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Biochemical blood profile in water buffaloes: alterations related to natural infection by *Trypanosoma* spp.

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ABSTRACT

Blood and biochemical profiles of animals can be used to evaluate the physiological state of an individual and relate it to that individual's health. In many countries, water buffaloes are currently evaluated using blood parameters from other bovine species. To accurately interpret the biochemical results from individual animals, species-specific reference ranges should be established. One hundred and twenty-eight water buffaloes, without clinical signs of disease and of different ages, were analyzed, and reference intervals were calculated (95% CI; $p < 0.05$). The data were analyzed according to three age groups (calves, young animals, and adults) and the infection status (infected or uninfected with *Trypanosoma* spp.). All the biochemical values of total serum proteins, aspartate aminotransferase, gamma globulin, urea nitrogen, calcium, and phosphorus were included; these are parameters that are generally affected by parasites or by infection with hemotropic agents. Natural infection with *Trypanosoma* spp. gave rise to differences ($p < 0.05$) in terms of increased calcium and decreased total protein, alkaline phosphatase, and aspartate aminotransferase. Thus, it can be inferred that the infection follows a chronic course in the affected animals, in this case, young animals or young animals.

Keywords: blood parameters, *Bubalus bubalis*, blood chemistry, *Trypanosoma* spp., hemotropic microorganism.

Perfil bioquímico do sangue em búfalos: alterações relacionadas à infecção natural por *Trypanosoma* spp.

RESUMO

Introdução: Os perfis sanguíneos e bioquímicos de animais podem ser usados para avaliar o estado fisiológico de um indivíduo e relacioná-lo com a saúde desse indivíduo. Em muitos países, os búfalos são atualmente avaliados usando parâmetros sanguíneos de outras espécies bovinas. Para interpretar com precisão os resultados bioquímicos de animais individuais, os intervalos de referência específicos da espécie devem ser estabelecidos.

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Método: Foram analisados 128 búfalos, sem sinais clínicos da doença e de diferentes idades, e calculados os intervalos de referência (IC 95%; p < 0,05). Os dados foram analisados de acordo com três faixas etárias (bezerros, novilhos e adultos) e o estado de infecção (infectado ou não por Trypanosoma spp.). Todos os valores bioquímicos de proteínas séricas totais, aspartato aminotransferase, gamaglobulina, nitrogênio ureico, cálcio e fósforo foram incluídos; estes são parâmetros que geralmente são afetados por parasitas ou por infecção com agentes hemotrópicos. Resultados: A infecção natural por Trypanosoma spp. originaram diferenças (p < 0,05) em termos de aumento de cálcio e diminuição de proteína total, fosfatase alcalina e aspartato aminotransferase. Com isso, pode-se inferir que a infecção segue um curso crônico nos animais acometidos, neste caso, animais jovens ou novilhos.

Palavras-chave: parâmetros sanguíneos, *Bubalus bubalis,* química do sangue, *Trypanosoma* spp., microrganismo hemotrópico.

INTRODUCTION

Determination of blood and serum biochemical parameters of animals is essential for confirming clinical diagnoses and estimating the severity of diseases (Piccione 2010). Additionally, these parameters are commonly used as useful indicators of the health and nutritional status of individuals of many species. They therefore help in diagnosing metabolic diseases, managing infertility and resolving low productivity among farm animals (Abd Ellah *et al.* 2014). It is unequivocal that several factors such as age affect the metabolism and, therefore, the blood profile of animals (Çetin *et al.* 2014).

Nonetheless, we have not been able to find many studies investigating the blood composition of the water buffalo (*Bubalus bubalis*). It is known that the genetic makeup of these animals is unique and that it gives them the ability to graze freely at night, thus avoiding high daytime temperatures, and enabling them to manage the stress of temperature differences and sparsity of forage during droughts. When well-fed, buffaloes can produce twice as much milk as any other animal adapted to Latin America (Young *et al.* 2019).

Given the above, the present study was carried out to describe the alterations to hematological and blood chemistry parameters that are present in animals naturally infected with *Trypanosoma* spp., and to assess their variation at different ages and physiological stages.

The buffalo population in Latin America is estimated to be approximately 3,800,000 (Almaguer Pérez 2017). In Colombia, it has grown remarkably, reaching a current number of around 338,567 animals in 2019 (Instituto Colombiano Agropecuario 2022). Water buffaloes are hardy animals that adapt very easily to geographical conditions that are difficult for cattle. Despite their phenotypic resemblance to cattle, they are genetically closer to sheep and goats than to cattle (Young *et al.* 2019). They are economically important due to their multipurpose production capacities, providing meat, milk, and work in areas where modernization is of limited use to agricultural production (Cruz 2007).

Buffaloes are widely distributed in the tropics of the Americas and on the Asian and African continents. They are susceptible to high temperatures and high

rates of solar radiation due to the dark coloration of their skin. These factors give rise to decreased production, and in some reports, these have been found to be associated with alterations at hematological, physiological, reproductive, biochemical, and oxidative levels (Pandey *et al.* 2015).

