Animal Health

Original

Relationship Between the Hematological Values in Hemogram, and the Parasitic Burden Found in Grazing Pelibuey Sheep

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Received: September, 2023; Accepted: October, 2023; Published: December, 2023.

ABSTRACT

Background: Cuba's subtropical climate favors the circulation of agents that cause gastroenteric parasitic diseases, a serious health problem in sheep raising. **Aim.** To determine the relationship between hematologic variables and the parasitic burden on different farms. **Materials and methods:** This research study took place between January and June, 2021. A total of 294 adult Pelibuey sheep were included in the study, all of which had a coprological test, and hemogram with differential. One-way ANOVA was used to study the variables per production unit. HSD Tukey multiple comparison test was used as well. Pearson correlation was performed to determine the relationship between the variables included; the hematological variables with the greatest impact on parasites were compared. **Results:** The animals considered resilient showed their hematological values within the normal ranges, despite the different parasitic burdens with *Haemonchus contortus.* The correlation analysis of highly parasitized animals showed a positive correlation between the mean corpuscular hemoglobin concentration (CHCM) and the hemoglobin concentration (r'=0.812**). The Hb concentration showed a positive correlation with the hematocrit (Hct) (r=0.775**), whereas the eggs per feces gram test was negative (r= -0.430**). **Conclusions:** The correlation coefficient for hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, lymphocytes, and neutrophiles was more visible in the animals infested with a parasitic burden below 800 eggs per gram.

Keywords: resilient animals, hemogram in sheep, hematological values in sheep, parasitic burden (*Source: MESH*)

Citations (APA)
Torres Rodríguez, A., Izquierdo Pérez, N., Arenal Cruz, A., & López Zaldivar, Y. (2023). Relationship Between the Hematological Values in Hemogram, and the Parasitic Burden Found in Grazing Pelibuey Sheep *Journal of Animal Prod*, *35*(3). https://rpa.reduc.edu.cu/index.php/rpa/article/view/e4575

INTRODUCTION

Parasitic diseases affect the productivity of grazing sheep and are considered one of the main problems faced by this species worldwide (Charlier *et al*., 2020). Parasitic infections are the leading cause of economic losses in Latin America and other livestock regions of the tropics and subtropics worldwide (Miller *et al.*, 2012), where climatic factors favor the development of parasitic infections (Carvalho *et al*., 2012).

Apparently healthy animals with regular parasitic loads throughout the year stop gaining weight, and many of them experience a decline in productivity, causing serious economic losses for farmers, as noted by Noriega-Burbano (2018).

Cuba's climate is subtropical, permitting year-round circulation of the causative agents of parasitic diseases, especially those responsible for gastroenteric parasitosis. These infections pose a serious health problem in sheep farming, as they reduce productivity efficiency, cause anemia, weight loss, stunted growth, and can even lead to death (Soca *et al*., 2005).

The use of blood is an effective diagnostic tool for identifying an animal's physiological responses, as it can reveal important information about its nutritional health, well-being, and physical condition (Couta, 2010). According to Voigt (2003), the most common application of a hematology laboratory is to monitor an animal's overall health, assess its general ability to transport oxygen, and evaluate its capacity to defend itself against infectious agents. In this way, the combination of medical history, physical examination, and other laboratory results helps create a comprehensive diagnosis of diseases caused by parasitic infections.

Blood is highly sensitive to environmental changes, so any quantitative and morphological change in blood cells is directly linked to the physiological or pathological state of the animal (Chandra *et al.*, 2012). The pathogenic actions of gastrointestinal parasites directly influence blood parameters, becoming noticeable in hemoglobin and hematocrit. The hemogram, due to the ease of obtaining a blood sample, its low cost, and the variety of data it provides, is one of the most widely used collateral analyses when laboratory diagnostic assistance is required (Couta, 2010).

Variations in the physiological state of animals affect hematological profiles. Gestation, lactation period, age, and sex have been mentioned in different animal species (cattle, sheep, goats, among others) as factors causing variations in normal hematological values (Guzmán & Callacná, 2013). Additionally, the levels of parasitic infestation by digestive strongyles are negatively correlated with hematological parameters such as hematocrit value (Hct). Measuring both Hct and hemoglobin (Hb) serves as a good indicator of the presence of hematophagous parasites (Morales *et al.*, 2001).

