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HEMATOLOGIC AND BIOCHEMICAL REFERENCE INTERVALS FOR SPANISH IBEX

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ABSTRACT: We studied blood samples from 529 Spanish ibexes (*Capra pyrenaica*) from different Andalusian mountain ranges in southern Spain, primarily from Sierra Nevada. For each sample, 13 hematologic and 32 biochemical parameters were analyzed. Within this database, we selected values obtained from live, free-ranging, physically restrained, clinically normal animals to determine reference intervals for these parameters. Distribution of values within each parameter was determined and differences in values between sex and age classes also were determined. We found significant differences in eight biochemical parameters among male and female ibexes. Significant differences in values for 20 hematologic and biochemical parameters between age classes also were found.
Key words: *Capra pyrenaica,* hematology, reference intervals, serum biochemistry.

INTRODUCTION

Current management of some wild mammal populations is based on more or less continuous monitoring of their demographic, physiologic, and genetic status. Blood and serum chemistry values may provide, when properly interpreted, a precise picture of the conditions of one animal at the moment of its sampling (i.e., nutritional status, disease condition, stress due to capture and handling) and may reflect habitat quality.

Therefore, in order to use these data for diagnostic purposes we need to compare them with reference or normal values. Data on hematologic and biochemical parameters for Spanish ibex (*Capra pyrenaica*) are scarce. Peinado et al. (1993) compared ranges of hematologic values between physically restrained and chemically immobilized animals. Data on effects of captivity on blood composition of ibex have been published by Peinado et al. (1995). In a recent report (Lavín et al., 1997) hematologic profiles of two Spanish ibex with acute haemonchosis were characterized. These studies were conducted on samples from the Puertos de Tortosa y Beceite National Game Reserve (northeastern Spain). Another study of hematologic parameters of ibex from Sierra Nevada Natural Park was recently published by Pérez et al. (1999).

These studies were based on a limited number of samples (especially healthy free-ranging physically restrained animals) and the results reflect differences in capture method, handling of animals, and sample transportation and laboratory procedures. Therefore, the aim of this study was to obtain reference ranges for hematologic and biochemical parameters of Spanish ibex from a large number of samples using uniform capture and handling protocols.

MATERIALS AND METHODS

More than 1,000 blood samples obtained from 375 male (27 <1 yr old, 192 1–3 yr old, 156 >3–10 yr old) and 154 female (32 <1 yr old, 50 1–3 yr old, 64 >3–10 yr old, and 8 >10 yr old) Spanish ibex were analyzed between March 1996 and June 1999. Animals came primarily from the Sierra Nevada (84.7%) and fewer animals came from other Andalusian ibex populations: Almería (3.2%), Málaga (8.1%), Cádiz (1.7%), and Jaén (2.3%). Age was determined by tooth replacement and horn segment

TABLE 1. Reference hematologic values for Spanish ibex from Andalusia.

Parameter[a]	*n*	Mean±SD	Min.	Max.	90% CI	G_1[b]	G_2[c]
RBC (10^{12}/l)	213	15.88±2.80	4.13	22.35	15.56–16.20	−1.135	2.738
Hematocrit (%) (calculated)	215	46.4±7.4	12.3	69.6	45.6–47.3	−0.834	3.668
Hematocrit (%) (packed cell volume)	207	42.8±6.1	11.0	55.0	42.1–43.5	−1.757	6.512
Hemoglobin (g/dl)	215	15.8±2.3	3.7	20.1	15.6–16.1	−1.706	5.907
MCV (fl)	216	29.5±3.5	19.6	46.0	29.1–29.9	1.747	5.506
MCH (pg)	195	10.1±1.5	6.4	15.1	9.9–10.3	0.843	1.532
MCHC (%)	195	34.0±3.9	22.1	43.2	33.6–34.5	−0.405	−0.90
Leukocytes/ml	214	15,482±6,794	3,300	41,000	14,715–16,249	0.874	1.527
Bands (%)	215	2.5±2.7	0.0	14.0	2.2–2.8	1.519	2.566
Neutrophils (%)	215	38.2±14.3	0.0	87.0	36.6–39.8	0.227	0.189
Lymphocytes (%)	214	55.2±14.7	0.0	89.0	53.5–56.8	−0.725	1.604
Monocytes (%)	215	1.3±1.6	0.0	8.0	1.2–1.5	1.371	1.832
Eosinophils (%)	214	1.8±3.1	0.0	30.0	1.5–2.2	4.693	34.256

[a] Abbreviations: RBC=red blood cells, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration.
[b] G_1=skewness.
[c] G_2=kurtosis.

TABLE 2. Reference biochemical values for Spanish ibex from Andalusia.

