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Hematological profile of water buffaloes: age and sex-related variations

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Abstract

The aim of this study was to establish blood reference intervals (RIs) and describe the effects of age and sex. In this cross-sectional study, we analyzed 1225 water buffaloes with nonvisible clinical signs of disease stratified by age and sex. The confidence intervals were calculated for each reference limit (95%, $p<0.05$). The data were analyzed in three age groups (calves, young, and adults) and two sex groups (females and males). The differences between age group, sex, and all the parameters were compared, and differences between age group, sex, and hematological parameters were found ($p<0.05$). Males showed higher values for red blood cell account, white blood cell account, and thrombocyte count; females showed higher values for Mean corpuscular volume and neutrophil parameters. Calves showed more hemoconcentration than in the other age groups. Young animals showed less total protein, neutrophils, and thrombocytes and a higher Mean corpuscular volume and lymphocyte count; statistically significant relation were found ($p<0.05$) in decreasing red blood cells, hemoglobin, packed cell volume, platelet, and total proteins, and increases in eosinophils and lymphocytes. The current study provides RIs and demonstrates changes associated with age and sex.

Keywords: blood cells; bovid; hematology.

Introduction

The blood profile of animals is vital to confirm clinical diagnoses and estimate the severity of diseases (26). Furthermore, parameters are commonly employed as valuable indicators of the health and nutritional status of many species and thus help diagnose metabolic diseases and manage infertility as well as low productivity in farm animals (3). Several factors, namely age and sex, affect metabolism and, thereby, the blood profile of animals (8). However, studies investigating the blood indices of *Bubalus bubalis* could be found with less representative samples (1, 7). Given the above, the present study was undertaken to determine reference values for different blood counts and their variation with different ages, sexes, physiological stages, and natural infections. The buffalo population in Latin America is estimated at approximately 4 million (23). Colombia's buffalo population has had noticeable growth, reaching a current number of approximately 338,567 animals in 2019 (17). *B. bubalis* are rustic animals and adapt quickly to difficult geographical conditions for cattle. They are economically important for their multipurpose production capacities, providing meat, milk, and work in areas where technification is seen as limited for agricultural production (9). Buffalo has an important global distribution in the tropics of the American, Asian, and African continents.

Hematological parameters are frequently used tools to diagnose the different pathologies affecting buffaloes.

Due to their anatomical resemblance to cattle, the ranges established for cattle are usually used in Colombian laboratories, which is not recommended due to variations in the concentration of erythrocytes, platelets, leukocytes, and hemoglobin and the mean corpuscular cell volume reported by some authors (8, 15, 22). To establish RIs in the hematology of buffaloes in South American tropical environments, age, sex, and environmental and geographical conditions should be taken into account (1, 33). Population growth in buffaloes has not been accompanied by noticeable growth in research about these animals. Some studies on the species have been conducted at the national level, which was intended to establish parameters or reference ranges for physiological constants and the identification of disease-producing etiological agents such as *Trypanosoma* spp. (24), pulmonary and gastrointestinal parasites and vector-borne diseases (18, 19, 28).

Thus, the present study aimed to determine the reference values for different blood counts and to study their variation with different ages, sexes, physiological stages, and natural infections.

Materials and methods

Study design and area

Ethical approval for the study was granted by the CES University Ethics Committee (approval number 128), and the research was conducted in November 2017.

The study area was located at a minimum latitude of 7.971111 and a maximum latitude of 8.09583; a minimum longitude of -75.5075° and a maximum longitude of -75.400278°; and an average of 20 msw and a maximum of 150 msw with an average temperature of 27 °C and precipitation of 1500 to 2000 mm, distributed in a bimodal model from April to May and October to November 2019. All the animals lived in the same environmental conditions. The choice of study area was based on the Colombian population of water buffaloes. According to the last livestock census of 2019 at that time, the buffalo population in Colombia is approximately 338,567 animals. Most buffaloes in Colombia are principally located in Cordoba (25.5%) and Antioquia (16.1%). Now the Colombia's buffalo population has had noticeable growth, rising to 563,372 animals located in the same distribution area (17).

