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HEMATOLOGY AND SERUM CHEMISTRY OF COTTONTAIL RABBITS OF SOUTHERN ILLINOIS

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ABSTRACT: In 1983 and 1984 blood was collected from 79 cottontail rabbits (*Sylvilagus floridanus*) confined to an outdoor enclosure in southern Illinois to establish reference values for hematology and serum chemistry. Packed cell volume, sodium, potassium, chloride, glucose, calcium, carbon dioxide, blood urea nitrogen, creatinine, uric acid, cholesterol, albumin, bilirubin, alkaline phosphatase, aspartate transaminase, alanine aminotransaminase, total protein, albumin/globulin ratio, and osmolality were measured. Sex and age (adult versus juvenile) of rabbit as well as season (June to September versus October to May) and method of capture (trap versus shot) variously affected most hematology and serum chemistry variables.

Key words: Hematology, serum chemistry, cottontail rabbits, *Sylvilagus floridanus*, survey.

INTRODUCTION

Hematology and serum chemistry are becoming increasingly important diagnostic tools in veterinary and wild animal medicine; however, baseline values are required to use these techniques to assess disease in a particular species. Hematological and serological reference values in laboratory lagomorphs including New Zealand white rabbits (*Oryctolagus cuniculus*), Dutch belted and Polish white hares (*Lepus europaeus*), and black-tailed jackrabbits (*L. californicus*) are well documented (Mitruka and Rawnsley, 1981). Reference values for eastern cottontail rabbits (*Sylvilagus floridanus*) are not as complete, although values have been reported for packed cell volume (PCV), blood urea nitrogen (BUN), cholesterol (CHOL), albumin (ALB), bilirubin (BILI), total protein (TP), and the albumin/globulin ratio (A/G) in wild and captive cottontails (Jacobson and Kirkpatrick, 1974; Jacobson et al., 1978a, b; Warren and Kirkpatrick, 1978). The objective of our research was to establish values for PCV, ALB, BUN, CHOL, BILI, TP, A/G, sodium (Na), potassium (K), chloride (Cl), glucose (GLU), calcium (Ca), carbon dioxide (CO_2), creatinine (CREA), uric acid (UA), alkaline phosphatase (AP), aspartate transaminase (AST), alanine aminotransaminase (ALT), and osmolality (OSMOL) in cottontails confined to an outdoor enclosure in southern Illinois.

MATERIALS AND METHODS

Cottontail rabbits, captured in the surrounding area, were stocked in a 1.5 ha outdoor enclosure in southern Illinois (37°41'N, 89°15'W) beginning in January of both 1983 and 1984 (Lepitzki, 1986). The habitat within the enclosure was old field dominated by various grasses and blackberry (*Rubus alleghaeniensis*) bushes. Rabbit populations were allowed to increase until a removal regime was instituted in May 1983 and in June 1984. A maximum density of 31.5 rabbits/ha (46 rabbits) was reached in June 1984. A commercial rabbit pellet food ration was provided *ad libitum* throughout the study to supplement natural vegetation.

At bi-weekly intervals, a maximum of five animals, preferably young of the year (juveniles), were collected from the pen for concurrent parasite and disease investigations (Lepitzki, 1986). Live-traps, baited with apple, were checked in the morning after the rabbits' nightly activity had ceased (Lepitzki, 1990). Animals live-trapped were brought to the laboratory in the trap, anesthetized with 40 mg/kg ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, New Jersey 07950, USA) and killed by exsanguination via cardiac puncture.

If no animals were captured after a couple nights of trapping, rabbits were collected by shooting with a 0.22 caliber rifle. Blood from animals that were shot was collected from bullet wounds and the heart in order to collect as large

TABLE 1. Hematology and serum chemistry of cottontail rabbits collected in southern Illinois in 1983 and 1984 showing independent variable predictors from the most parsimonious models.