Blood parameters are frequently used tools for diagnosing different pathological conditions that affect buffaloes. Because of the anatomical similarity between buffaloes and cattle, laboratories in different Latin American countries routinely use the ranges established for cattle. However, this is not recommendable, given the variations in erythrocyte, platelet, leukocyte, and hemoglobin levels, and in agglomerated cell volume, that have been reported by some authors (Çetin *et al.* 2014; Hilali *et al.* 2006; Martini *et al.* 2019). To analyze the blood parameters of naturally infected buffaloes, tropical conditions in South America need to be evaluated, considering the animals' ages and the environmental and geographical conditions (Abd Ellah *et al.* 2014; Torres–Chable *et al.* 2017). The growth in the buffalo population has not been accompanied by any notable growth in research on these animals.

Hemotropic microorganisms such as *Trypanosoma* spp. affect many animals, including buffaloes, and parasites of this genus are transmitted through vectors such as *Tabanus* and *Stomoxys*, among others (Desquesnes *et al.* 2013). The diseases caused by these microorganisms are characterized as acute in young animals and pregnant females, which can die in only a few weeks. However, the form of these diseases in endemic areas is characterized as chronic, causing anemia and renal and liver platelet disorders, which result in animals that present poor body condition, cachexia, and death due to multiorgan damage (Birhanu *et al.* 2015; Desquesnes *et al.* 2013; Hilali *et al.* 2006).

The objective of this study was to determine the blood chemistry values in buffaloes (*Bubalus bubalis*) of different ages that were naturally infected with hemotropic microorganisms such as *Trypanosoma* spp.

MATERIALS AND METHODS

Ethics committee approval

The present study was approved by the ethics committee of CES University, Colombia, in 2018, before the beginning of the study.

Study design and area

The study area was located between the latitudes of 7 .971111 and 8.09583 degrees, and between the longitudes of 75.400278 and 75.5075 degrees. The average altitude was 20 meters above sea level (masl) and the maximum was 150 masl. The average annual temperature was 27 °C and the annual precipitation ranged from 1500 to 2000 mm, distributed bimodally, generally from April to May and from October to November. All the animals lived under the same environmental conditions.

The choice of the study area was based on the Colombian population of water buffaloes. According to the latest livestock census of 2019, the buffalo population in Colombia is around 338,567 animals. Most of the buffaloes in Colombia are found in the departments of Cordoba (25.5%) and Antioquia (16.1%) (Instituto Colombiano Agropecuario 2022). Those areas with the greatest concentrations of buffaloes in Colombia were chosen.

Reference population

A local census was taken in Cordoba and Antioquia, where the largest populations are located, and the total was found to be 55,307 animals. The sample size was calculated from this study population, using the methodology for estimating the global point frequency of a disease in large populations, with a confidence level of 95%, an expected proportion of 50%, and an expected absolute error of 3%. Through this, a sample size of 1068 animals, adjusted to 1225, was obtained. From this sample, a subsample calculation was performed on the total population of 1225 animals with a confidence level of 95%, an expected standard deviation of 30%, and absolute precision of 5%, thereby obtaining a sample size of 125 animals, adjusted to 128.

Fifty-one farms participated in this study, by signing an authorization for the ethics committee. The samples were taken over a period of about eight months. Animals were randomly included according to age and group. All animals that showed lethargy, gestational status, low body condition, and/or externally visible lesions, or that had been treated with antibiotics or antiparasitic in the last three months, were excluded. Blood samples were collected from 128 animals, with a mean age of four years (range: 1-16 years). All these animals were currently vaccinated and dewormed and none of them showed signs of disease during the month before and after blood withdrawal.

Preanalytical procedures

Blood samples of 7 mL were collected aseptically with homogenization, from the coccygeal vein, using vacutainer tubes. Some tubes contained the anticoagulant ethylenediaminetetraacetic acid (EDTA) and others did not have anticoagulants (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, UK). Sample collections were done using an 18-gauge needle and holder. After collection, the blood samples were inverted eight to ten times, refrigerated, and transported to the laboratory of the Colombian Institute of Tropical Medicine (ICMT), located in Envigado, Antioquia, Colombia. When the tubes arrived, the samples were left at room temperature and then analyzed.

Analytical procedure

To visualize hemotropic microorganisms through standard methods (Palmer *et al.* 2015)), all samples were immediately processed for evaluation by means of blood smears. All tubes showing coagulation, hemolysis, lipemia, or jaundice were excluded.

Biochemical blood analysis

From the samples in the tubes without anticoagulant, the serum was separated at 3,000 rpm for 10 minutes, and then 500 µl of sample from each animal was deposited in the automated analyzer A15 (Biosystems®). This automated analyzer integrates biochemistry and turbidimetry reagents, with management software that makes it possible to control the samples in relation to the reagents. It enables measurement of the parameters of the basic chemical profile of kidney and liver function such as phosphorus, calcium, alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatinine (CRE), blood urea nitrogen (BUN) and gamma-glutamyl-transferase (GGT). These parameters were chosen

in accordance with the literature, which shows the most important biochemical values for making veterinary diagnoses, in order to demonstrate the functioning of the main organs in the animal (Barger and MacNeill 2015).

To visualize hemotropic microorganisms, a blood smear was made using a drop of blood from the EDTA tube, which was stained with Hemacolor® (15) and then observed under a 100x microscope by two independent laboratory bacteriological observers. Both observers were blinded to the results (Walker *et al.* 1990).

