\circ}C$ <12 hr after collection for later analysis. Only samples not showing macroscopic signs of hemolysis were used for biochemical analyses.

Red blood cell (RBC) count, white blood cell (WBC) count, platelet count, and hemoglobin concentration were determined with an electronic impedance semi-automated analyzer (Sysmex F-800; Toa Medical Electronics, Hamburg, Germany). Packed cell volume (PCV) was measured using a standard micro-hematocrit method with a centrifuge (Haematospin 1400, Hawksley, Sussex, UK) at $14,000 \times G$ for 6 min. Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) were calculated from RBC, hemoglobin concentration, and hematocrit. A differential leukocyte count was performed by identifying 200 leukocytes on blood smears stained with a commercially available Diff-Quick-like stain (Química Clínica Aplicada, Tarragona, Spain). Biochemical variables were determined with two automatic analyzers (Cobas Mira; Roche, Rotkreuz, Switzerland, and Olympus AU400; Olympus, Mainz, Germany), except for sodium and potassium, which were measured by flame photometry (Corning 410C; Corning Medical, Medfield, Corning, USA), and for cortisol, which was analyzed with a commercially available enzyme-linked immunosorbent assay kit (EIA-1887; DRG Instruments, Marburg/Lahn, Germany). Protein electrophoretic fractions were determined by the method described by Lastras et al. (2000), and the fractions analyzed were those described for the Spanish ibex by Peinado et al. (1993) and Cuenca et al. (1996).

Distribution of measurements obtained for each variable was assessed for normality. Non-normal variables were log-transformed and normality reassessed. Outliers were deleted when justified by experimental data and when indicated by statistical study. Using the PROC

GLM procedure of the SAS System for Windows V8 (SAS Institute, Cary, North Carolina, USA), a multivariate analysis of variance was performed on the normal data in order to detect statistical differences between groups. A nonparametric analysis of variance (ANOVA) was performed on abnormal data using the PROC NPAR1WAY ANOVA procedure in the same statistical software. For each variable, factors considered in the model were method of capture, sex and season, and interactions between these factors. In view of the unbalanced distribution of animals, group least square means were used. The minimum accepted significance level was $P < 0.05$.

RESULTS

Tables 1 and 2 show the hematologic and biochemical parameters for the 70 Spanish ibexes captured via drive-net and box-trap; the recommended central 95% interval is provided (Lumsden, 1998; Walton, 2001), as well as sample size, mean, standard deviation, coefficient of variation, and range. Animals captured via drive-net showed lower MCHC, neutrophil count, and concentration of total serum bilirubin, urea, and aspartate aminotransferase (AST) than those captured via box-trap; conversely, they had higher monocyte and eosinophil counts and higher triglyceride and potassium concentrations.

DISCUSSION

No captured-related mortality was recorded for ibex caught either by drive-net or box-trap; therefore, these methods of capture seem to be safe for this species. Drive-net showed a better performance than box-trap, because preparation and execution of drive-net capture needed fewer days than box-trap. Nevertheless, more workers are needed to capture Spanish ibex with drive-net.

Hematologic and biochemical values for ibexes captured via drive-net differed on several counts from those previously reported for this species when captured with other methods: namely, higher concentrations of RBC, PCV, WBC, eosino-

phils, triglyceride concentration, chloride, sodium, α -1, α -2, and gamma globulin fractions; and lower values for MCV, MCHC, hemoglobin concentration, urea, CK, albumin, and A:G ratio (Peinado et al., 1993; Pérez et al., 1999, 2006). Aspartate aminotransferase concentration was found to be higher or lower according to different authors (Peinado et al., 1993; Pérez et al., 2006).

Values obtained from box-trap capture showed several differences in comparison with those already reported for this species using the same method of capture: namely, higher values for RBC, PCV, WBC, cholesterol concentration, AST, CK, α -1 and α -2 electrophoretic fractions; and lower values for creatinine concentration and A:G ratio (Peinado et al., 1993). However, Peinado et al. used only a few animals, and no information was given concerning sex or time of retention in the box after capture. In our study, ibexes may have spent between 10 hr to 20 hr in the trap, possibly leading to higher stress levels when compared with previous studies.