Reference population

A local census was taken in Cordoba and Antioquia, where the largest populations are located, with a result of 55,307 animals. With this study population, the sample size was calculated using the methodology for estimating the overall point prevalence of a disease in large populations, with a confidence level of 95%, an expected proportion of 50%, and an expected absolute error of 3%, obtaining a sample size of 1068, adjusted to 1225 animals. Fifty-one farms participated in the study and signed the authorization for the ethics committee. The samples were taken in eight months. Animals were randomly included according to age group. The ages were classified as calves (less than 1 year), young animals (1-2 years), and adults (more than 2 years). Sex was classified as male and female. All animals that had lethargy were gestating, had poor body condition and/or externally visible lesions, or had been treated with antibiotics or antiparasitic in the last three months were excluded if they had any of those conditions. Blood samples were collected from 1225 animals with a mean age of four years (range: 1-16 years). All the animals were vaccinated and dewormed six months prior to the blood sample collection. All animals showed no signs of illness and did not have medication for the samples. Additionally, they had not received any medications during the month that preceded sampling.

Preanalytical procedures

A total of 7 ml of blood was collected in each tube aseptically of the coccygeal vein using vacutainer tubes with the anticoagulant ethylene diamine tetra acetic acid (EDTA) (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, UK) using an 18-gauge needle and a holder by the veterinarian of each farm present in the study. After collection, the blood samples were inverted 8–10 times, refrigerated, transported to the laboratory of the Colombian Institute of Tropical Medicine (ICMT), located in Antioquia, Colombia, and then analyzed at room temperature. The process took one month to have all the blood samples.

Analytical procedures

Using standard methods, all samples were processed immediately to evaluate hematological values and blood smear parameters and visualize hemotropic microorganisms (2). All blood samples that showed coagulation, hemolysis, lipemia, or icterus were excluded.

Hematological analysis

The hematology samples were analyzed using the Abacus team (Abacus Vet Junior, Diatron MI Ltd, Budapest, Hungary) (27), calibrated for the bovine species, to determine 15 hematological parameters, including the three populations and the direct differential count. The equipment was calibrated each time with the blanks and calibrators provided by the manufacturers. The following analytes were recorded: RBC count, HGB concentration, PCV (or HCT), MCV, MCH, MCHC, RDW, platelet (PLT) count, WBC count, and levels

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of neutrophils, lymphocytes, monocytes, eosinophils, basophils, fibrinogen, and TP total protein. Absolute and relative counts were made.

The packed cell volume (PCV) was measured using the standard microhematocrit method (19). The remaining plasma of centrifugation was deposited in a refractometer (Atago Co. Ltd., Tokyo, Japan), with which the analysis of plasma total proteins was performed as the standard process indicated (34). To perform the differential blood cell count, a blood spread was performed with a drop of blood from the tube with EDTA, stained with Hemacolor® dye, and then observed under a microscope with a magnification of 100x (2).

The differential WBC counts were performed manually in air-dried Giemsa-stained blood smears by two independent bacteriologist observers from the laboratory. Both observers were blinded to the results of the automated differential WBC counts and carried out manual WBC counts based on a 200-cell differential count with the mean used for further statistical analysis. Neutrophil-to-lymphocyte ratios (NLRs) were calculated by dividing absolute neutrophil counts by absolute lymphocyte counts. Blood smears were also examined for the presence of PLT clumps and blood microorganisms (34).

The fibrinogen was analyzed using the Clauss fibrinogen assay as a protocol normally used in the ICMT laboratory, according to the method already described (31).

Statistical analysis

RIs were determined according to the guidelines of the American Society for Veterinary Clinical Pathology (ASVCP) (sample size: >50) (13). The Kolmogorov–Smirnov test was used to assess the normal distribution with $p<0.05$ values (Reference Value Advisor) (14). The Dixon and Tukey tests (3 x IQR, 1.5 x IQR) were used to identify outliers and suspected outliers (Reference Value Advisor)(24). The means and standard deviations were calculated from the data obtained and then statistically evaluated by measures analysis of variance. Significant results were subjected to the Tukey–Kramer multiple comparisons test. The 95.0% reference intervals were calculated by removing the upper and lower 2.5% of the interval for each hematological constituent to give the 2.5 and 97.5 percentiles as described. The outliers were removed for each parameter evaluated and given a different number of n to analyze. Confidence intervals were calculated for each reference limit. The 90% confidence intervals (CI) were calculated for each reference limit to determine whether their precision was sufficient for clinical use. The analysis was performed using untransformed data. The statistical analysis was performed using Student's unpair t-test (32) and the Kruskal–Wallis test (11) as non-parametric tests. Differences at $p< 0.05$ were considered significant.