Statistical analysis

The RIs were determined in accordance with the American Society for Veterinary Clinical Pathology (ASVCP) guidelines (sample size: > 50) (Friedrichs *et al.* 2012). The Kolmogorov–Smirnov test was used to evaluate the normal distribution of values with $p < 0.05$ (reference value advisor). The Dixon and Tukey test (3 x IQR, 1.5 x IQR) was used to identify outliers and suspicious outliers (reference value advisor). The 95% reference intervals were calculated by eliminating the top and bottom 2.5% of the range for each hematological parameter, to give the 2.5 and 97.5 percentiles. The 90% confidence interval (CI) was calculated for each reference limit to determine whether its precision was sufficient for clinical use. The analysis was performed using untransformed data. The correlation analysis was performed using the Student's t-test (Student 1908) and Levene's test (Gastwirth *et al.* 2009). A significant value of $p < 0.05$ was taken.

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

RESULTS

Description of the population and study area

The animals were randomly included according to age and group. All animals showing lethargy, gestational status, low body condition, and/or externally visible lesions, or that had been treated with antibiotics or antiparasitics in the last three months, were excluded. One hundred and twenty-eight animals were included in the study, of which 75.8% (97/128) were female and 24.2% (31/128) were male; 50% (64/128) were young animals, 19.5% (25/128) were calves and 4.7% (6/128) were adults.

Blood biochemistry

The blood biochemistry results are presented in tables 1, 2, and 3. Table 1 shows the RI for the entire population included in the study. Table 2 shows RIs according to age group. Table 3 shows the results from *Trypanosoma* spp.-positive animals. Blood smear of positive animals is shown in figure 1 with red blood cell alteration descriptions on table 4. Normal ranges used in cattle are shown as a normal way to analyze biochemistry parameters in water buffalos in Colombia.

Calcium, phosphorus, and creatinine values were similar between ages and cattle ranges, showing uniformity between standard deviation and means. Ureic nitrogen showed lower ranges, approximately half compared to cattle values, we have not found significant differences between ages.

TABLE 1. Blood biochemical parameters of the total buffalo population

Reference intervals for the total population, Kolmogorov-Smirnov normality test

Measurements			Descriptive statistics								RI computation				
Analytes	Conventional units	SI units	Initial no.	Extreme values withdrawn	Final no.	Meanb	SDb	Median	Min	Max	Normality test p-valueb,c	Symmetry p-valueb,d	Distributione	LRL of RIg	URL of RIg
Calcium	mg/dL	mmol/L	128	22	106	9.3	2.15	9.38	3.63	15.42	0.063	-0.154	G	4.201	13.7738
Phosphorus	mg/dL	mmol/L	128	10	118	7.3693	1.92	7.36	2.21	12.13	0.2	0.032	G	3.5185	11.198
Ureic nitrogen	mg/dL	mmol/L	128	19	109	14.9	6.02	14.2	6.6	6.775	0.08	0.77	G	6.775	30.375
Creatinine	mg/dL	µmol/L	128	1	127	1.655	0.44396	1.63	0.58	2.89	0.2	0.115	G	0.854	2.614
AST	U/L	U/L	127	9	118	109.34	33.498	110	16	204	0.2	0.059	G	32.75	187.2
GGT	U/L	U/L	118	16	102	20.01	7.672	19	8	42	0.035	0.411	NG	8	35.43
Alkaline phosphatase	U/L	U/L	128	36	92	123.46	51.177	112.5	60	256	0	0.953	NG	61	251.1

Descriptive statistics and RIs for blood chemical analytes. SD, standard deviation; G, Gaussian; NG, non-Gaussian; Max, maximum; Min, minimum; LRL, lower referral limit; and URL, upper referral limit. Confidence intervals were calculated for each RI, as recommended by PetitClerc and Solberg (1987).
Source: own elaboration.

TABLE 2. Blood biochemical parameters of the population according to age

Reference intervals for the adult population, Kolmogorov-Smirnov normality test

Measurements			Descriptive statistics								RI computation				
Analytes	Conventional units	SI units	Initial no.	Extreme values withdrawn	Final no.	Meanb	SDb	Median	Min	Max	Normality test p-valueb,c	Symmetry p-valueb,d	Distributione	LRL of RIg	URL of RIg
Calcium	mg/dL	mmol/L	128	22	106	9.26	1.99	9.36	4.12	13.36	0.063	-0.154	G	5.64	13.19
Phosphorus	mg/dL	mmol/L	128	10	118	7.38	1.88	7.4	2.21	11.1	0.2	0.032	G	4.64	10.45
Ureic nitrogen	mg/dL	mmol/L	128	19	109	16.15	5.46	15.5	8	33.5	0.08	0.77	G	9.6	28.2
Creatinine	mg/dL	µmol/L	128	1	127	1.72	0.39	1.73	0.92	2.47	0.2	0.115	G	0.84	2.65
AST	U/L	U/L	127	9	118	107	24	110	45	155	0.2	0.059	G	55	144
GGT	U/L	U/L	118	16	102	20	7	19	8	36	0.035	0.411	NG	8	33
Alkaline phosphatase	U/L	U/L	128	36	92	117	43	114	60	214	0	0.953	NG	64	202

Reference intervals for young/reared population, Kolmogorov-Smirnov normality test