In Cuba, sheep farming has evolved from a means of family subsistence to a livestock exploitation system, with the goal of meeting the growing demands of the population. This paradigm shift leads to the modification of the research profiles of the research and development institutions in the field, resulting in the breakdown of rigid work schemes (Arece *et al*., 2015).

For the control of diseases in sheep, more sensitive techniques have been introduced in coprological diagnosis in recent years, such as the McMaster and Mini-FLOTAC techniques (Rinaldi *et al*., 2014; Casado *et al*., 2020).

McMaster is a globally recognized and widely used technique in veterinary laboratories, as it can be modified based on the volume of feces to be examined. This technique also allows for the modification of the flotation solution to be used, depending on the parasite being diagnosed (Bosco *et al*., 2014).

The existing diagnostic framework studies today are few and limited. So the relationship between egg count in feces has not been studied comprehensively, only its correlation with hemoglobin and microhematocrit as hematological parameters. The relationship between egg count and the complete hemogram could provide invaluable information about the animal's health status, ensuring more accurate and confirmatory diagnoses of the presence of gastrointestinal nematodes.

Considering the criteria previously described by different authors, this study pursued following aim: To establish the relationship between the hematological values of the hemogram and the parasitic load in Pelibuey sheep production units under grazing conditions.

MATERIALS AND METHODS

This research study took place between January and June 2021. A total sheep population of 294 adult male Pelibuey animals, aged over 12 months and under 5 years, was studied. All had undergone deworming more than six months prior, with irregularities in antiparasitic treatment. *Haemonchus contortus* accounted for more than 95% of the helminths found in the coprological analysis of third-stage larvae.

Seven production units were established, and in each, 42 randomly selected animals that appeared healthy were chosen; all belonging to the municipality of Jimaguayú. All the animals were under similar grazing conditions.

Each production unit was evaluated for EPG, Hct, Hb, MCHC, platelet count, total leukocytes, and leukocyte differential.

Sample collection

Hematology was performed by extraction using a hypodermic needle from the jugular vein, and the sample collection was carried out in the morning. A 10% ethylenedinitrilotetraacetic acid (EDTA) solution was used as an anticoagulant in 5 mL vials at a ratio of 15 µL per mL of blood. The samples were properly homogenized, identified, and refrigerated for transport.

Hematological analysis

Hematocrit determination

For hematocrit determination, the protocol described in the Manual of Techniques for Clinical Laboratory, Havana, 1969, was followed.

Hemoglobin concentration determination

The determination of hemoglobin concentration was carried out using the Drabkin cyanmethemoglobin technique (ICSH standard, 1996).

Mean corpuscular hemoglobin concentration (MCHC)CHCM

The method used by Benjamin in 1991 was followed for the determination of this corpuscular constant.

Differential blood cell count

A smear was made for each blood sample to perform the differential count of white cells using the May-Grunwald-Giemsa technique, according to the Clinical Laboratory Manuals (Colina *et al.*, 1989).

Total leukocyte count

For the total leukocyte count, a hemocytometer (Neubauer-ruled cell counting chamber) was used, following the 2% acetic acid technique (Colina *et al.*, 1989).

Platelet count on slides

The platelet count was performed on the smear used for the differential leukocyte count, using the May-Grunwald-Giemsa technique. For the count, 20 fields were examined, tallying the total number of platelets and reporting the counted number per 10^9/L (Suaríaz *et al.*, 2004).

Egg per gram (EPG) count

To determine the level of infestation in the animals, fecal samples were collected directly from the rectum. All samples were bagged, identified, and refrigerated for transport to the parasitology laboratory at the Faculty of Agricultural Sciences of the University of Camagüey, where EPG was performed using the McMaster technique (Cringoli *et al.*, 2004).