Values for Spanish ibex, when compared with other wild ungulates such as Southern chamois (*Rupicapra pyrenaica*) captured via drive-net, were different in several cases: namely, higher values for RBC, WBC, lymphocytes, monocytes, neutrophils, eosinophils, glucose concentration, creatinine, and total proteins and their fractions; and lower values for PCV, hemoglobin, MCV, MCH, platelets, cortisol concentration, cholesterol, triglycerides, lactate, urea, CK, LDH, AST, ALT, AP, sodium, and A:G ratio (López-Olvera et al., 2006).

Catecholamines, released by the adrenal medulla due to sympathetic stimulation, cause smooth muscle contraction in the spleen capsule and release erythrocytes from the spleen into the bloodstream (Jain, 1993). This release causes an increase in RBC, hemoglobin concentration, and PCV (Jain, 1993; Ganong, 2004). Moreover, spleen erythrocytes have a

TABLE 1. Hematologic values for Spanish ibex (*Capra pyrenaica*) captured via drive-net and box-trap.

Variable ^a	Drive-net					Box-trap				
	<i>n</i>	Mean±SD	CV (%)	Central 95 % interval	Range	<i>n</i>	Mean±SD	CV (%)	Central 95 % interval	Range
RBC (×10 ¹² /l)	25	17.88±3.46	19.33	11.78–22.98	11.15–24.02	44	16.44±3.51	21.33	9.57–20.45	7.57–24.70
PCV (l/l)	26	0.40±0.08	19.58	0.27–0.49	0.25–0.56	44	0.39±0.09	22.23	0.23–0.48	0.22–0.55
Hemoglobin (g/l)	25	140.00±21.24	15.17	99.2–167.6	94–183	43	139.42±25.66	18.40	92.4–173.6	82–176
MCV (fl)	26	23.03±3.42	14.84	19.22–27.59	14.17–32.18	42	23.25±2.62	11.27	19.67–28.12	17.71–29.28
MCH (pg)	26	8.02±0.93	11.62	6.85–9.58	6.34–10.20	42	8.3±0.8	9.67	7.3–9.7	7.1–10.4
MCHC (g/dl) ^b	25	34.52±1.63	4.74	31.62–36.88	31.43–37.37	44	35.76±2.10	5.86	32.68–39.11	32.1–42.31
Platelets (×10 ⁹ /l)	26	229.00±164.77	71.95	67.25–533.50	36–656	44	165.14±121.01	73.28	35.45–347.45	11–676
WBC (×10 ⁹ /l)	26	14.82±5.04	34.04	7.18–21.98	5.8–25.0	44	14.61±4.70	32.15	8.70–21.17	5.0–27.8
Lymphocytes (×10 ⁹ /l)	26	9.69±4.19	43.29	4.60–14.89	3.28–18.70	44	8.37±3.52	42.01	3.68–12.90	2.34–20.16
Monocytes (×10 ⁹ /l) ^b	26	0.36±0.24	65.44	0.04–0.80	0.03–0.85	44	0.25±0.18	69.21	0.01–0.55	0.00–0.77
Neutrophils (×10 ⁹ /l) ^b	26	4.20±2.44	58.25	1.46–8.57	1.01–9.13	44	5.74±2.43	42.40	3.00–9.83	2.28–14.49
Band neutrophils (×10 ⁹ /l)	26	0.00±0.02	372.12	0.00–0.03	0.0–0.7	44	0.00±0.02	535.95	0–0	0.0–0.1
Eosinophils (×10 ⁹ /l) ^b	26	0.57±0.37	64.60	0.18–1.31	0.15–1.37	44	0.23±0.27	117.29	0.0–0.9	0.00–1.05
Basophils (×10 ⁹ /l)	26	0.00±0.01	509.90	0.00–0.00	0.00–0.05	44	0.00±0.01	663.32	0–0	0.00–0.06

^a RBC = red blood cells; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; WBC = white blood cells.