Measurements			Descriptive statistics								RI computation				
Analytes	Conventional units	SI units	Initial no.	Extreme values withdrawn	Final no.	Meanb	SDb	Median	Min	Max	Normality test p-valueb,c	Symmetry p-valueb,d	Distributione	LRL of RIg	URL of RIg
Calcium	mg/dL	mmol/L	128	22	106	8.95	2.55	8.73	3.63	15.42	0.063	-0.154	G	4.28	13.4
Phosphorus	mg/dL	mmol/L	128	10	118	7.45	2.06	7.27	3.55	12.13	0.2	0.032	G	4.3	11.18
Ureic nitrogen	mg/dL	mmol/L	128	19	109	14.8	6.13	13.9	6.8	31.2	0.08	0.77	G	7.14	26.4
Creatinine	mg/dL	µmol/L	128	1	127	1.58	0.45	1.58	0.92	2.17	0.2	0.115	G	0.58	2.89
AST	U/L	U/L	127	9	118	113	36	111	16	205	0.2	0.059	G	67	187
GGT	U/L	U/L	118	16	102	21	8	21	9	42	0.035	0.411	NG	10	32
Alkaline phosphatase	U/L	U/L	128	36	92	123	51	109	61	255	0	0.953	NG	69	237

Reference intervals for steers, Kolmogorov-Smirnov normality test

Measurements			Descriptive statistics								RI computation				
Analytes	Conventional units	SI units	Initial no.	Extreme values withdrawn	Final no.	Meanb	SDb	Median	Min	Max	Normality test p-valueb,c	Symmetry p-valueb,d	Distributione	LRL of RIg	URL of RIg
Calcium	mg/dL	mmol/L	128	22	106	9.96	1.45	10.13	5.79	12.99	0.063	-0.154	G	7.9	12.06
Phosphorus	mg/dL	mmol/L	128	10	118	7.19	1.75	7.53	2.29	10.13	0.2	0.032	G	4.66	9.13
Ureic nitrogen	mg/dL	mmol/L	128	19	109	13.1	6.44	9.3	6.6	26.7	0.08	0.77	G	6.7	21.6
Creatinine	mg/dL	µmol/L	128	1	127	1.71	0.49	1.61	0.94	2.71	0.2	0.115	G	0.97	2.45
AST	U/L	U/L	127	9	118	107	43	110	23	204	0.2	0.059	G	33	176
GGT	U/L	U/L	118	16	102	18	8	15	8	35	0.035	0.411	NG	9	34
Alkaline phosphatase	U/L	U/L	128	36	92	132	62	122	61	256	0	0.953	NG	62	243

Descriptive statistics and RI for blood chemical analytes. SD, standard deviation; G, Gaussian; NG, non-Gaussian; Max, maximum; Min, minimum; LRL, lower referral limit; and URL, upper referral limit. Confidence intervals were calculated for each RI, as recommended by PetitClerc and Solberg (1987).
Source: own elaboration.

TABLE 3. Biochemical blood parameters of the buffalo population, according to age, compared with the standard evaluation values from the A15 Biosystems® equipment

Reference intervals for the total population					Standards	
Measurements			RI computation		Values in cattle	
Analyte	Conventional units	SI units	LRL of RI[g]	URL of RI[g]	LRL of RI[g]	URL of RI[g]
Calcium	mg/dL	mmol/L	4.201	13.7738	7.9	10
Phosphorus	mg/dL	mmol/L	3.5185	11.198	4.6	9
Ureic nitrogen	mg/dL	mmol/L	6.775	30.375	21.4	55.6
Creatinine	mg/dL	µmol/L	0.854	2.614	0.5	1.1
AST	U/L	U/L	32.75	187.2	48	100
GGT	U/L	U/L	8	35.43	20	48
Alkaline phosphatase	U/L	U/L	61	251.1	29	99

LRL, lower referral limit; and URL, upper referral limit, for both species.
Source: own elaboration.

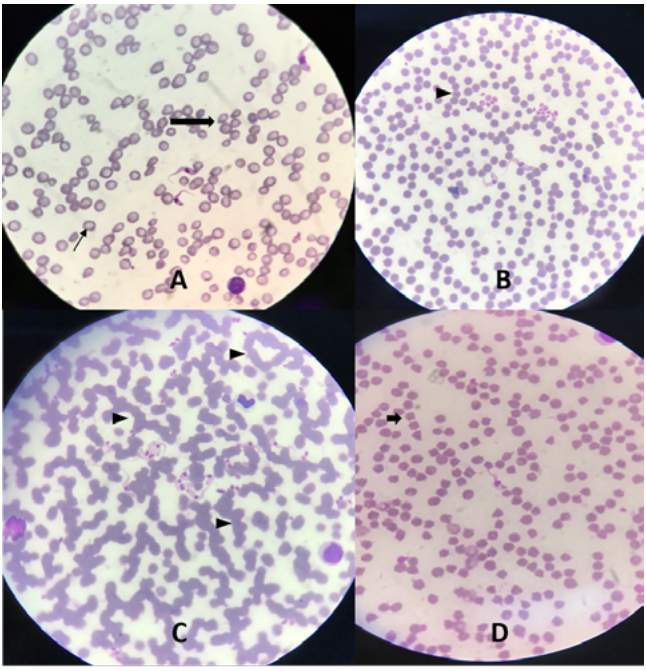

FIGURE 1. Blood smear representation of positive animals. A. Arrow showing red blood cells with hypochromia, abnormal forms. *Trypanosoma* spp. presence B. Rouleaux formation and *Trypanosoma* sp. C. Arrow showing rouleau formation and *Trypanosoma* sp. clotted. D. Arrow schistocytes and *Trypanosoma* sp.

Source: own elaboration.