Parameter[a]	*n*	Mean±SD	Min.	Max.	90% CI	G_1[b]	G_2[c]
Glucose (mg/dl)	211	126.1±66.0	2.6	297.0	118.6–133.6	−0.092	−0.243
Cholesterol (mg/dl)	212	53.0±21.8	23.0	174.0	50.5–55.4	1.881	5.425
Triglycerides (mg/dl)	208	37.1±37.8	1.7	238.0	32.8–41.4	2.861	9.477
Uric acid (mg/dl)	181	0.4±1.2	0.0	16.1	0.2–0.5	11.904	152.431
Blood urea (mg/dl)	210	44.4±15.5	15.7	113.0	42.6–46.1	1.941	5.695
Creatinine (mg/dl)	211	1.7±0.7	0.2	6.0	1.6–1.7	1.862	10.113
AST (IU/l)	209	235.3±212.4	26.6	1,758.0	211.0–260.0	8	19.472
ALT (IU/l)	211	48.4±52.3	6.6	468.0	42.4–54.3	4.362	24.759
LDH (IU/l)	207	1,509.0±901.4	313.0	5,605.0	1,405.5–1,612.6	2.150	5.512
CK (IU/l)	204	748.0±944.5	35.2	6,630.0	638.7–857.3	3.197	12.810
TBIL (mg/dl)	110	0.5±0.6	0.1	4.0	0.4–0.6	3.304	13.647
DBIL (mg/dl)	103	0.2±0.2	0.0	0.9	0.2–0.3	1.462	2.635
GGT (IU/l)	210	51.0±25.3	14.2	241.0	48.1–53.8	4.439	25.334
AMY (IU/l)	95	482.8±739.5	2.9	3,280.0	356.8–608.9	1.807	2.693
ALP (IU/l)	211	588.1±528.9	37.6	3,638.0	527.9–648.2	2.918	10.817
CES (ng/ml)	69	49.9±27.6	1.4	112.0	44.4–5.4	0.235	−1.094
Proteins (g/dl)	214	7.2±1.1	4.3	13.7	7.1–7.3	1.217	4.965
Albumin (g/dl)	209	47.5±7.8	17.9	65.9	46.6–48.4	−0.548	1.065
Alpha 1 (g/dl)	209	6.8±1.8	0.1	14.1	6.6–7.0	0.896	2.813
Alpha 2 (g/dl)	209	12.1±3.5	2.1	29.3	11.7–12.5	0.754	4.656
Beta (g/dl)	209	6.5±3.0	2.3	21.0	6.1–6.8	2.656	8.312
Gamma (g/l)	208	26.9±7.7	4.3	57.1	26.1–27.8	0.633	2.085
A/G ratio (g/dl)	187	0.9±0.3	0.5	1.8	0.9–1.0	0.719	0.250
Ca	175	10.6±2.0	5.9	18.7	10.3–10.8	0.806	1.824
Phosphates	177	6.9±2.8	2.8	25.9	6.6–7.3	2.166	11.644
Fe	201	161.2±64.8	25.5	497.0	153.7–168.8	1.925	7.160
Chloride	203	97.4±14.9	58.7	133.0	95.7–99.1	−1.086	1.563
Mg	202	3.0±0.8	1.0	7.6	2.9–3.1	1.495	7.282
Na	137	145.2±8.2	116.7	171.6	144.0–146.3	0.868	2.506
K	137	7.0±2.6	3.1	15.4	6.7–7.4	1.110	1.006
Estradiol	11	1.0±0.0	1.0	1.0	1.0–1.0		
Cortisol	140	82.5±45.6	15.0	215.0	76.1–88.9	1.024	0.608

[a] Abbreviations: AST=aspartate aminotransferase, ALT=alanine aminotransferase, LDH=lactate dehydrogenase, CK=creatine kinase, TBIL=total bilirubin, DBIL=direct bilirubin, GGT=glutamyl transferases, AMY=amylase, ALP=alkaline phosphatase, CES=cholinesterase, A/G=albumin/globulin.

[b] G_1=skewness.

[c] G_2=kurtosis.

counts (Fandos, 1991). Blood samples were obtained by jugular venipuncture of ibexes captured for management purposes using corral traps (Pérez et al., 1997). Altitude at which animals were captured ranged from 1,400 m to 2,500 m above sea level.

Live animals were physically restrained and their eyes covered for protection and stress reduction. Ethylenediaminetetraacetic acid was used as anticoagulant and serum was obtained by centrifugation (4,750xG for 10 min). Sera were refrigerated at 4 C and analyzed within 24 hr at a single laboratory. Cytohematologic analyses were performed with a Cell-Dyn 610™ autoanalyzer (Abbott Científica, S.A., Madrid, Spain). Parameters measured are shown in Table 1.