^b Means observed between drive-net and box-trap captured animals are statistically ($P<0.05$) different.

TABLE 2. Biochemical values for Spanish ibex (*Capra pyrenaica*) captured via drive-net and box-trap.

Variable ^a	Drive-net				Box-trap					
	n	Mean±SD	CV (%)	Central 95 % interval	Range	n	Mean±SD	CV (%)	Central 95 % interval	Range
Cortisol (nmol/l)	26	251.36±123.55	49.15	138.71–520.64	115.14–651.92	43	227.73±136.32	59.86	93.27–526.85	79.57–723.05
Glucose (mmol/l)	26	9.78±4.17	42.62	5.87–14.13	2.39–25.26	44	10.92±3.96	36.28	4.70–16.27	2.10–17.81
Cholesterol (mmol/l)	26	1.36±0.46	34.14	0.85–1.79	0.78–3.14	44	1.21±0.31	25.57	0.8–1.8	0.70–2.03
Triglycerides (mmol/l) ^b	26	0.71±0.25	35.41	0.39–1.15	0.29–1.20	44	0.43±0.27	62.79	0.09–0.93	0.07–1.15
Total bilirubin (μmol/l) ^b	26	3.35±1.28	38.07	1.45–5.39	0.17–5.64	40	4.46±2.08	46.68	1.37–8.05	1.03–8.72
Lactate (mmol/l)	26	15.42±7.88	51.09	5.78–26.31	5.42–34.07	44	12.35±8.91	72.19	2.58–29.21	2.18–35.13
Creatinine (μmol/l)	26	125.70±20.16	16.04	104.09–165.75	96.36–167.96	44	119.06±27.28	22.91	78.32–150.28	72.49–194.48
Urea (mmol/l) ^b	26	3.16±2.17	68.44	0.75–5.86	0.53–8.24	44	7.03±5.11	72.64	1.88–16.35	1.09–25.94
CK (IU/l)	26	160.69±55.09	34.28	101.5–250.25	93–353	40	278.53±265.47	95.31	101.50–797.05	63–1318
LDH (IU/l)	26	627.73±113.08	18.01	476.75–815.75	452–999	40	625.39±157.98	25.26	460.15–923.30	433.0–1029.7
AST (IU/l) ^b	26	62.88±18.53	29.47	40.50–87.75	25–110	40	75.75±39.31	51.89	43.95–145.50	43–220
ALT (IU/l)	26	13.19±6.29	47.65	2.75–22.00	2–23	42	15.62±6.83	43.72	6.05–24.00	3–35
AP (IU/l)	26	192.38±115.86	60.22	75.75–354.50	72–597	44	199.77±103.10	51.61	55.6–365.7	48–442
Chloride (mmol/l)	26	114.73±4.08	3.55	109.5–122.2	109.4–123.7	44	112.77±4.82	4.28	104.63–120.51	101.1–121.1
Sodium (mmol/l)	24	154.54±8.53	5.52	139.45–169.40	138–173	43	155.15±7.44	4.80	145.10–167.99	133–172
Potassium (mmol/l) ^b	26	5.40±1.39	25.71	4.05–7.80	3.7–9.1	44	4.65±1.26	27.16	2.85–6.73	2.7–8.1
Total protein (g/l)	26	86.44±7.73	8.94	76.50–100.03	73.0–110.2	44	86.97±10.19	11.72	70.6–96.0	48.9–110.7
Albumin (g/l)	26	39.91±5.37	13.45	30.08–46.98	26.35–47.71	44	39.99±6.54	16.36	29.23–46.09	9.89–47.76
α-1-globulin (g/l)	26	4.69±0.68	14.48	3.85–5.84	3.40–5.98	44	4.94±0.64	12.94	3.95–6.26	3.28–6.57
α-2-globulin (g/l)	26	8.16±0.72	8.79	6.97–9.07	6.87–9.40	44	8.34±0.98	11.73	6.8–9.8	6.62–11.59
β-globulin (g/l)	26	4.51±1.22	27.12	3.04–6.14	2.84–8.70	44	4.94±0.79	15.99	3.61–6.52	3.48–7.00
γ-globulin (g/l)	26	29.17±6.44	22.08	20.02–41.87	18.31–44.84	44	28.73±6.46	22.47	20.06–42.95	18.40–48.25
A:G ratio	26	0.86±0.21	24.40	0.54–1.21	0.44–1.34	44	0.87±0.19	21.42	0.52–1.08	0.26–1.23