The aspartate aminotransferase (AST) values changed notably between ages. Adults generally presented a lower range of 55 (U/L), compared with young/reared animals that presented a range of 67 (U/L). Calves, on the other hand, had a level of 33 (U/L). Regarding the upper range, there was a large range of 187 (U/L) among young/reared animals, followed by calves with 176 (U/L) and adults with 144 (U/L). General population of the study shown higher RI, compared with cattle RI.

GGT values show no differences according to age and cattle, PA shows a higher range compared to cattle values. The upper range increase in almost all the population of the study until 251 mg/dL, and in cattle normal range is until 99 mg/dL. According to the values used in cattle for PA, we have seen differences in the upper range going to 243 mg/dL in young animals. In cattle, the normal range is 20 to 48 mg/dL, but in this study, we found 61 to 251.1 mg /dl, howing a big difference.

Results from blood smears and capillary concentration

This test detected that 18% (23/128) were positive for *Trypanosoma* spp. without clinical signs. All tests were negative for other hemotropic organisms. Among the female buffaloes tested, 52.17% (12/23) were positive, and among the males, 47.82% (11/23). The age group that was most frequently positive was young/reared animals with 69.56% (16/23), then calves with 17.39% (4/23), followed by adult female buffaloes 26.1% (6/23), and bulls 4.3% (1/23).

A significant difference ($p > 0.05$) between the sexes was found regarding positivity to *Trypanosoma* spp. Males contracted the disease more naturally. There was no relationship between age group and infection in this research.

Changes to biochemical parameters in buffaloes naturally infected with *Trypanosoma* spp.

A statistically significant relationship ($p < 0.05$) was found regarding the blood calcium levels in infected animals. These levels were found to be increased in infected animals, with a mean of 18.06 versus a mean of 9.05 in healthy animals. For alkaline phosphatase, higher values were found in animals that were positive for the infection, compared with the rest of the population. For alkaline phosphatase, a mean of 86.8 was found in infected animals, compared with 12.2 in healthy animals. For GGT, a mean of 14.43 was found, compared with 20.2 overall. However, GGT values were observed not to be lower than the lower end of the ranges for the total population analyzed and cattle values

For UN values and ranges, we have seen an evident increase in the ranges and mean compared with not infected animals of the study.

The distribution in relation to the rest of the population can be seen in figure 2. For calcium, not only there was an increase in the mean shown, but also the ranges stood out from the upper end of the ranges for the total population, as seen in figure 3. Total proteins showed a decreasing trend with infection by *Trypanosoma* spp., compared with the uninfected population, data not included in tables.

There was no statistically significant relationship between the presence of the infectious agent and the other analytes evaluated.

