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Effect of Infestation Level of *Eimeria spp.* on Hematological Parameters and Blood Biochemistry (Albumins, Globulins and Total Proteins) in Alpaca

Daniela Guadalupe Zea-Ticona[1], Harnold Segundo Portocarrero-Prado[1],Uri Harold Perez-Guerra[1], Ali William Canaza-Cayo[2,3], Oscar Efrain Cardenas-Minaya[4], José Eduardo Ramírez-Aruquipa[4], Ronnie Mask Condori-Morales[5], Francisco Halley Rodríguez-Huanca[1]

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ABSTRACT

Background: Eimeriosis causes inflammation of the intestinal mucosa, leading to excessive mucus, epithelial shedding, and bleeding. It affects newborns and offspring aged 2 to 3 weeks, causing 80% of diarrhea cases and 43.3% mortality in offspring between 1 and 2 months. This study evaluated the prevalence and parasitic load of *Eimeria* and its effect on hematological parameters and blood biochemistry (albumin, globulin and total protein) in alpacas.

Methods: Fecal and blood samples were collected by jugular venipuncture from 42 alpacas aged 1 to 2 months at two time points (T1 and T2, 30 days apart). Fecal samples were analyzed using the modified McMaster method and blood samples were examined using a colorimetric-spectrophotometric technique. A t-test compared time points and Pearson's correlation tested associations between variables.

Result: The mean parasitic load was 8485.71 Oocysts per gram of feces (OPG) at T1 and 2400.00 at T2. Blood analysis results were: Hematocrit (Hto) (%): 31.86 (T1) and 13.47 (T2); Red blood cells (RBC) ($\times 10^6$/µL): 9.39 and 3.27; White blood cells (WBC) ($\times 10^3$/µL): 11.47 and 9.38; Neutrophils (Neu) (%): 50.14 and 49.98; Monocytes (Mon): 15.45 and 16.33. Hto, RBC and WBC values were altered, while total protein (TP) (4.44 and 4.80), Albumin (3.57 and 4.00) and Globulim (0.87 and 0.80) were below normal ranges. A negative correlation was found between RBC and OPG (-0.42) and a positive correlation between parasitic load and neutrophils (0.31). Monocytes and OPG showed a moderate inverse correlation (-0.39 and -0.43). *Eimeria spp.* negatively affected hematological parameters but had no impact on albumin, globulin and total protein levels.

Key words: Alpacas, Blood biochemistry, *Eimeria lamae, Eimeria macusaniensis*, Offspring.

INTRODUCTION

Alpaca production in Peru provides economic support to a large part of the rural population (Moya and Torres, 2008). Puno is the region with the highest alpaca production in the country with 58% of the national population (1,681,919) (FAO, 2005). Poor breeding conditions have led to health problems in the population (Ballweber, 2009). Parasitism has become a common threat to the health and productivity of alpacas (Barrientos, 2017).

Eimeriosis, caused by protozoa belonging to the genus *Eimeria*, family *Eimeriidae* and phylum apicomplexa, is one of the most prevalent parasitic diseases affecting cattle globally (Cardim *et al.,* 2018). The parasite causes inflammation of the intestinal mucosa, excessive mucus production and peeling of the epithelium with the presence of blood, affecting the absorption of nutrients and therefore the productive performance of alpacas (Ramírez *et al.,* 1998). Eimeriosis affects the well-being of newborns (2 and 3 weeks of age), causing up to 80% of cases of diarrhea and death of 43.3% of offspring between 1 and 2 months of age (Lucas *et al.,* 2016). Between 4 and 5 months of age, the prevalence in offspring can reach up to 90%, subsequently the levels decrease and alpacas become carriers (Rojas, 2004).

Hematology and blood biochemistry play an important role in the assessment of the nutritional status and general

[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional del Altiplano, Av. Floral 1153, Puno, Perú.
[2]Facultad de Ciencias Agrarias, Escuela Profesional de Ingeniería Agronómica, Universidad Nacional del Altiplano, Av. Floral 1153, Puno, Perú.
[3]Departamento de Estatística, Instituto de Ciências Exatas e Tecnológicas, Universidade Federal de Lavras, Código postal 3037, CEP 37200-900, Lavras, MG, Brazil.
[4]Dirección de Desarrollo Tecnológico Agrario, Instituto Nacional de Innovación Agraria (INIA), Estación Experimental Agraria Illpa, Puno, Perú.
[5]Carrera Profesional de Medicina Veterinaria y Zootecnia, Universidad Nacional del Madre de Dios, Madre de Dios, Perú.

Corresponding Author: Francisco Halley Rodríguez-Huanca, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional del Altiplano, Av. Floral 1153, Puno, Perú. Email: halleyal@gmail.com ORCIDs: 0000-0002-3967-0410, 0009-0006-6599-2970, 0000-0001-8301-9759, 0000-0001-8321-8651, 0000-0002-4189-4747, 0000-0003-2138-9863, 0000-0003-1206-8873, 0000-0002-5389-1885.