The values measured in the project were tabulated into a database made using Excel software and analyzed using IBM SPSS statistical software V.22.

Results

Description of the study population

One thousand two hundred and twenty-five animals located on 51 farms were included in this study, of which 75.8% (929/1225) were female buffaloes and 24.2% (296/1225) were male buffaloes. The most common age group was young animal buffaloes with 51.6% (632/1225), followed by adults with 29.6% (363/1225) and calves with 17.4% (213/1125).

Hematology

Hematology results are presented in the following tables: Table 1 shows the RI for all the populations included in the research study, followed by Table 2, which shows RIs according to age groups, and Table 3, in which the RI was divided by the sex of the animals in the study.

Several parameters showed no differences between RIs according to sex in the following parameters: PCV, MCHC, MCH, RDW, and TP. Males showed higher RBC, WBC, lymphocyte count, and thrombocyte count; females showed higher MCV and neutrophil parameters.

Several differences were found between age groups. Calves showed the highest hemoconcentration, with high ranges in PCV and RBC count compared to young animals and adults with higher MCV. White cell lines on calves showed a higher concentration derivate of a high range of lymphocytes, and thrombocyte ranges were higher than in the other age groups. On the other hand, young animals showed less total protein, fewer neutrophils and thrombocytes, and a higher WBC count and lymphocyte count than the adult population Tables 4,5 and 6.

No differences were found between levels of hemoglobin, MCHC, MCH, RDWc, monocytes, eosinophils, basophils, and fibrinogen associated with age group.

Discussion

To the best of the author's knowledge, the present study is the first attempt to establish RIs for hematologic analytes in water buffalo (*Bubalus bubalis*) in Colombia, and South America with this sample size and in accordance with the recently published ASVCP QALS committee guidelines for the determination of RIs in veterinary species (4) comparing the different ranges used in cattle. The literature contains articles that used different sizes (21, 29).

The stages of lactation or gestation could not be compared, and animals with gestation status were not included, which could affect leukocyte counts except for a few weeks peripartum, although without influencing hemoglobin concentration (20, 25, 30).

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Table 1. Total buffalo population blood parameters. Descriptive statistics and RIs for hematologic analytes determined by Abacus Junior Vet. except for differential WBC counts performed manually by standard blood smear for all the populations. SD standard deviation. G indicates Gaussian; NG non-Gaussian; Max. maximum; Min. minimum. Confidence intervals were calculated for each RI as recommended by PetitClerc and Solberg (1987).

Hematology Reference Intervals - Total buffalo population. Normality test Kolmogorov–Smirnov															
Measurand	**Conventional Units**	**SI Units**	**Initial n**	**Removed outliers n**	**Final n**	**Mean**	**SD**	**Median**	**Min**	**Max**	**Normality test p value <0.05**	**Symmetry test p value**	**Distribution**	**2.5th percentil (90%CIs)**	**97.5th percentil (90%CIs)**
PCV	%	L/L	1121	25	1096	39.2219	7.22799	39	19	58	0.000	0.313	NG	27	55
RBC conc.	10^6/µL	1012/L	1122	25	1097	7.8016	1.7805	7.63	2.8	12.72	0.000	0.045	NG	4.5735	11.8475
Hemoglobin	g/dL	g/L	1122	30	1092	12.9541	2.11	12.9	7.4	18.5	0.014	0.186	NG	9.0325	17.4675
MCV	fL	fL	1122	1	1121	43.74	6.813	43	24	63	0.000	0.214	NG	32	57
MCHC	g/dL	g/L	1117	12	1105	34.3138	2.45836	34.3138	29	41.4	0	0.263	NG	30.165	39.4
MCH	pg	pg	1121	29	1092	16.8289	2.41906	16.8	9.8	24.2	0.000	0.211	NG	12.9325	21.8
RDWc	%	%	1122	12	1110	20.14	2.35374	19.7	15.6	25.8	0.000	0.468	NG	16.6	24.5
TS/TP	g/dL	g/L	1121	19	1102	70.85	7.006	70	52	92	0.000	0.13	NG	58	84
WBC conc.	10^3/µL	109/L	1122	30	1092	12.4884	3.73442	12.04	2.55	23.16	0.051	0.44	G	6.4425	20.5
Neutrophil (heterophil)	%	%	1114	12	1102	37.1	9.491	36	11	62	0.000	0.326	NG	20	57
Lymphocyte	%	%	1115	8	1107	57.35	10.461	58	30	85	0.000	-0.309	NG	36.7	75
Lymphocyte	10^3/µL	109/L	1121	46	1075	7.6133	3.22802	7.23	2.67	17.02	0	0.64	NG	3.1	15.153
Monocyte	%	%	1115	447	668	2.93	1.028	3	2	6	0	0.992	NG	2	5
Monocyte	10^3/µL	109/L	1120	462	658	0.555	0.19278	0.5	0.31	1.12	0	0.915	NG	0.32	1.04
Eosinophil	%	%	1115	827	288	6.53	1.638	6	5	11	0	1.076	NG	5	11
Basophil	%	%	1111	0	1111	0	0	0	0	0	0	0	G	0	0
Platelet conc. (thrombocytes)	10^3/µL	10^9/L	1112	98	1014	147.1458	54.93	147.1458	20.8	320	0	1.004	NG	64	287
Fibrinogen	g/L	g/L	1118	504	614	5.64	1.895	6	4	12	0	0.974	NG	4	10