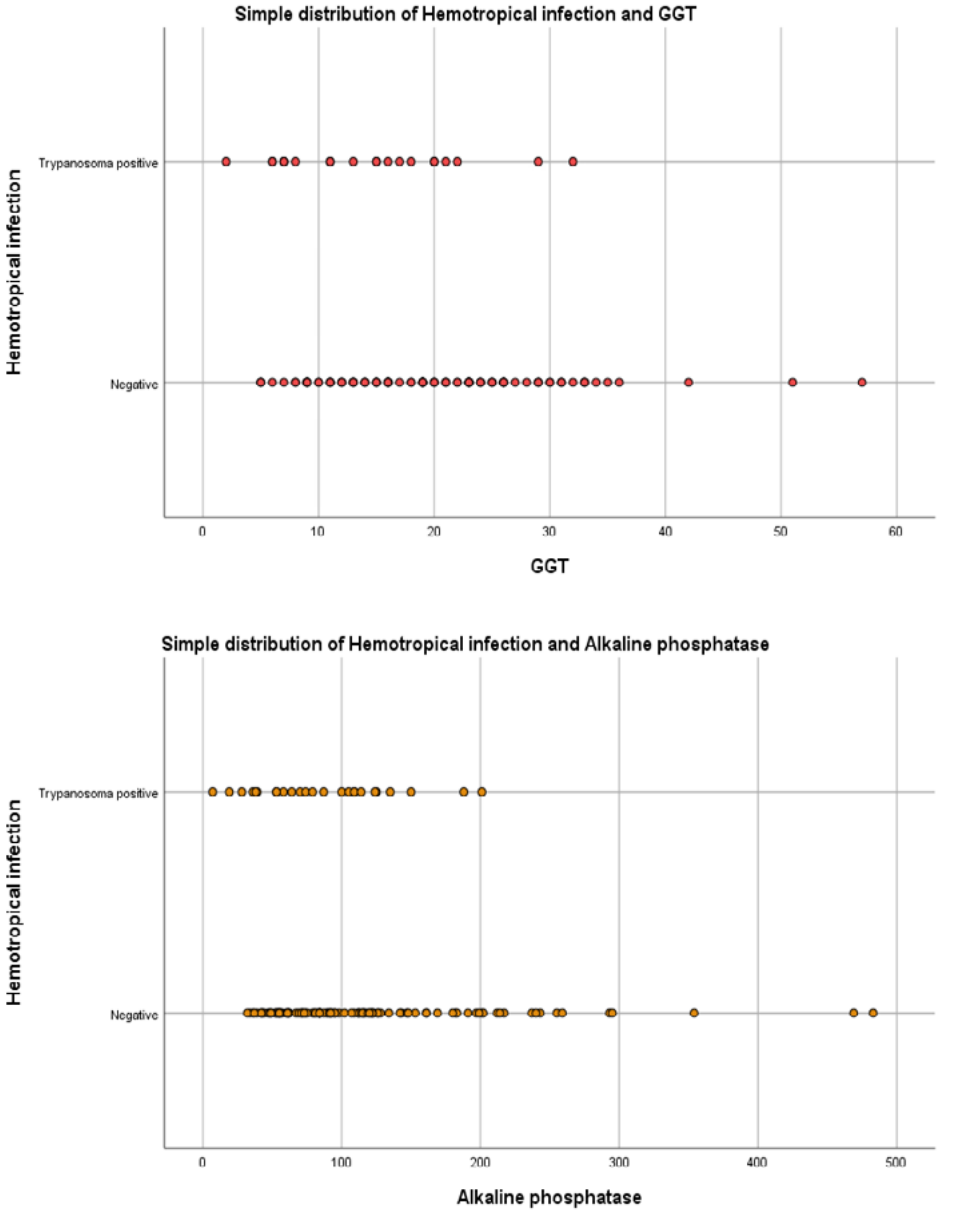

FIGURE 2. Displacement of the analytes alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) in the population infected by *Trypanosoma* spp. Note that although the values do not decrease in relation to the range of the general population, the location of the cases is observed to be among the lower values.

Source: own elaboration.

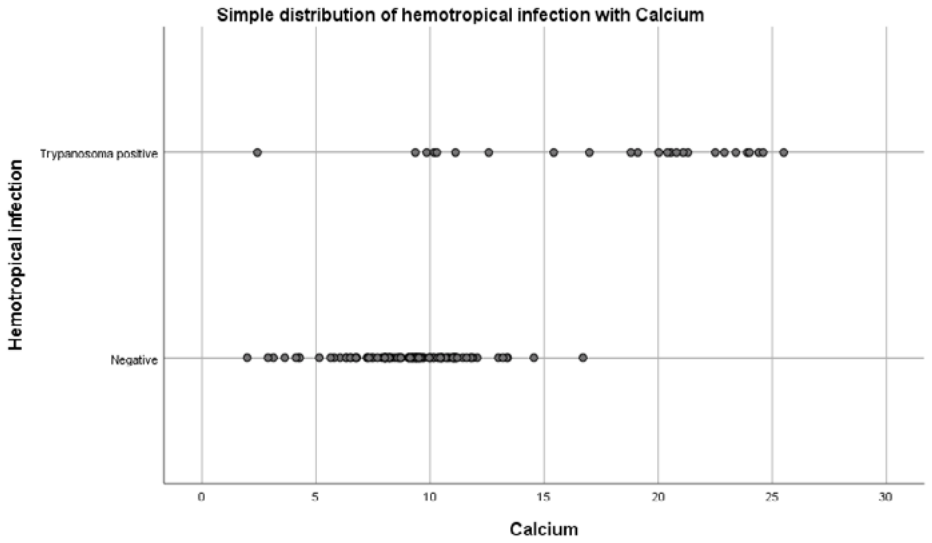

FIGURE 3. Simple distribution diagram. Distribution of calcium findings in blood in animals naturally infected by *Trypanosoma* spp. It can be seen that animals with positivity generally had a shift to the right, through which an increase in analyte values was demonstrated.

Source: own elaboration.

DISCUSSION

To our knowledge, the present study was one of the attempts to establish RIs for blood biochemistry analytes in water buffalo (*Bubalus bubalis*) in South America, done in accordance with the recently published ASVCP QALS committee guidelines for the determination of RIs in veterinary species (Arnold *et al.* 2019), in comparison with the different ranges used in cattle.

Overall, the data gave us a unique opportunity to make comparisons between the naturally infected animals and the results of healthy animals. Variations between data sets were minimized by comparing ages and natural infection with *Trypanosoma* spp. For the RI analysis, animals that were positive for *Trypanosoma* spp. were analyzed through blood smears. The stages of lactation or gestation could not be compared all the animals in gestation were excluded. The data of this study give the possibility to see some differences according to Buffalo species and cattle.

Most of the previous textbooks and studies reporting on RIs in water buffaloes in South America have not specified the biological, clinical, and geographical characteristics for establishing RIs.

The need for buffalo-specific RIs is increasingly discussed within veterinary medicine. In several countries, values taken from cattle are routinely used for analyzing the physiological and health status of water buffaloes. However, in our study, we found several differences according to the species: most of the parameters had a wider range than in cattle, as shown in table 3. All these values for buffaloes were

compared with the parameters given by the A15 Biosystems® analyzer, which is used for cattle. Among the animals in the study, 50% were young animals and 25% were Mediterranean buffalo. The rest of the animals were crosses and mixtures of age groups.

Within tables 1 and 2, calcium and phosphorus showed uniformity of results between sexes and ages and also similar to the values found in cattle. Similar to what was evaluated by (Abd Ellah *et al.* 2014; Hilali *et al.* 2006), where they found values of 9.7-12.4 mg/dL and in our study of 4-13 mg/dL showing a slight decrease in the lower range but the upper is comparable.

In the case of creatinine, the values were much higher than those reported in the literature for cattle with a range between 0.8-2.6 mg/dL, this result is highly similar to that found by (Abd Ellah *et al.* 2014; Amle *et al.* 2014), where they show ranges of 1-2 mg/dL. dL in the study population in South Africa, but compared to that of cattle, which ranges from 0.5-1.1 mg/dL in healthy animals, it is relatively high. Urea nitrogen presented lower ranges than those reported in cattle and a greater amplitude in the measurement range with units of 21.4-55.6 mg/dL and a standard deviation of 6.4, although this deviation decreased a little in adult animals.