Statistical analysis

To determine the behavior of parasitic load and hematological values across production units, a simple ANOVA was performed, and the Tukey HSD multiple comparison test was used to compare means. Linear correlation was determined using Pearson's correlation coefficient, and the Student's t-test was used to differentiate parasitic loads with values greater and less than 800 EPG. All data were processed using GraphPad Prism version 8.0 (2016, GraphPad Software, USA).

RESULTS AND DISCUSSION

The average blood values of the investigated animals are shown in Table 1, and were within the normal range according to the reference established in Cuba, and were similar to those reported by Barrionuevo (2018) for Creole sheep in Ecuador.

Table 1. Results obtained from the leukogram and parasitological analysis in each farm

Production units with 42 animals	**Statistical values**	**McM Eggs per gram**	**Platelets ($X10^9$/L)**	**Lymphocytes (%)**	**Neutrophils (%)**
1	Means	1109.43 b,c	236.80 a	56.7 a,b	40.29 a,b
	SE	223.50	5.32	2.21	1.933
2	Means	1540.00 c,d	265.5 2 a,b	58.5 a,b	40.39 a,b
	SE	278.13	7.75	1.54	1.65
3	Means	1117.38 b,c	245.61 a	62.2 b	36.74 a,b
	SE	267.71	6.25	1.41	1.44
4	Means	2503.75 d	296.72 b	61.6 b	35.78 a,b
	SE	386.89	10.38	1.85	1.92
5	Means	105.60 a	248.80 a	61.1 a,b	36.07 a,b
	SE	22.04	7.77	1.68	1.74
6	Means	472.13 a,b	247.88 a	64.5 b	33.72 a
	SE	170.05	6.58	1.55	1.39
7	Means	353.33 a,b	293.47 b	53.8 a	42.86 b
	SE	97.14	17.94	.1.98	1.94

Different scripts in the columns indicate significant differences, according to the Tukey HSD test.

Da Rosa Rossi (2017) and Guanín (2019) reported that leukocytes have a value of 6.63×10^9/L, compared to the amount reported by Couto Hack (2010) in blood analyses of Merino sheep, which presented a value of 9.30×10^9/L. Neutrophils show a value of 25.97%, while Couto (2010) reported an average of 39.10% for Mexican Creole sheep (within the normal range of 40 to 50%) Pedreira *et al*. (2007) and Couto (2010) report neutrophil values ranging from 40 to 50%, suggesting that this value is within the normal range.

Escribano (2019) reports that susceptible infested animals show an increase in platelet count. This is due to the pathophysiology of haemonchosis as a hemorrhage-inducing condition, not just the blood-draining action of the nematode. Additionally, the parasites secrete anticoagulant substances that cause bleeding after feeding, leading to a mild reactive thrombosis secondary to the hemorrhage.

The finding of high platelet counts is secondary to the hyperproduction of thrombopoietin. This secondary thrombocytosis can occur after a hemorrhagic process or in association with it, especially when the hemorrhage causes iron deficiency anemia. It is important to emphasize that animals living in the low tropics are more predisposed to contact with parasites, including gastrointestinal parasites, which significantly affect small ruminants. In most cases, they can cause substantial losses of serum iron and even chronic anemia, which could lead to secondary thrombocytosis (Puicón, 2018).

The values obtained for lymphocytes were within the normal range for the species. Production unit No.6 reported the highest values at 64.5%, followed by production unit No.3 with 62.2%, indicating mild lymphocytosis.

Guanín (2019), in a study conducted in Mexico with adult Creole sheep, obtained an average lymphocyte value of 47.48%. However, Partida Luna *et al*. (2012) reported maximum values of up to 66.67%.

Valente and Delgado-Camarena (2017) reported that constant antigenic challenge and the absence of eosinophilia in the presence of lymphocytosis suggest the establishment of an adaptive response that confers resistance to infection in the host, findings that align with our study. Authors such as Greeg (2003) and Pedreira *et al*. (2007) reported eosinophil values ranging from 0.1% to 0.4%. However, Sandoval *et al*. (2007) found eosinophilia in parasitized animals, with these cells representing 17% of the differential leukocyte count. Soch *et al*. (2011) also found an increase in eosinophils, which was associated with low altitudes and temperatures.