^a CK creatine kinase; LDH = lactate dehydrogenase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; AP = alkaline phosphatase; A:G = albumin:globulins.
^b Means observed between drive-net and box-trap captured animals are statistically ($P<0.05$) different.

higher MCV, and consequently a lower MCHC than circulating erythrocytes, because they mature in the spleen after release from the bone marrow (Cross et al., 1988). Spanish ibex captured via drive-net showed a lower MCHC than those captured via box-trap, although no changes in RBC, hemoglobin concentration, or PCV were observed. Therefore, this difference is probably unrelated to catecholamine stimulation.

Catecholamines also induce leukocytosis, with neutrophilia or lymphocytosis, and mild eosinophilia. However, corticosteroids induce leukocytosis with neutrophilia, lymphopenia, and eosinopenia; maximum levels are reached four to six hours after exposure to the stressor. Monocyte count variation is species-dependent (Jain, 1993; Duncan et al., 1994; Taylor, 2000). Therefore, in the Spanish ibexes captured via box-trap, higher values for neutrophils and a lower eosinophil count suggest a more-established and prolonged corticosteroid-induced stress response, probably due to time spent inside the box.

Aspartate aminotransferase is a nonspecific, but sensitive, marker of soft tissue damage, frequently used to complement CK changes that indicate muscular damage (Kramer and Hoffmann, 1997). Larger increases in CK and AST have been related to a stronger stress response to capture and handling (Kent et al., 1980; Kock et al., 1987a; Chapple et al., 1991). Higher AST concentrations in the ibexes captured via box-trap could indicate greater muscular damage due to capture stress in these animals. Although the observed values for CK seem to follow the same trend, the differences for this parameter were not statistically significant, probably due to wide variability.

Small ruminants show an increase in serum bilirubin concentration due to fasting or anorexia, hemolytic anemia, liver failure, or systemic disease (Haskell and Anttila, 2001). Serum triglyceride concentration is mainly diet related

(Bruss, 1997), whereas urea increases are due to dehydration and cortisol-induced protein catabolism (Finco, 1997). The lower serum triglyceride and higher serum concentrations of total bilirubin and urea observed in the ibexes that were captured via box-trap could be attributed to time spent in the box and the resultant fasting.

Both exercise and massive muscular necrosis can increase serum potassium concentration (Carlson, 1997), whereas glucocorticosteroids increase urinary excretion (Bia and DeFronzo, 1981). The more-intense physical activity of the ibex captured via drive-net (possibly increasing potassium concentration), and the effect of glucocorticoids on ibexes captured via box-trap (possibly reducing it), may account for the significant differences found in our study.

To summarize, differences found between the capture methods in the hematologic and biochemical values would seem to indicate that, for this species, the drive-net is physiologically less stressful than the box-trap. Time spent in the box by the animals before removal could account partially for the apparently higher stress induced by box-trap. To our knowledge, this is the first time that drive-nets have been used to capture this species. When using a new method of capture, it is important to assess animal safety in terms of physiologic compromise. Moreover, drive-net is a collective capture method, because it is possible to capture more than one animal at a time, and shows a better performance than box-trap. For Spanish ibex, drive-net capture has been proven, over more-common capture techniques, to be a useful and secure technique. Further studies are needed to provide a more-accurate account of the effects arising from the influence of variables such as habitat, as well as to evaluate the nutritional and pathologic status of captured animals.

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