AST values were lower than those described in cattle. But they are comparable to those found by (Abd Ellah *et al.* 2014; Fidelis Junior *et al.* 2016), where they show a range of 78-132 mg/dL. Consistent with the findings in the present study, but reinforcing the difference with those described in livestock ranging from 48-100 mg/dL. Regarding GGT, in general, it presented uniformity and correlation with the values reported in cattle. Also reported in the same buffalo hematology articles.

Trypanosomiasis is caused by the genus *Trypanosoma* spp. It is a vector-borne disease that commonly affects water buffaloes and cattle in tropical and subtropical regions. Although buffaloes that showed signs of disease, or had evidence of parasitic infections, were omitted from our study, natural infections were present and were usually asymptomatic. Furthermore, parasitemia is not always visible on blood smears examined under a microscope (Desquesnes *et al.* 2013). Therefore, a subset of the animals in our sample population may have had *Trypanosoma* spp. infection. The differences in the state of infection due to *Trypanosoma* spp. could be another reason why the blood biochemistry values reported in our study differed from those of other studies. This could also explain the differences observed between the farms used in our study.

In alkaline phosphatase, differences are observed with the established ranges in cattle, extreme data were found up to 243 mg/dl without any sign of disease, and these high data were found mainly in young animals. The study data is 61-251.1 mg/dL and those reported in cattle are 29-99 mg/dL. Marking a remarkable difference between the species. However, relating studies of the species we find that higher data are found within the species than those reported with ranges of 0-488 mg/dL(Abd Ellah *et al.* 2014)

Regarding the alterations found in animals naturally infected by *Trypanosoma* sp. We found a very marked increase in urea nitrogen. Comparing with the rest of the evaluated population. Although it is a little more related to the ranges managed in cattle, it is convenient to review the physiological process of the

infection that can lead to the alteration and increase of the enzyme, similar conditions were found in infection by *T. congolence* in buffaloes (Reddy *et al.* 1988). In turn, changes in the decrease of AST and GGT were evident with respect to the general population. Similar to what (Fidelis Junior *et al.* 2016), reports, where the experimental infection in cattle with *Trypanosoma vivax* shows a significant decrease in both enzymes.

According to these findings, it should be considered to separate the ranges of blood biochemistry between the species of buffalo and cattle, since in certain analytes they present a constant difference in the studies and it must be taken into account to evaluate the degree of real alteration that they may present in the veterinary clinic. In addition, it is necessary to review physiological changes related to infection by *Trypanosoma* sp. and urea nitrogen, as a possible marker of infection.

CONCLUSIONS

In summary, we measured the blood biochemical variables of healthy water buffaloes on various farms according to their age and presence or absence of natural infection with *Trypanosoma* spp., and provided RIs and various other statistical data for each variable. It should be considered to separate the ranges of blood biochemistry between the species of buffalo and cattle. In addition, it is necessary to review physiological changes related to infection by *Trypanosoma* sp. and urea nitrogen, as possible markers of infection.

The information obtained in this study will be particularly useful for veterinarians and others involved in buffalo production.

CONFLICT OF INTERESTCONFLICT OF INTEREST

We declare that the authors do not have a conflict of interest.

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REFERENCES

Abd Ellah MR, Hamed MI, Ibrahim DR and Rateb HZ. 2014. Serum biochemical and hematological reference intervals for water buffalo (*Bubalus bubalis*) heifers. Journal of the South African Veterinary Association. 85(1):7. Available in: http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S1019-91282014000100001

Adewoyin AS and Nwogoh B. 2014. Peripheral blood film. A review. Annals of Ibadan Postgraduate Medicine. 12(2):71-79. Available in: https://pubmed.ncbi.nlm.nih.gov/25960697/

Almaguer–Pérez Y. 2007. El búfalo, una opción de la ganadería. REDVET. 81-23. Available in: https://www.redalyc.org/pdf/636/63612734014.pdf

Amle M, Patodkar V, Department of Veterinary Physiology, KNP College of Veterinary Science, Shirval, Satara, Maharashtra, India, Shelar R and Birade H. 2014. Serum Biochemical Levels of Repeat Breeder Cross Bred Cows under Rural Condition of Satara District of Maharashtra. International Journal of Advanced Veterinary Science and Technology. 3(1):109-113. https://doi.org/10.23953/cloud.ijavst.193

Arnold JE, Camus MS, Freeman KP, Giori L, Hooijberg EH, Jeffery U, Korchia J, Meindel MJ, Moore AR, Sisson SC, Vap LM and Cook JR. 2019. ASVCP Guidelines: Principles of Quality Assurance and Standards for Veterinary Clinical Pathology (version 3.0): Developed by the American Society for Veterinary Clinical Pathology's (ASVCP) Quality Assurance and Laboratory Standards (QALS) Committee.