Escribano (2019) reported that for circulating eosinophils, the value is only affected in susceptible animals, which could be related to susceptibility. It is necessary to highlight the resistance of sheep to *H. contortus*, the level of circulating eosinophils, and those present in the abomasal mucosa, demonstrating a close tissue association.

In our study, monocytes, eosinophils, and Stabkerniger (stab) neutrophils showed normal values, but within minimal ranges and with great dispersion, resulting in extreme variability coefficients without diagnostic significance.

In Table 2, other hematological indicators and the results of the McMaster technique for the parasitic load of the studied sheep are presented.

Table 2. Other hematological indicators and results of the parasitic load (EPG)

Production units with 42 animals	Statistical values	Mc Master EPG	Total leukocytes ($x10^9$/L)	CHCM (g/L)	Hematocrit (%)	Hemoglobin (g/L)
1	Means	1109.43	10.00	303.12	.30.0	90.95
	SE	223.50	0.43	5.07	0.8	2.60
2	Means	1540.00	9.35	298.41	31	92.17
	SE	278.13	0.47	5.95	1.10	3.23
3	Means	1117.38	10.30	304.59	31	94.30
	SE	267.71	0.40	7.13	0.91	3.09
4	Means	2503.75	8.62	303.55	28.3	87.30
	SE	386.89	0.25	8.47	0.86	3.98
5	Means	105.60	9.32	326.63	31.7	102.48
	SE	22.04	0.45	6.42	0.64	1.53
6	Means	472.13	9.28	329.38	29.9	96.67
	SE	170.05	0.37	6.19	0.93	2.34
7	Means	353.33	8.75	344.18	25.8	89.04
	SE	97.14	0.39	0.54	0.86	2.99

Díaz-Anaya *et al*. (2014) observed a prevalence of *Haemonchus spp*. of 31.3%. Only 6% of the animals showed hematocrit (Hct) values below normal, while 6.5% had normal hemoglobin values in the presence of the helminth. The values of erythrocyte count and mean corpuscular volume did not show alterations in the presence of infection. Likewise, no relationship was observed between hematological parameters and the presence of *Haemonchus* sp. eggs in sheep feces.

Hack (2016) found that more than 75% of sheep showed normal total leukocyte counts, 22% had leukocytosis, and 2% showed leukopenia.

Guanín (2019) reported that leukocytes showed a value of 6.63×10^9/L, compared to the leukocyte count determined by Couto Hack (2010) in blood analyses of Merino sheep, which presented a value of 9.30×10^9/L.

For hematocrit (Hct), Polizopoulou (2010) stated that in cases of anemia with dehydration, sudden increases in hematocrit can occur due to splenic contraction, masking lower-than-average hematocrit values. Similarly, this can happen with physiological leukocytosis. The observation of reactive lymphocytes and lymphocytes with azurophilic granulation is associated with chronic infectious events or those that have triggered a very strong immune response.

A decrease in erythrocyte count, hemoglobin (Hb), and hematocrit (Hct) in sheep during an experimental infection with *Haemonchus contortus* indicates a normocytic and normochromic anemia, which is common in infestations with hematophagous parasites (González Viscailuz, 2017). It coincides with Morales *et al.* (2002) who stated that Hct value and Hb concentration are directly related to the number of juvenile and adult *H. contortus* forms in the abomasum.

Díaz-Anaya *et al*. (2014) reported that only 12 individuals, both females and males, infected with *Haemonchus* sp. showed hematocrit (Hct) and hemoglobin (Hb) values below physiological normality, but without a decrease in total leukocyte count or mean corpuscular volume. A large number of females and males infected with the parasite showed hematocrit (Hct) and hemoglobin (Hb) values within normal ranges (51 and 63, respectively). In this study, 63.7% of the sheep presented normal hematocrit (Hct) values in the absence of the parasite, while 25.4% showed normal Hct even in the presence of *Haemonchus* sp. Only 6% of the animals exhibited Hct values below normal levels in the presence of the parasite. In the case of hemoglobin (Hb), 62.2% presented normal values in the absence of the parasite, while 6.5% showed abnormal Hb values in the presence of the helminth.