Variable	Units	Predictors	df	F	*P*	Subclass*	*n*	Mean (SD)	Range
Packed cell volume (PCV)	%	Season + Method + Age	3,75	24.71	0.0001	summer shot A	1	39.6 —	—
						summer shot J	15	28.6 (6.5)	18.0–39.2
						summer trap A	3	38.3 (2.9)	35.7–41.5
						summer trap J	25	35.8 (5.5)	20.2–42.9
						winter shot A	9	41.4 (4.4)	34.2–49.0
						winter shot J	3	35.9 (7.9)	30.3–44.9
						winter trap A	10	44.6 (3.2)	37.8–48.9
						winter trap J	13	40.2 (3.2)	34.3–46.4
Sodium (Na)[a]	mEq/l	Age	1,69	5.62	0.0205	A	21	140.9 (3.6)	134–148
						J	50	137.9 (5.3)	123–149
Potassium (K)[b]	mEq/l	Method	1,61	35.73	0.0001	trap	52	6.0 (1.3)	2.8–8.4
						shot	11	8.3 (0.7)	7–9.5
Chloride (Cl)	mEq/l	Sex + Season	2,69	12.00	0.0001	F summer	18	100.2 (5.4)	89–113
						F winter	18	95.0 (5.1)	84–107
						M summer	17	105.0 (5.9)	93–115
						M winter	19	100.4 (7.5)	89–114
Glucose (GLU)	mg/dl	Season	1,70	6.02	0.0166	summer	35	212.3 (72.3)	42–348
						winter	37	254.3 (72.6)	38–398
Calcium (Ca)	mg/dl	Method	1,70	11.52	0.0011	trap	52	10.5 (1.2)	6.4–13.1
						shot	20	11.9 (2.1)	7.9–15.0
Carbon dioxide (CO_2)	mEq/l	none	—	—	—	—	24	19.0 (6.0)	7–26
Blood urea nitrogen (BUN)[c]	mg/dl	Method + Season + Method*Season	3,67	7.66	0.0002	shot summer	8	14.1 (4.8)	8–21
						shot winter	12	17.3 (4.8)	11–27
						trap summer	26	27.3 (11.5)	10–47
						trap winter	25	19.2 (6.9)	7–38
Creatinine (CREA)	mg/dl	Age	1,68	14.53	0.0003	A	22	1.4 (0.3)	0.9–2.0
						J	48	1.0 (0.4)	0.1–2.0
Uric acid (UA)[d]	mg/dl	Method	1,69	11.83	0.0010	trap	52	1.5 (0.6)	0.4–3.3
						shot	19	2.2 (1.1)	0.9–4.8
Cholesterol (CHOL)[e]	mg/dl	Age	1,68	5.90	0.0178	A	21	29.2 (10.8)	14–57
						J	49	37.9 (14.7)	16–86
Albumin (ALB)[f]	g/dl	Season + Age	2,68	16.52	0.0001	summer A	3	2.7 (0.4)	2.3–3.0
						summer J	31	2.7 (0.2)	2.3–3.1
						winter A	18	3.1 (0.2)	2.8–3.5
						winter J	19	2.9 (0.2)	2.5–3.4

TABLE 1. Continued.

Variable	Units	Predictors	df	F	P	Subclass*	n	Mean (SD)	Range
Bilirubin (BILI)[g]	mg/dl	Method + Sex	2,65	11.63	0.0001	shot F	12	0.3 (0.07)	0.2–0.4
						shot M	5	0.2 (0.05)	0.2–0.3
						trap F	22	0.2 (0.08)	0.1–0.3
						trap M	29	0.1 (0.09)	0.0–0.3
Alkaline phosphatase (AP)[h]	U/l	Age	1,67	7.62	0.0074	A	22	70.0 (67.8)	18–276
						J	47	106.1 (40.5)	47–249
Aspartate transaminase (AST)[i]	U/l	Season	1,58	14.32	0.0004	summer	30	136.1 (72.1)	34–293
						winter	30	77.7 (44.2)	20–202
Alanine aminotransaminase (ALT)[j]	U/l	Method	1,67	5.81	0.0187	trap	52	82.8 (51.0)	19–296
						shot	17	120.6 (70.4)	24–258
Total protein (TP)	g/dl	Age + Method	2,35	14.67	0.0001	A shot	6	7.0 (1.3)	4.8–8.3
						A trap	8	5.9 (0.9)	4.8–7.8
						J shot	2	6.0 (0.1)	5.9–6.1
						J trap	22	5.0 (0.7)	3.8–6.1
Albumin/globulin (A/G)	ratio	Sex + Age	2,35	5.60	0.0078	F A	8	1.0 (0.3)	0.5–1.5
						F J	11	1.2 (0.2)	1.0–1.5
						M A	6	1.2 (0.4)	0.6–1.7
						M J	13	1.3 (0.2)	1.0–1.6
Osmolality (OSMOL)[k]	mOsm/l	none	—	—	—	—	37	278.8 (8.2)	248–296

* Summer = rabbits captured between June and September; winter = rabbits captured between October and May; shot = rabbits that were shot; trap = rabbits that were live-trapped; A = adult; J = juvenile, young-of-the-year; M = male; F = female.