Metabolites, hormones, enzymes, proteins, and ions analyzed are shown in Table 2. Most biochemical measurements were made using an autoanalyzer (BT 2245™, Biotechnica Instruments, Rome, Italy) but some were made manually using an ES 36™ spectrophotometer (Sclat Tecnologic, Barcelona, Spain). Total proteins were measured using BioSystems™ kits (BioSystems-Atom, Barcelona, Spain) and pro-

TABLE 3. Values of select parameters that differed significantly between male and female ibex.

Parameter[a]	Mann-Whitney *U*	*P*	Males (*n*)	Mean±SD	Females (*N*)	Mean±SD
Hematocrit (packed cell volume) (%)	6,127.00	0.006	122	43.7±5.8	82	41.6±6.3
Cholesterol (mg/dl)	3,298.50	0.000	127	47.9±17.9	82	60.4±25.2
Triglycerides (mg/dl)	3,873.50	0.006	124	33.6±36.6	81	41.5±39.0
CK (IU/l)	3,937.50	0.040	127	672.4±853.1	75	884.0±1,083.9
ALP (IU/l)	6,953.00	0.000	129	659.7±549.2	79	440.2±403.2
Proteins (g/dl)	6,614.00	0.003	128	7.4±1.2	83	7.0±0.9
Alpha 2 (g/dl)	4,222.50	0.044	125	11.9±3.1	81	12.6±3.9
Chloride (g/dl)	3,475.00	0.001	126	94.5±16.8	75	102.1±9.7
Mg (mg/dl)	3,812.00	0.019	126	2.8±0.7	76	3.2±0.9

[a] Abbreviatoins: CK=creatine kinase, ALP=alkaline phosphatase.

teins were fractioned by cellulose acetate electrophoresis and bands were measured by a 434 photodensitometer™ (Digiscan, Barcelona, Spain).

Statistical analyses, mainly non-parametric tests, were performed by using BMDP (Dixon, 1990; Berkeley, California, USA) and SYSTAT (London, UK) packages. To obtain reference ranges for the parameters studied, we selected values from live and clinically normal animals immediately after capture.

RESULTS

Reference hematologic and biochemical values for Spanish ibex are summarized in Tables 1 and 2. High variability in white blood cell (WBC), monocyte, and eosinophil counts and some biochemical parameters were found (Tables 1, 2). Values obtained for the different hematologic and biochemical parameters did not fit normal distribution as values obtained for G_1 (skewness) and G_2 (kurtosis) indicate (Tables 1, 2). There were some significant differences in values obtained from males and females (Table 3) and in some parameters related to age (Table 4).

DISCUSSION

According to Borjesson et al. (2000), data reported as means, standard deviations, and ranges do not adequately address parameters that are not normally distributed, do not provide usable reference values, and are not appropriate for all sample sizes. For certain parameters showing greater biologic and analytic variation, such as leukocyte counts and WBC differential counts, intervals obtained using the 90th percentile provide tighter reference intervals (Solberg, 1999). This confidence level has been chosen in our study to characterize reference intervals.

Captive ibex had higher hematocrit values, hemoglobin concentration, and higher erythrocyte counts than the same animals when captured suggesting these increases are due to effects of stress on these parameters (Peinado et al., 1995). Differences in RBC counts may be attributable to stress (splenic contraction), hormonal influences, hydration status, dietary differences, adaptations to a desert environment (Borjesson et al., 2000), or adaptation to a high mountain environment.

Total leukocyte count varies with species and is influenced by age (Jain, 1993), but also may reflect stress-induced corticosteroid or epinephrine release due to capture, handling, and transport, as well as disease and allergic reactions (Bubenik and Brownlee, 1987; Marco et al., 1997; Borjesson et al., 2000). In most species for which reference ranges have been established, the neutrophil/heterophil count is a useful indicator of infection (Gascoyne and Hawkey, 1992). Lymphocytes may increase proportionally in chronic infections, whereas neutrophils may increase during acute infections (Bubenik and Brownlee, 1987).

Previous hematologic data obtained

TABLE 4. Values of select parameters that differed significantly between age classes. Mean values±standard deviation for each age class.