Morales *et al*. (2001) state that hematological parameters such as hematocrit (Hct), hemoglobin (Hb), erythrocyte count, and mean corpuscular volume can be used as indirect indicators of resistance to parasitic infection, particularly to hematophagous species such as *H. contortus*. A prevalence of *Haemonchus* sp. of 31.3% was observed. Only 6% of the animals showed hematocrit (Hct) values below normal, while 6.5% had abnormal hemoglobin (Hb) values in the presence of the helminth. The values of erythrocyte count and mean corpuscular volume did not show alterations in the presence of infection; no relationship was observed between hematological parameters and the presence of *Haemonchus* sp. eggs in sheep feces.

Díaz-Anaya *et al*. (2014) In their study, 63.7% of the sheep showed normal hematocrit (Hct) values in the absence of the parasite, while 25.4% presented normal Hct even in the presence of *Haemonchus* sp., and 6% of the animals exhibited Hct values below normal levels in the presence of the parasite. In the case of hemoglobin (Hb), 62.2% presented normal values in the absence of the parasite, while 6.5% showed abnormal Hb values in the presence of the helminth. The values of hemoglobin (Hb), hematocrit (Hct), erythrocyte count, and mean corpuscular volume in sheep were estimated, pointing to a normocytic normochromic anemia in animals that showed reduced values for the first two parameters mentioned. It is important to note that the parasitic load was not determined, as this study was only qualitative in nature (absence/presence).

Mejía Vasquez (2018) reported normal values for mean corpuscular hemoglobin concentration (CHCM) ranging from 310.8 to 340.8 g/L in a study on Creole sheep (*Ovis aries*).

Hemoglobin (Hb) values were within the reported range for the species; however, farms No.4 and 7 had low values of 87.30 g/L and 90.24 g/L, respectively, while farm No.5 reached higher values at 102.48 g/L. This situation may be due to better or worse adaptation to the parasitic load. Under warm climate conditions, the concentrations of hematological parameters in animals are modified. The higher hemoglobin (Hb) concentration in the treatment without shade may be associated with an adaptation mechanism in lambs to supply the necessary amount of water to the circulatory system, allowing cooling through evaporation (Kamal *et al.*, 2016).

The difference in parasitic load among the different production units was determined using coproscopic examination techniques (McMaster) for diagnosis in the studied flocks. Production unit No.4 had the highest parasitic load with 2,503.75 eggs per gram (EPG), while farm No.5 presented values of 105.6 EPG

Castro Arnaez (2019) conducted research on sheep using the McMaster technique. The lowest egg per gram (EPG) value obtained was 0, the highest was 49,100, and a total of 65.9% of the animals had an EPG lower than 1,000. Among the microhematocrits performed, the lowest value obtained was 7%, and the highest was 43%. Herrera *et al*. (2013) reported that 15.7% of ruminants had parasitic loads of 700 or more eggs per gram (EPG) of fecal matter, which were considered high.

Correlation between hematological values and parasitic load when the count is less than 800 eggs per gram of feces

Figure 1 shows the data on the correlation between hematological values and parasitic load found in animals with fewer than 800 eggs per gram (EPG). As observed, the correlation coefficients obtained are very subtle concerning the presence of EPG, being positive for mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb), and lymphocytes, while negative for hematocrit, neutrophils, and total leukocytes. No significant correlation was found between parasitic load and eosinophils, stab cells, platelets, or monocytes.

There is a close relationship between leukocyte profile and plasma glucocorticoid levels during physiological stress. These hormones can act by increasing the number and percentage of neutrophils (neutrophilia), while decreasing lymphocytes (lymphopenia or lymphocytopenia). The neutrophil-to-lymphocyte (N/L) ratio is used as a complementary measure of the animal's stress response. As a response to increased glucocorticoid levels during stress, circulating lymphocytes are retained in lymphoid organs and skin, causing a reduction in their presence in the bloodstream (Romero *et al.*, 2011).