Table 2. Blood parameters of the female buffalo population. Descriptive statistics and RIs for hematologic analytes determined by Abacus Junior Vet. except for differential WBC counts performed manually by standard blood smear for all the populations. SD standard deviation. G indicates Gaussian; NG non-Gaussian; Max. maximum; Min. minimum. Confidence intervals were calculated for each RI as recommended by PetitClerc and Solberg (1987).

Hematology RI-FEMALE. Normality test Kolmogorov–Smirnov															
Measurands			**Descriptive Statistics**										**RI Computation**		
Measurand	**Conventional Units**	**SI Units**	**Initial n**	**Removed outliers n**	**Final n[a]**	**Mean[b]**	**SD[b]**	**Median**	**Min**	**Max**	**Normality test p value[b,c]**	**Symmetry test p value[b,d]**	**Distribution[e]**	**LRL of RI[g]**	**URL of RI[g]**
PCV	%	L/L	818	0	818	38.74	6.96	38	19	58	0.000	0.313	NG	27	54
RBC conc.	10^6/µL	10^{12}/L	817	0	817	7.54	1.73	7.34	2.8	12.71	0.000	0.045	NG	4.53	11.62
Hemoglobin	g/dL	g/L	816	0	816	12.81	1.98	12.8	7.4	18.3	0.014	0.186	NG	9.15	17
MCV	fL	fL	835	0	835	45	7	45	28	63	0.000	0.214	NG	32	58
MCHC	g/dL	g/L	826	0	826	34.4	2.38	34.5	29.5	41	0	0.263	NG	30.1	39.3
MCH	pg	pg	809	0	809	17.18	2.38	17.1	10.5	24.2	0.000	0.211	NG	13	21.8
RDWc	%	%	827	0	827	19.97	2.42	19.3	15.6	25.8	0.000	0.468	NG	16.6	24.5
TS/TP	g/dL	g/L	822	0	822	72	7	70	52	90	0.000	0.13	NG	60	84
WBC conc.	10^3/µL	10^9/L	929	93	836	12.53	4.73	11.71	1.07	62.84	0.051	0.44	G	7.03	22.73
Neutrophil (heterophil)	%	%	822	0	822	38	9	37	11	62	0.000	0.326	NG	22	57
Lymphocyte	%	%	826	0	826	56	10	57	30	85	0.000	-0.309	NG	36	74
Lymphocyte	10^3/µL	10^9/L	800	0	800	7.22	3.19	6.56	2.67	17.02	0.000	-0.309	NG	3.03	14.76
Monocyte	%	%	487	0	487	3	1	3	2	6	0	0.992	NG	2	5
Monocyte	10^3/µL	10^9/L	520	0	520	0.54	0.19	0.49	0.3	1.12	0	0.915	NG	0.3	1.03
Eosinophil	%	%	239	0	239	7	2	6	5	11	0	1.076	NG	5	11
Basophil	%	%	239	0	239	0	0	0	0	0	0	0	G	0	0
Platelet conc. (thrombocytes)	10^3/µL	10^9/L	778	0	778	145.03	52.27	131.5	28	320	0	1.004	NG	64	277
Fibrinogen	g/L	g/L	455	0	455	6	2	6	4	12	0	0.974	NG	4	10