[a] One outlier, Na 119 mEq/l.

[b] Seven animals with K ≥ 10 mEq/l; out of SMA's range.

[c] One outlier, BUN 55 mg/dl.

[d] One outlier, UA 7.9 mg/dl.

[e] Two outliers, CHOL 136 mg/dl and 201 mg/dl.

[f] One outlier, ALB 4.3 mg/dl.

[g] Two outliers, BILI 0.5 mg/dl.

[h] Three outliers, AP ≥ 350 U/l; out of SMA's range.

[i] 11 animals with AST ≥ 300 U/l; out of SMA's range.

[j] Three animals with ALT≥ 300 U/l; out of SMA's range.

[k] One outlier, OSMOL 235 mOsm/l.

a volume as possible as quickly as possible. Depending on the location of the wound, more blood could be collected quickly from the wound than from the heart.

Blood was collected in heparinized capillary tubes (Red-Tips, Heparinized Micro-hematocrit capillary tubes, Fisher Scientific, Pittsburgh, Pennsylvania 15219, USA) for PCV determination and in test tubes. If the blood was collected in the field, test tubes were placed on ice and transported to the laboratory. Blood in the test tubes was allowed to clot at room temperature and then the serum was separated by low speed centrifugation (3,000 rpm, 5 min), pipetted into vials, and stored frozen (−20 C) until analyzed by a Technicon Sequential Multiple Analyzer (SMA II) (Technicon Corporation, Tarrytown, New York 10591, USA) at Memorial Hospital (Carbondale, Illinois 62901, USA). Whenever possible, serum was analyzed within 1 week of collection; however, some serum was frozen for an extended time before analysis. The extended storage did not seem to adversely affect the serological profiles. For example, even though 11 of the serum samples in 1983 were stored for over 6 weeks, none contained serological variables beyond the range of the rabbit serum analyzed within one week of collection.

A series of 1-way analyses of variance (ANOVA's) was used to determine if sex or age (adult versus juvenile) of rabbit or season (June to September versus October to May) or method of collection (shot versus trapped) influenced the serological variables (PROC GLM; SAS Institute Inc., 1979). If more than one independent variable (sex, age, season, or method) influenced a serological variable, 2-, 3-, and 4-way ANOVA's including interactions were subsequently constructed to yield the most parsimonious model. Data were pooled from both years after no year effects were found. Tukey's studentized range tests (SAS Institute Inc., 1979) were used to examine differences between means. Extreme variates visually spotted in the frequency distribution of the data (PROC UNIVARIATE; SAS Institute Inc., 1979) and determined to be statistical outliers by Grubb's test (Sokal and Rohlf, 1981), were removed from the data set prior to ANOVA. In all statistical analyses, a probability level of $P < 0.05$ was used. Non-parametric 1-way ANOVA equivalents (PROC NPAR1WAY WILCOXON, SAS Institute Inc., 1979) yielded the same statistical results. Adult rabbits were defined as not young of the year; the two seasons were long due to small sample sizes.

RESULTS

Blood from 79 rabbits was analyzed for PCV; sufficient quantities of serum was collected from 72 of these animals. Carbon dioxide, TP, A/G, and OSMOL were not determined for all rabbits; CO_2 was eliminated from the SMA II's analyzing sequence and the other three variables added in 1984. All animals appeared healthy upon post-mortem examination.