Veterinary Clinical Pathology. 48(4):542-618. https://doi.org/10.1111/vcp.12810

Barger AM and MacNeill AL. 2015. Clinical pathology and laboratory techniques for veterinary technicians. Available in: https://onlinelibrary.wiley.com/doi/book/10.1002/9781119421351

Birhanu H, Fikru R, Said M, Kidane W, Gebrehiwot T, Hagos A, Alemu T, Dawit T, Berkvens D, Goddeeris BM y Büscher P. 2015. Epidemiology of *Trypanosoma evansi* and *Trypanosoma vivax* in domestic animals from selected districts of Tigray and Afar regions, Northern Ethiopia. Parasites & Vectors: 8(1). https://doi.org/10.1186/s13071-015-0818-1

Çetin N, Bekyürek T and Çetin E. 2014. Effects of sex, pregnancy and season on some hematological and biochemical blood values in angora rabbits. Scandinavian Journal of Laboratory Animal Sciences. 36:155-162. https://doi.org/10.23675/SJLAS.V36I2.180

Cruz LC. 2007. Trends in buffalo production in Asia. Italian Journal of Animal Science. 6(sup2):9-24. https://doi.org/10.4081/ijas.2007.s2.9

Desquesnes M, Holzmuller P, Lai DH, Dargantes A, Lun ZR and Jittaplapong S. 2013. *Trypanosoma evansi* and Surra: A Review and Perspectives on Origin, History, Distribution, Taxonomy, Morphology, Hosts, and Pathogenic Effects. BioMed Research International. 1-22. https://doi.org/10.1155/2013/194176

Fidelis Junior OL, Sampaio PH, Machado RZ, André MR, Marques LC and Cadioli FA. 2016. Evaluation of clinical signs, parasitemia, hematologic and biochemical changes in cattle experimentally infected with Trypanosoma vivax. Revista Brasileira de Parasitologia Veterinária. 25(1):69-81. https://doi.org/10.1590/S1984-29612016013

Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton R M, Barnhart KF and Blanco–Chávez J. 2012. ASVCP reference interval guidelines: Determination of de novo reference intervals in veterinary species and other related topics. Veterinary Clinical Pathology. 41(4):441-453. https://doi.org/10.1111/vcp.12006

Gastwirth JL, Gel YR and Miao W. 2009. The Impact of Levene's Test of Equality of Variances on Statistical Theory and Practice. Statistical Science. 24(3). https://doi.org/10.1214/09-STS301

Hilali M, Abdel-Gawad A, Nassar A Abdel-Wahab A. 2006. Hematological and biochemical changes in water buffalo calves (Bubalus bubalis) infected with Trypanosoma evansi. Veterinary Parasitology. 139(1-3):237-243. https://doi.org/10.1016/j.vetpar.2006.02.013

Instituto Colombiano Agropecuario (ICA). 2022. Censo bufalino en Colombia. Censo pecuario. Available in: https://www.ica.gov.co/areas/pecuaria/servicios/epidemiologia-veterinaria/censos-2016/censo-2018

Martini V, Bernardi S, Russo V, Guccione J, Comazzi S and Roperto S. 2019. Blood lymphocyte subpopulations in healthy water buffaloes (Bubalus bubalis, Mediterranean lineage): Reference intervals and influence of age and reproductive history. Veterinary Immunology and Immunopathology. 211:58–63. https://doi.org/10.1016/j.vetimm.2019.04.007

Palmer L, Briggs C, McFadden S, Zini G, Burthem J, Rozenberg G, Proytcheva M Machin SJ. 2015. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. International Journal of Laboratory Hematology. 37(3):287-303. https://doi.org/10.1111/ijlh.12327

Pandey V, Nigam R, Jaiswal AK, Sudan V, Singh RK and Yadav PK. 2015. Haemato-biochemical and oxidative status of buffaloes naturally infected with *Trypanosoma evansi*. Veterinary Parasitology. 212(3-4):118-122. https://doi.org/10.1016/j.vetpar.2015.07.025

Piccione G. 2010. Reference values for some haematological, haematochemical, and electrophoretic parameters in the Girgentana goat. Turk J Vet Anim Sci. 34:197-204. https://doi.org/10.3906/vet-0902-1

Reddy GR, More T, Sharma SP and Singh LN. 1988. The oxidant defense system in water-buffaloes (*Babalus bubalis*) experimentally infected with Anaplasma marginate. Veterinary Parasitology. 27(3-4):245-249. https://doi.org/10.1016/0304-4017(88)90039-8

Student. (1908). The Probable Error of a Mean. *Biometrika*, *6*(1), 1. https://doi.org/10.2307/2331554

Torres–Chable OM, Ojeda–Robertos NF, Chay–Canul AJ, Peralta–Torres JA, Luna–Palomera C, Brindis–Vázquez N, Blitvich BJ, Machain–Williams C, García–Rejon JE, Baak–Baak CM, Dorman KS and Alegría–López MA. 2017. Hematologic RIs for healthy water buffaloes (*Bubalus bubalis*) in Southern Mexico. Veterinary Clinical Pathology. 46(3):436-441. https://doi.org/10.1111/vcp.12508

Walker HK, Hall WD and Hurst JW (Eds.). 1990. Clinical methods: The history, physical, and laboratory examinations (3rd ed). Butterworths.

Young R, Lefevre L, Bush SJ, Joshi A, Singh SH, Jadhav SK, Dhanikachalam V, Lisowski ZM, Iamartino D, Summers KM, Williams J L, Archibald AL, Gokhale S, Kumar S and Hume DA. 2019. A Gene Expression Atlas of the Domestic Water Buffalo (*Bubalus bubalis*). Frontiers in Genetics. 10: 668. https://doi.org/10.3389/fgene.2019.00668

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