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Table 3. Male buffalo population blood parameters. Descriptive statistics and RIs for hematologic analytes determined by Abacus Junior Vet. except for differential WBC counts performed manually by standard blood smear for all the populations. SD standard deviation. G indicates Gaussian; NG non-Gaussian; Max. maximum; Min. minimum. Confidence intervals were calculated for each RI as recommended by PetitClerc and Solberg (1987).

Hematology RI-MALE. Normality test Kolmogorov–Smirnov															
Measurands			**Descriptive Statistics**										**RI Computation**		
Measurand	**Conventional Units**	**SI Units**	**Initial n**	**Removed outliers n**	**Final n[a]**	**Mean[b]**	**SD[b]**	**Median**	**Min**	**Max**	**Normality test p value[b,c]**	**Symmetry test p value[b,d]**	**Distribution[e]**	**LRL of RI[g]**	**URL of RI[g]**
PCV	%	L/L	278	0	278	40.64	7.81	40	20	58	0.000	0.313	NG	25	56
RBC conc.	10^6/µL	10^{12}/L	280	0	280	8.57	1.71	8.49	4.12	12.72	0.000	0.045	NG	5.16	12.13
Hemoglobin	g/dL	g/L	276	0	276	13.38	2.42	13.3	7.4	18.5	0.014	0.186	NG	9	18
MCV	fL	fL	286	0	286	40	6	40	24	57	0.000	0.214	NG	30	53
MCHC	g/dL	g/L	279	0	279	34.05	2.66	33.7	29	41.4	0	0.263	NG	30.2	39.5
MCH	pg	pg	283	0	283	15.84	2.26	15.4	9.8	23.3	0.000	0.211	NG	12.5	21.2
RDWc	%	%	283	0	283	20.67	2.05	20.5	16.3	25	0.000	0.468	NG	16.9	24.4
TS/TP	g/dL	g/L	280	0	280	69	7	70	52	90	0.000	0.13	NG	58	84
WBC conc.	10^3/µL	10^9/L	296	10	286	14	4.73	13.44	2.55	53	0.051	0.44	G	8.14	24.69
Neutrophil (heterophil)	%	%	280	0	280	33	9	33	13	60	0.000	0.326	NG	20	54
Lymphocyte	%	%	281	0	281	62	10	63	32	84	0.000	-0.309	NG	39	76
Lymphocyte	10^3/µL	10^9/L	275	0	275	8.75	3.07	8.6	2.67	17	0.000	-0.309	NG	3.56	15.36
Monocyte	%	%	181	0	181	3	1	3	2	6	0	0.992	NG	2	6
Monocyte	10^3/µL	10^9/L	169	0	169	0.57	0.2	0.52	0.3	1.1	0	0.915	NG	0.3	0.97
Eosinophil	%	%	49	0	49	6	2	6	5	11	0	1.076	NG	5	11
Basophil	%	%		0		0	0	0	0	0	0	0	G	0	0
Platelet conc. (thrombocytes)	10^3/µL	10^9/L	236	0	236	154.13	62.55	134	20.8	317	0	1.004	NG	53	301
Fibrinogen	g/L	g/L	159	0	159	6	2	6	4	12	0	0.974	NG	4	10

Table 4. Calf buffalo population blood parameters. Descriptive statistics and RIs for hematologic analytes determined by Abacus Junior Vet. with the exception of differential WBC counts performed manually by standard blood smear for all the populations. SD standard deviation. G indicates Gaussian; NG non-Gaussian; Max. maximum; Min. minimum. Confidence intervals were calculated for each RI as recommended by PetitClerc and Solberg (1987).