Hematology and serum chemistry values along with the independent variables from the most parsimonious statistical models are presented in Table 1. Whenever an independent variable was a significant predictor, the serum variable has been divided into its constituent subclasses. Notice that for some variables, samples sizes within each subclass (e.g., PCV, summer shot Adult, $n = 1$) are extremely low and furthermore, sample sizes between subclasses are not equal; therefore, the comparison between means within each subclass is meaningless. Means within each class (e.g., PCV, comparisons between summer vs. winter, shot vs. trap, Adult vs. Juvenile) are statistically significant ($P < 0.05$, Tukey's) in all cases although caution should be advised due to unequal sample sizes and overlapping ranges.

Carbon dioxide and OSMOL were the only serological variables not influenced ($P > 0.05$) by sex or age of rabbit, or season or method of collection (Table 1). Other serological variables and PCV were variously affected by sex, age, season, and method. Some were affected by more than one independent variable and a significant interaction between two independent variables was found in one case (BUN, Table 1).

In general, values for serological variables in the present study were similar to reference values for domestic rabbits (*O. cuniculus*) (Mitruka and Rawnsley, 1981) although GLU, AP, AST, and ALT were higher in cottontails (Table 2). Comparisons between our study and other studies on cottontails are more difficult because of the data presentation in published works. Nonetheless, the range of values for PCV, BUN, CHOL, ALB, BILI, TP, and A/G in this study always overlapped the ranges

TABLE 2. Hematology and serum chemistry of cottontail rabbits collected in southern Illinois in 1983 and 1984 with reference values for domestic rabbits and other cottontails.

Variable	Units	Southern Illinois				Domestic rabbits[a]		Other cottontails	
		n	Mean	SD	Range	Mean	SD	Range	Range of means
Packed cell volume (PCV)	%	79	37.2	6.7	18–49			11.0–54.0[b]	24.3–45.8[c]
Sodium (Na)	mEq/l	71	138.7	5.0	123–149	144	1.3		
Potassium (K)	mEq/l	63	6.4	1.5	2.8–9.5	6.1	0.2		
Chloride (Cl)	mEq/l	72	100.2	6.9	84–115	103	1.3		
Glucose (GLU)	mg/dl	72	233.9	75	38–398	132	13		
Calcium (Ca)	mg/dl	72	10.9	1.6	6.4–15	9.8	1.1		
Carbon dioxide (CO_2)	mEq/l	24	19.0	6.0	7–26				
Blood urea nitrogen (BUN)	mg/dl	71	21.0	9.8	7–47	18.4	4.7	4–132[b]	7.3–39.6[c]
Creatinine (CREA)	mg/dl	70	1.1	0.4	0.1–2.0	1.6	0.4		
Uric acid (UA)	mg/dl	71	1.7	0.8	0.4–4.8	2.6	0.9		
Cholesterol (CHOL)	mg/dl	70	35.3	14.1	14–86	25.6	12.1		34–55.8[d]
Albumin (ALB)	g/dl	71	2.9	0.3	2.3–3.5	3.2	0.3	1.6–8.6[b]	3.5–6.0[c]
Bilirubin (BILI)	mg/dl	68	0.18	0.09	0–0.4	0.31	0.04		0.2–1.5[c]
Alkaline phosphatase (AP)	U/l	69	94.6	53.1	18–276	10.2	2.7		
AST	U/l	60	106.9	66.2	20–293	70.2	11.3		
ALT	U/l	69	92.1	58.2	19–296	64.1	6.2		
Total protein (TP)	g/dl	38	5.6	1.1	3.8–8.3	6.8	0.4	3–12[b]	5.0–10.3[c]
Albumin/globulin (A/G)	ratio	38	1.2	0.3	0.5–1.7	0.9	0.2		0.69–2.24[b]
Osmolality (OSMOL)	mOsm/l	37	278.8	8.2	248–296				

[a] 200 New Zealand White (*Oryctolagus cuniculus*); Mitruka and Rawnsley, 1981; [b] Jacobson et al., 1978b; [c] Jacobson et al., 1978a; [d] Warren and Kirkpatrick, 1978.

of other published values for cottontails (Table 2).

DISCUSSION

A study attempting to establish reference values for hematology and serum chemistry in free-ranging populations of wild animals is replete with problems; however, to the wildlife disease expert or wildlife manager, the information derived from these types of studies are invaluable when it is desired to differentiate between diseased and healthy individuals and populations. Blood samples are not always collected under the best of conditions or with the ideal technique nor is it always possible to analyze the samples immediately. This study attempts to bridge the gap between the laboratory situation in which all nutritional and stress factors are controlled and blood is collected with the best of techniques and the field situation in which animals are stressed from pursuit and capture, and most likely, blood is collected from the animal which has just been shot.