In Figure 2, the correlation between the different elements of the matrix behaved similarly to Figure 1, but in this case, with higher values due to the animals having more than 800 eggs per gram (EPG).

It is noteworthy that in the correlation analysis of animals with a high parasitic load, a positive correlation was found between mean corpuscular hemoglobin concentration (MCHC) and hemoglobin concentration (r=0.812**). The Hb concentration showed a positive correlation with Hct (r=0.775**), while it had a negative correlation with EPG (r=-0.430**).

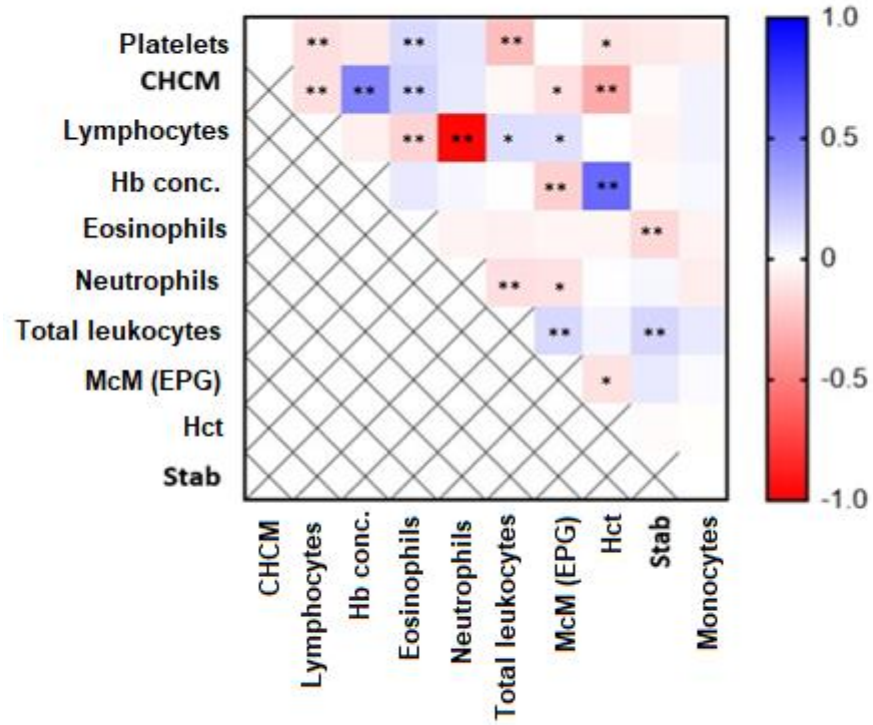

Figure 1. Correlation between hematological values and parasitic load when the count is less than 800 eggs per gram of feces
Legend: CHCM: Mean corpuscular hemoglobin concentration: Hemoglobin Concentration, McM: McMaster technique = EPG. Hct: hematocrit, Stab: Stabkerniger (count of immature neutrophils). * p < 0.05 ** p < 0.01.

Figure 2 shows that, unlike previous findings, none of the correlations between parasitic load and hematological variables were significant. This indicates that these animals manage to maintain hematological values within reference intervals despite harboring high parasitic loads and appearing outwardly healthy, coinciding with what was reported by Noriega Burbano (2018).

Kelkele *et al.* (2012) proposed a negative correlation between hematocrit value and EPG, also suggesting that blood loss is caused by the parasite's hematophagous action.

Rowe *et al*. (2008) state that there is an effect linking the pathogenic actions of gastrointestinal parasites with blood values, becoming particularly noticeable in hemoglobin (Hb) and hematocrit (Hct) levels in sheep, which leads to anemia, anorexia, weight loss, depression, and death. The results do not match those obtained in this study, as these authors did not use groups.