Parameter[a]	Age class				Kruskal Wallis	P
	<1 yr	1–3 yr	>3–10 yr	>10 yr		
Hemoglobin (g/dl)	15.2±2.0	16.0±1.9	16.2±2.6	14.2±3.9	1.443	0.003
MCV (fl)	30.0±5.1	28.4±2.6	30.2±2.6	31.5±3.0	20.977	0.000
MCH (pg)	9.9±2.0	10.0±1.1	10.4±1.4	10.0±1.7	9.940	0.007
Leukocytes/ml	17,114±7,490	17,058±4,874	12,542±4,874	14,229±12,495	25.880	0.000
Bands (%)	2.4±2.4	2.4±2.6	2.6±2.9	3.3±5.0	7.795	0.020
Lymphocytes (%)	57.4±14.3	58.0±12.6	50.7±16.5	52.7±10.8	13.851	0.001
Monocytes (%)	1.6±2.0	1.5±1.5	0.8±1.2	2.3±1.5	6.424	0.040
Cholesterol (mg/dl)	73.5±28.9	44.1±11.6	48.0±13.2	45.0±12.7	25.764	0.000
Triglycerides (mg/dl)	41.6±28.9	34.7±42.6	37.3±39.6	29.3±15.2	12.533	0.002
ALT (IU/l)	57.5±45.7	55.7±69.5	33.4±18.7	38.1±51.1	24.987	0.000
LDH (IU/l)	1,968±1,148	1,394±851	1,320.5±627	1,384±713	14.530	0.001
CK (IU/l)	934±1,025	791±1,105	578±647	591±471	10.147	0.006
TBIL (mg/dl)	0.83±0.90	0.40±0.41	0.33±0.27	0.37±0.28	6.027	0.049
ALP (IU/l)	792±778	624±458	421±294	253±100	25.133	0.000
Total proteins (g/dl)	6.8±1.0	7.2±1.0	7.6±1.3	7.3±0.7	16.141	0.000
Albumin (g/dl)	47.2±8.3	50.0±6.7	45.4±7.1	37.9±12.8	13.988	0.001
Gamma (g/dl)	25.3±8.8	25.0±6.3	30.0±6.6	36.4±10.4	25.626	0.000
Ca (mg/dl)	11.3±1.1	10.3±2.0	10.3±2.5	9.7±2.3	13.823	0.001
Phosphorus (mg/dl)	8.7±3.3	6.1±2.1	6.5±2.1	5.8±4.2	15.198	0.001
Mg (mg/dl)	3.2±0.9	2.8±0.8	3.1±0.7	2.8±0.5	7.261	0.027

[a] Abbreviations: ALT=alanine aminotransferase, LDH=lactate dehydrogenase, CK=creatine kinase, TBIL=total bilirubin, ALP=alkaline phosphatase.

from Spanish ibex (Peinado et al., 1993, 1995; Pérez et al., 1999) are within ranges determined in this study. Hematologic and biochemical values obtained for Spanish ibex are relatively similar to those reported for Alpine ibex (*Capra ibex*) (Cook et al., 1986; de Meneghi et al., 1990; Sartorelli et al., 1991), but differ from those determined for other wild bovids, such as the mouflon (*Ovis ammon*) (Marco et al., 1997) and bighorn sheep (*Ovis canadensis*) (Franzman, 1972; Borjesson et al., 2000).

Blood urea values obtained in our study were high compared to those reported for other *Capra* species, like the Alpine ibex (de Meneghi et al., 1992). These values may influenced by protein intake, thus reflecting habitat quality (Franzman, 1972). Creatine kinase values were significantly higher in females and total protein levels were significantly higher in males (Table 4). De Meneghi et al. (1990) argued that significant sex related changes in this muscle enzyme are consistent with higher mobility of females within their home range. Creatinine is produced in active muscle tissue. Its production is directly related to the muscular mass (Wolkers et al., 1994). We also found significantly higher values for alkaline phosphatase (ALP) in male ibex. Plasma creatinine level, urea/creatinine ratio and alkaline phosphatase level were considered the most useful parameters for evaluating condition of red deer (*Cervus elaphus*) (Wolkers et al., 1994). Our results are consistent with the fact that during most of the year male and female ibexes use habitats with different quality.

Values for 20 parameters were significantly different among age classes (Table 4). We found hemoglobin concentration, mean corpuscular volume, and mean corpuscular hemoglobin concentration increased with age, and counts of leukocytes and lymphocytes decreased in animals from 3–10 yr of age. These data suggest maturation of both circulatory and immunologic functions. Counts of leukocytes and lymphocytes increased in aged animals (>10 yr old).

Young animals had significantly higher ALP values and lower total protein concentrations. Alkaline phosphatase levels are generally higher in young animals because of bone growth (Marco et al., 1997). In our study, maximum values of ALP were in young animals when most epiphyses are not yet fused. These age-related changes have been reported for other wild ungulates such as bighorn sheep (Borjesson et al., 2000). Young animals had relatively high levels of creatine kinase which may be due to greater susceptibility to stress-induced events such as capture and handling and capture myopathy (Vassart et al., 1992; Marco et al., 1997).

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