The rabbits in this study were free-ranging but confined to a large, outdoor, natural enclosure. Food was provided to supplement the natural vegetation. Both trapping and shooting were used to collect animals. Both juvenile and adult rabbits were collected in proportions to what would be present in the wild; more juveniles than adults during summer, the exact opposite during winter as juveniles become adults. And lastly, sample sizes in each subclass tended to be small and are not equal to the sample sizes in other subclasses. Nonetheless, some trends are apparent.

Some of the trends we noticed have been documented by others or are explainable; however, many have not been previously reported and we have difficulty providing explanations. The higher levels of GLU in our cottontails in comparison with domestic rabbits (Table 2) could be due to stress and/or exercise (Medway et al., 1969; Benjamin, 1981). The significant ($P < 0.05$) elevation in GLU we saw during winter was unexpected because the animals were being fed. As expected (Medway et al., 1969; Benjamin, 1981), juvenile rabbits had higher ($P < 0.05$) AP levels than adults; why cottontails had higher levels than domestic rabbits is unknown. Differences due to season for AST (summer higher, $P < 0.05$), method for ALT (shot higher, $P < 0.05$), and species for both variables (Tables 1 and 2) can not be explained although Mitruka and Rawnsley (1981) reported these serum variables varied widely in rabbits they tested.

Differences due to season, sex, and method have been reported for PCV (Jacobson et al., 1978a, b). We found the same seasonal (winter higher, $P < 0.05$) and method (trapped higher, $P < 0.05$) trends (Table 1). We did not find males to have higher PCV than females as previously reported (Jacobson et al., 1978a). Our adults did however have higher ($P < 0.05$) PCV's than did juveniles (Table 1).

Jacobson et al. (1978a) found lower ALB levels in spring (16 Mar–14 Apr) while we saw lower ($P < 0.05$) levels during summer (Table 1). We also saw an age class difference (adult higher, $P < 0.05$). We duplicated the trends for higher ($P < 0.05$) BUN in trapped cottontails noted by Jacobson et al. (1978b) and nutritionally-reolism due to stress has been used by others to explain elevated BUN in trapped (Jacobson et al., 1978b) and nutritionally-restricted cottontails (Warren and Kirkpatrick, 1978), nutritional stress could not explain the seasonal difference (summer higher, $P < 0.05$) in our study (Table 1) because the animals were being fed continuously. Jacobson et al. (1978a) also noted an elevated BUN in spring.

We found elevated ($P < 0.05$) BILI in female cottontails (Table 1); Jacobson et al. (1978a) also noted a similar trend. A seasonal difference in TP reported by Jacobson et al. (1978a) was not apparent in our study. Age class differences in TP we found (juveniles higher, $P < 0.05$) may be explained through lower production of protein due to young age (Benjamin, 1981). The aspirated blood from bullet wounds

most likely contained more protein due to localized trauma as reflected in animals we shot having higher ($P < 0.05$) TP than those trapped (Table 1). We cannot explain the higher ($P < 0.05$) A/G ratio we saw in males and juveniles or the elevated ($P < 0.05$) CHOL in juveniles (Table 1). As even minimal hemolysis results in elevated K (Benjamin, 1981) it was not unexpected to have our shot animals with higher ($P < 0.05$) K than those trapped (Table 1). Increased ($P < 0.05$) levels of Ca and UA in animals we shot (Table 1) are not explainable as are elevated ($P < 0.05$) levels of Na and CREA in adults and increased ($P < 0.05$) levels of Cl in males and in animals we collected during the summer (Table 1).

Clearly, more hematology and serum chemistry data on cottontail rabbits must be collected before precise and accurate explanations of variability can be formulated. We reinforced some trends noted by others and we found many new trends, most of which we could not explain. It is of utmost importance for future investigators to record all intrinsic (e.g., sex, age, breeding condition, stress level, nutritional status) and extrinsic (e.g., month, method) factors that could affect these data. Without a sound knowledge of what is normal and what can influence hematological and serological variables, abnormality and disease can not be assessed using these useful techniques.

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