Hematology RI-CALF. Normality test Kolmogorov–Smirnov															
Measurands			**Descriptive Statistics**										**RI Computation**		
Measurand	**Conventional Units**	**SI Units**	**Initial n**	**Removed outliers n**	**Final n[a]**	**Mean[b]**	**SD[b]**	**Median**	**Min**	**Max**	**Normality test p value[b,c]**	**Symmetry test p value[b,d]**	**Distribution[e]**	**LRL of RI[g]**	**URL of RI[g]**
PCV	%	L/L	172	0	172	42.69	8.88	42	20	58	0.000	0.313	NG	27	58
RBC conc.	10^6/µL	10^{12}/L	559	0	559	7.8	1.556	7.76	4.35	12.72	0.000	0.045	NG	5.14	12.57
Hemoglobin	g/dL	g/L	556	0	556	12.93	1.92	13.1	8.6	18.5	0.014	0.186	NG	9.12	18.2
MCV	fL	fL	187	0	187	38	5	37	30	59	0.000	0.214	NG	31	53
MCHC	g/dL	g/L	183	0	183	34.11	2.56	33.5	31	41.4	0	0.263	NG	31	39.9
MCH	pg	pg	185	0	185	15.23	2.23	14.6	10.5	22.5	0.000	0.211	NG	12.1	22
RDWc	%	%	184	0	184	21.67	2.01	21.75	16.5	25.8	0.000	0.468	NG	17.12	25.2
TS/TP	g/dL	g/L	181	0	181	68	7	68	52	90	0.000	0.13	NG	58	80
WBC conc.	10^3/µL	10^9/L	197	10	187	15.14	4.25	14.72	5.87	28.94	0.051	0.44	G	7.52	24.8
Neutrophil (heterophil)	%	%	185	0	185	32	8	31	12	54	0.000	0.326	NG	18	51
Lymphocyte	%	%	182	0	182	63	8	64	36	84	0.000	-0.309	NG	42	76
Lymphocyte	10^3/µL	10^9/L	181	0	181	9.88	3.03	9.8	3.1	17	0.000	-0.309	NG	3.78	16.06
Monocyte	%	%	128	0	128	3	1	3	2	6	0	0.992	NG	2	5
Monocyte	10^3/µL	10^9/L	92	0	92	0.53	0.19	0.46	0.3	0.98	0	0.915	NG	0.3	0.96
Eosinophil	%	%	24	0	24	6	2	6	5	11	0	1.076	NG	5	11
Basophil	%	%		-187	187	0	0	0	0	0	0	0	G	0	0
Platelet conc. (thrombocytes)	10^3/µL	10^9/L	116	0	116	191.8	69.2	180	45	319	0	1.004	NG	97	316
Fibrinogen	g/L	g/L	104	0	104	6	2	6	4	10	0	0.974	NG	4	10

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Table 5. Young animal buffalo population blood parameters. Descriptive statistics and RIs for hematologic analytes determined by Abacus Junior Vet. with the exception of differential WBC counts performed manually by standard blood smear for all the populations. SD standard deviation. G indicates Gaussian; NG non-Gaussian; Max. maximum; Min. minimum. Confidence intervals were calculated for each RI as recommended by PetitClerc and Solberg (1987).

Hematology RI-YOUNG ANIMAL. Normality test Kolmogorov–Smirnov															
Measurands			**Descriptive Statistics**										**RI Computation**		
Measurand	**Conventional Units**	**SI Units**	**Initial n**	**Removed outliers n**	**Final n[a]**	**Mean[b]**	**SD[b]**	**Median**	**Min**	**Max**	**Normality test p value[b,c]**	**Symmetry test p value[b,d]**	**Distribution[e]**	**LRL of RI[g]**	**URL of RI[g]**
PCV	%	L/L	560	0	560	38.83	6.81	39	20	58	0.000	0.313	NG	25.18	53.5
RBC conc.	10^6/µL	10^{12}/L	559	0	559	7.8	1.56	7.76	3.31	12.57	0.000	0.045	NG	4.89	11.04
Hemoglobin	g/dL	g/L	556	0	556	12.93	1.92	13.1	7.4	18.3	0.014	0.186	NG	9	16.8
MCV	fL	fL	567	0	567	43	6	42	29	63	0.000	0.214	NG	35	57
MCHC	g/dL	g/L	560	0	560	34.37	2.52	34.45	29.5	41	0	0.263	NG	30	39.45
MCH	pg	pg	557	0	557	16.7	2.25	16.3	11.6	23.6	0.000	0.211	NG	11.3	21.7
RDWc	%	%	561	0	561	19.91	2.05	19.5	15.6	25.6	0.000	0.468	NG	16.7	24.2
TS/TP	g/dL	g/L	559	0	559	70	4	70	52	90	0.000	0.13	NG	58	84
WBC conc.	10^3/µL	10^9/L	632	64	568	13.36	4.96	12.81	1.07	62.84	0.051	0.44	G	6.52	23.63
Neutrophil (heterophil)	%	%	562	0	562	36	9	36	11	62	0.000	0.326	NG	20	56
Lymphocyte	%	%	564	0	564	59	10	59	30	85	0.000	-0.309	NG	38	76
Lymphocyte	10^3/µL	10^9/L	543	0	543	8.01	3.02	7.87	2.67	17	0.000	-0.309	NG	3.3	15.15
Monocyte	%	%	345	0	345	3	1	3	2	6	0	0.992	NG	2	5
Monocyte	10^3/µL	10^9/L	391	0	391	0.55	0.2	0.5	0.3	1.12	0	0.915	NG	0.3	1.04
Eosinophil	%	%	128	0	128	6	1	6	5	11	0	1.076	NG	5	10
Basophil	%	%	540	0	540	0	0	0	0	0	0	0	G	0	0
Platelet conc. (thrombocytes)	10^3/µL	10^9/L	540	0	540	143.55	52.59	131	20.8	320	0	1.004	NG	53	273
Fibrinogen	g/L	g/L	297	0	297	6	2	5	4	12	0	0.974	NG	4	10