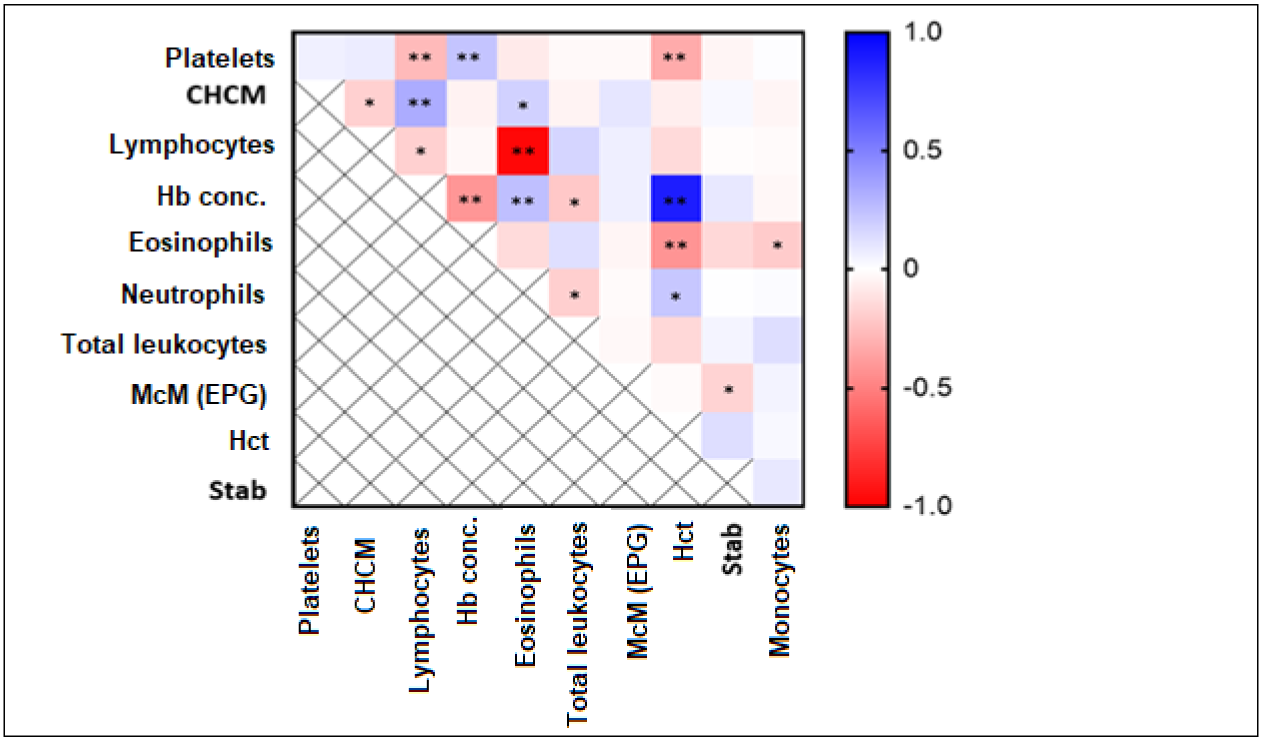

Figure 2. Correlation between hematological values and parasitic load when the count is greater than 800 eggs per gram of feces

Legend: CHCM: Mean corpuscular hemoglobin concentration, Conc Hb: Hemoglobin Concentration, McM: McMaster technique = EPG. Hct: hematocrit, Stab: Stabkerniger (count of immature neutrophils). * $p < 0.05$ ** $p < 0.01$.

Figures 3 and 4 show that only the indicators for mean corpuscular hemoglobin concentration (MCHC) and hemoglobin (Hb) exhibited significant differences between animals with fewer than 800 eggs per gram (EPG) and those with more than 800 EPG. These animals, despite carrying substantial parasitic loads, maintain hematological values within normal ranges. This suggests that we may be dealing with resilient animals capable of sustaining acceptable productive levels despite harboring high parasitic burdens. Clinically, these animals appear healthy, as reported by Noriega Burbano (2018)