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condition of populations (Campbell, 2012). Therefore, this research is aimed at determining whether parasitosis *Eimeria spp*. influence hematological and biochemical components, since these are necessary to diagnose and assess severity of infection. Blood tests are also useful for detecting the presence of antibodies, evaluating the immune response of parasitized alpaca offspring and identifying possible secondary complications. The objective of this study was to determine the effect of infestation level of *Eimeria spp*. on hematological parameters and blood biochemistry (albumins, globulins and total protein) in alpacas.

MATERIALS AND METHODS

The study was carried out in the Quimsachata annex of the Instituto Nacional de Innovacion Agraria (INIA), located between the districts of Santa Lucia and Cabanillas in the provinces of Lampa and San Román, in the Puno region at an altitude of 4300 meters above sea level, a dry agroecological zone. Two fecal and two blood samples, taken 30 days apart were collected from 42 alpacas (regardless of sex), aged 1 to 2 months. Stool samples (3 to 8 g) were collected directly from the rectum of the pups placed in polyethylene bags, blood was obtained directly from the jugular vein and collected in in sterile tubes and labeled. The samples of blood and stools were transferred in a separate box with cooling gel to the laboratories of the Facultad de Medicina Veterinariay Zootecnia at the Universidad Nacional del Altiplano Puno for analysis. The weight of the animals was recorded before sampling using a digital scale.

The diagnosis of *Eimeria sp*. infection based on the detection of oocysts in host feces using flotation techniques. The five species of *E. lamae, E. alpacae, E. punoensis, E. macusaniensis* and *E. ivitaensis* were determined using the qualitative Flotation method and the modified McMaster quantitative method, respectively (Capello *et al.,* 2020). *Eimeria* in Soth American Camelids have such distinctive characteristics that they can be clearly distinguished without the need for incubation (Dubey, 2018), which is why incubation was not necessary in the present study. The OPG of each sample was separated into 3 groups: low infestation (50 to 200 OPG), moderate infestation (200 to 800 OPG) or high infestation (> 800 OPG) (Arauco *et al.,* 2021). Hematocrit, erythrocyte count, leukocyte count and differential leukocyte count were estimated using the colorimetric-spectrophotometric technique with a fully automated hematology cell counter (Shawn, 2014). The samples were evaluated within 42 hours of sampling as recommended by Hadžimusić (2023). The Biuret method was used to determine total protein. The spectrophotometric colorimetric technique was used to determine the quantity of albumins (Coila and Quiñones, 2014).

After confirming the normality of the data, a Student “t” significance test was used to compare the effect of OPG levels on hematological parameters and the Pearson correlation coefficient was used to measure the statistical relationship between the variables Red blood cells (RBC), Neutrophils (Neu), Monocytes (Mon) and Oocysts per gram of feces (OPG).

RESULTS AND DISCUSSION

Eimeria oocyst count

There are few studies that show a relationship between parasite load and blood and biochemical parameters in baby alpacas. Is is very important to understand the effects that these parasites have on biochemical parameters and weight because thee newborns are the most susceptible.

Table 1 shows that the mean OPG at time 1 (T1) ranged from 1111.9 to 7373.8 OPG, with a minimum load of 0 OPG and a maximum load of 36,700 OPG for *E. lamae*, a total average of 14,511.9 OPG and a maximum of 69,800 for all *Eimeria*. For time 2 (T2) the average parasite load was between 2390.5 and 19.1 with a minimum of 0 and a maximum of 27,600 OPG for *E. lamae* having a total average of 5,769 *Eimeria* and a maximum of 44,700 OPG.

The prevalence of *Eimeria* shown in Table 1 are similar what was reported by Rodríguez *et al.* (2012), who found a

Table 1: Summary levels of *Eimeria* load in naturally infected alpaca animals.

Time	Parasites	Mean, OPG	D.E., OPG	Mín, OPG	Máx, OPG
Time 1	*E. macusaniensis*	1111.9	2364.7	0	14000
	E. alpacae	4647.6	7182.3	0	32600
	E. lamae	7373.8	9930.9	0	36700
	E. punoensis	1378.6	4011.2	0	25000
	E. ivitaensis	0	0	0	0
	Total OPG	14511.9	18471.4	0	69800
Time 2	*E. macusaniensis*	540.5	1207.9	0	7100
	E. alpacae	959.5	2079.4	0	9800
	E. lamae	1859.5	4644.4	0	27600
	E. punoensis	2390.6	4093.4	0	19600
	E. ivitaensis	19.1	123.4	0	800
	Total OPG	5769.0	9716.7	0	44700

E: *Eimeria.*

Table 2: Weight, OPG and hematological parameters at T1 and T2 in alpaca calves from the quimsachata annex.