Table 6. Adult buffalo population blood parameters. Descriptive statistics and RIs for hematologic analytes determined by Abacus Junior Vet. with the exception of differential WBC counts performed manually by standard blood smear for all the populations. SD standard deviation. G indicates Gaussian; NG non-Gaussian; Max. maximum; Min. minimum. Confidence intervals were calculated for each RI as recommended by PetitClerc and Solberg (1987).

Hematology RI-ADULTS. Normality test Kolmogorov–Smirnov															
Measurands			**Descriptive Statistics**										**RI Computation**		
Measurand	**Conventional Units**	**SI Units**	**Initial n**	**Removed outliers n**	**Final n[a]**	**Mean[b]**	**SD[b]**	**Median**	**Min**	**Max**	**Normality test p value[b,c]**	**Symmetry test p value[b,d]**	**Distribution[e]**	**LRL of RI[g]**	**URL of RI[g]**
PCV	%	L/L	323	0	323	37.79	6.44	37	19	58	0.000	0.313	NG	27	52
RBC conc.	10^6/µL	10^{12}/L	324	0	324	6.9	1.45	6.75	2.8	12.32	0.000	0.045	NG	4.24	10.65
Hemoglobin	g/dL	g/L	324	0	324	12.5	2	12.3	7.5	18.1	0.014	0.186	NG	9.15	17.2
MCV	fL	fL	326	0	326	49	5	49	32	60	0.000	0.214	NG	36	57
MCHC	g/dL	g/L	321	0	321	34.45	2.24	34.7	29	40.2	0	0.263	NG	30.5	38.8
MCH	pg	pg	309	0	309	18.28	1.89	18.3	11	24.2	0.000	0.211	NG	13.8	21.9
RDWc	%	%	324	0	324	19.61	2.58	18.7	16	24.5	0.000	0.468	NG	16.6	24.3
TS/TP	g/dL	g/L	322	0	322	75	6	76	58	90	0.000	0.13	NG	64	84
WBC conc.	10^3/µL	10^9/L	354	28	326	10.94	3.99	10.12	4.47	42.78	0.051	0.44	G	6.3	20.27
Neutrophil (heterophil)	%	%	314	0	314	49	9	41	16	62	0.000	0.326	NG	24	59
Lymphocyte	%	%	320	0	320	51	10	52	30	79	0.000	-0.309	NG	33	70
Lymphocyte	10^3/µL	10^9/L	311	0	311	5.63	2.5	4.85	2.67	16.72	0.000	-0.309	NG	2.86	12.35
Monocyte	%	%	165	0	165	3	1	3	2	6	0	0.992	NG	2	5
Monocyte	10^3/µL	10^9/L	185	0	185	0.53	0.2	0.48	0.3	1.11	0	0.915	NG	0.3	1.07
Eosinophil	%	%	135	0	135	7	2	6	5	11	0	1.076	NG	5	11
Basophil	%	%	324	0	324	0	0	0	0	0	0	0	G	0	0
Platelet conc. (thrombocytes)	10^3/µL	10^9/L	319	0	319	139.88	45.65	130	28	311	0	1.004	NG	64	257
Fibrinogen	g/L	g/L	194	0	194	6	2	6	4	12	0	0.974	NG	4	10

Most textbooks and previous studies reporting RIs in water buffaloes in South America do not specify a representative sample according to the biological, clinical, or geographic characteristics for the establishment of RIs. In addition, a limitation of some studies is that they only provide a statistical description of data without a statistically representative sample of a country that cannot represent RIs for the population.