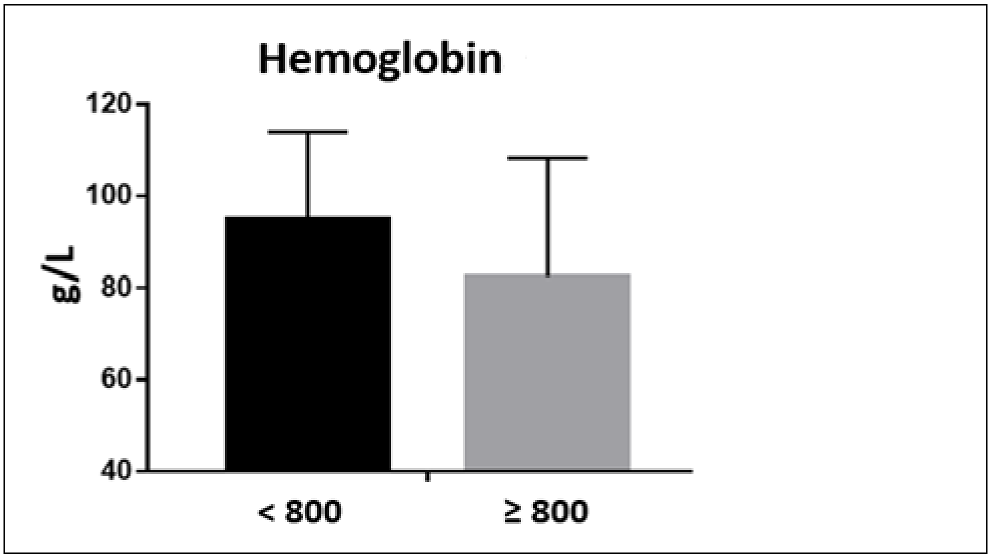

Figure 3. Comparison of Hb between animal populations with fewer than 800 eggs per gram (EPG) and those with more than 800 EPG as parasitic loads. ($p < 0.01$) according to Student's t-test

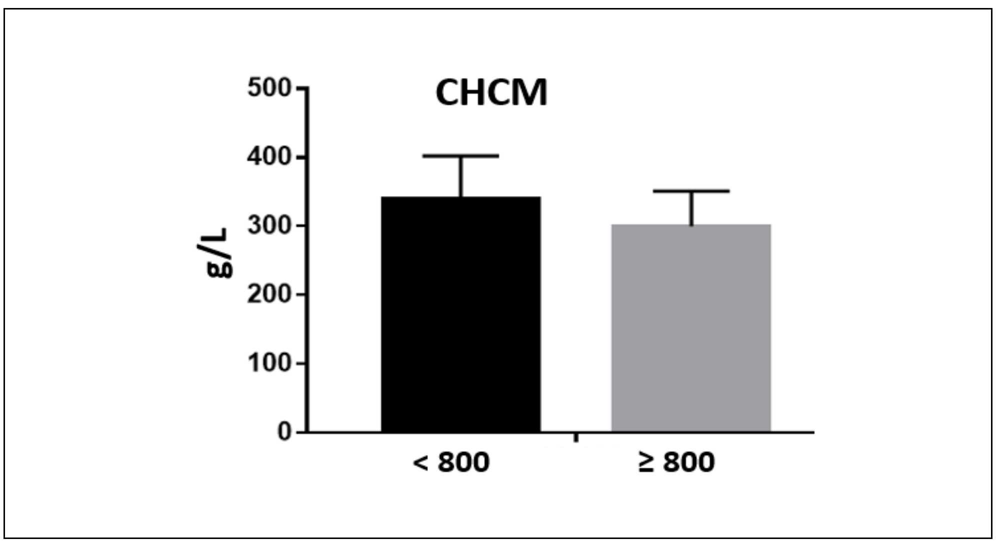

Figure 4. Comparison of MCHC between animal populations with fewer than 800 EPG and those with more than 800 EPG as parasitic loads
($p < 0.01$) according to student-t

CONCLUSIONS

The correlation coefficient for hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, lymphocytes, and neutrophils becomes more evident in animals infested with a parasitic load of fewer than 800 eggs per gram.

Resilient animals with significant hematophagous parasitism do not show alterations in hematological indicators.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: ATR, NIP, AAC, YLZ; data analysis and interpretation: ATR, NIP, AAC, YLZ; redaction of the manuscript: ATR, NIP, AAC, YLZ.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.