Previous investigations have measured hematologic values for water buffalo in various countries in the Eastern Hemisphere, including Australia, Egypt, India, and Italy (1, 25, 33). Most hematologic measurements vary between countries.

All hematologic means inferred from Colombian data differ from the corresponding means reported from other countries (21). One reason for these changes could be geographic location. Previous studies have shown that hematological variables are influenced by geography (11) and by infection (18). Alternatively, the differences could be attributed to the age and sex of the animals as a factor of influence for hematological analytes (29).

One limitation of the study is the potential presence of ticks and other vectors on the animals, which could transmit vector-borne diseases. These infections might have influenced the normal values of the blood parameters, potentially confounding the results. The presence of such vectors introduces variability that could impact the accuracy of the blood parameter measurements, making it challenging to distinguish between changes caused by underlying diseases and those resulting from vector-borne infections (24). This factor needs to be considered when interpreting the study's findings.

The study showed that males had higher RBC, WBC, lymphocyte, and thrombocyte values, and females showed higher MCV and neutrophil parameter values. Differences were found in the age groups: calves showed more hemoconcentration, with high ranges of PCV, MCV, and RBC counts, compared with young animals and adults. White cell lines for calves showed more concentration derivates of a high range of lymphocytes, and thrombocyte ranges were higher than in the other age groups. These findings can be explained by the fact that hematopoietic activity is higher in young animals (12) and the loss of hormonal production in older animals (5).

Young animals showed less total protein, fewer neutrophils and thrombocytes, and higher WBC and lymphocyte counts than the adult population, suggesting that exposure to several microorganisms helps produce more lymphocytes in the WBC count, improves resistance in an infective environment, and produces antibodies (32). The results are consistent with other research on water buffaloes, where the values are related and show the depletion of the cell population when the animals get older (6).

Additionally, the different reference populations did not necessarily contain animals of the same breed or lactation status. Previous studies have demonstrated that hematologic measurements can vary according to the lactation (10) and reproductive status (29) of the species under investigation. Other factors that could account for the observed differences are different management practices, the undefined health and nutritional status of the studied animals, temporal variations, differences in the analytical methods used to collect blood, and differences in the analyzers used to measure hematological variables. Reticulocytes were not detected in any water buffaloes analyzed in this study or other bovine studies. Fibrinogen showed lower values compared with previous studies (32).

The need for buffalo-specific RIs is increasingly being discussed in veterinary medicine. In several countries, cattle values are normally used to analyze the physiological and health statuses of water buffaloes, but in this study, were found differences according to the species; for example, RDWc was found to have high ranges, indicating the relationship with a larger amount of red blood cells, as in other studies of buffaloes (16). Additionally, a higher concentration of WBC counts was found to be associated with a high presence of neutrophils in the studied population. All these values were compared with the parameters given by the analyzer used for bovines and cattle.

In summary, hematologic variables were measured in healthy water buffaloes from several farms according to age and sex, and provided RIs and various other statistical data for each hematologic variable. The information obtained in this study will be particularly helpful to veterinarians and other individuals involved in buffalo breeding. Future research is needed to explore the relationship between vector impacts and blood parameters in buffaloes. Understanding this connection could provide valuable insights into the health and well-being of buffalo populations, potentially leading to improved management and treatment strategies. Comprehensive studies should be conducted to examine how different vectors affect various blood parameters, considering factors such as seasonal variations and geographic locations. This research is crucial for developing effective measures to mitigate the adverse effects of vector-borne diseases in buffaloes.

Conflict of interests

The authors declare no competing interests.

Preprint

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Author contributions

All the authors contributed equally to the research process.

Availability of data and materials

All data required could be accessed by the corresponding author.

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