Time	Weight, kg	OPG	Hto%	RBC, $\times 10^6$/ µL	WBC, $\times$ 10^3/µL	Neu%	Lin%	Mon%	Eosi%	Bas%
Time 1	11.52[a]	8485.7[a]	31.86[a]	9.39[a]	11.47a 50.14	29.81	15.45	4.24	0.33	
Time 2	15.04[b]	2400.0[b]	13.47[b]	3.27[b]	9.38[b]	49.98	29.19	16.33	4.26	0.26
Probability	<0.001	0.002	<0.001	<0.001	0.027	0.788	0.274	0.080	0.928	0.480

Means with different letters are significantly different (p<0.05).

OPG: Oocysts per gram of feces; Hto : Hematocrit; RBC: Red blood cells; WBC: White blood cells; Neu : Neutrophils; Lin : Lymphocytes; Mon : Monocytes; Eosi : Eosinophils; Bas: Basophils.

high load of *E. lamae* in Bolivia. Our results are also similar to Camareno (2014), who found a higher load for *E. punoensis* and *E. alpacae*. Quina (2015) repored 21,977 OPG in alpaca babies. Merzenich (2022) showed that the OPG in alpaca babies were higher than those of adults in Quimsachata. The results of this and other research differ due to differences in the climate, sanitary conditions and the different locations studied. The presence of *Eimeria* in young animals is related to a very poor immune response (Pérez *et al.,* 2014).

Weight and hematological parameters

In Table 2, the average weight of the alpacas at T1 was 11.52 kg and at T2 15.04 kg ($p<0.05$). The parasitic load at T1 was 8484.71 OPG. At T2 the parasitic load decreased to 2400.00 OPG ($p<0.05$). The average hematocrit value at T1 was 31.86%. at T2 the hematocrit decreased to 13.47% ($p<0.05$). The average number of erythrocytes was 9.39 $\times$ 10^6/µL at T1 and 3.27 $\times$ 10^6/µL at T2 ($p < 0.05$). The total leukocyte count at T1 was 11.47 $\times 10^3$/µL. At T2 the average value of white blood cells decreased to 9.38 $\times$ 10^3/µL, as did the parasitic load ($p<0.05$). There was no significant differences in cell differentiation.

In Table 2, the result of the average hematocrit value at T1 was similar to values recorded by Barrios *et al.* (2016), who measured an average hematocrit value of 29.36% in calves under 1 month old. The average hematocrit value found at T2 was lower than T1. The difference could be due to alpaca calves experiencing anemia due to the parasitic infection. Watson (2015) mentioned that iron deficiency is linked to blood loss due to parasitic infestation, hemostatic deficiencies and bleeding lesions in the digestive system: all of which lead to anemia. The erythrocyte values shown in Table 3 are slightly below the normal values reported by Barrios *et al.* (2016) who mention reference levels of 9.75 to 16.4 $\times 10^6$/µL in healthy alpaca calves. It could be deduced that the average values of erythrocytes in alpaca babies are lower because South American Camelids (CS) are partially anemic until they reach 3 to 4 months of age. This results from a process in which the fetal Hemoglobin cells are replaced by large adult Hemoglobin cells. This exchange ends at 3 months of age (Abaigar, 1993). Eimeriosis cause animals to have diarrhea due to poor absorption by destroyed intestinal villi, chlorine secretion and increased intestinal permeability, giving rise to dehydration (García and Rivera, 2017). This accompanied by anemia can cause a false increase in total erythrocyte volume, resulting in the erythrocyte levels being slightly lower than normal for this species (Weiss and Wardrop, 2010).

Table 3 shows that, the parasite load did not influence the weight of alpacas ($p>0.05$). The hematocrit results did not change with differing parasitic loads ($p>0.05$). There were fewer red blood cells at T1 (9.10 $\times 10^6$/µL and 9.39 $\times 10^6$/µL) in alpaca with moderate and high OPG loads ($p>0.05$). Samples taken at T2 showed below normal RBC indicating an anemic process. Red blood cell values in animals with a high parasite load were slightly elevated

Table 3: Hematological parameters by level of infection in alpaca calves from the Quimsachata Annex.

Time	n	OPG load	Weight, kg	Hto,%	RBC, $\times 10^6$/ µL	WBC, $\times 10^3$/ µL	Neu,%	Lin ,%	Mon,%	Eosi,%	Bas,%
Time 1	4	Low	11.12	32.25	9.58	9.15	52.00	26.50^{a}	17.75	3.25	0.00
	4	Moderate	12.45	29.5	9.1	10.32	49.25	30.25^{b}	16.75	3.5	0.25
	34	High	11.46	32.09	9.39	11.88	50.03	30.15^{ab}	15.03	4.44	0.38
Probability			0.702	0.216	0.928	0.391	0.420	0.032	0.103	0.123	0.303
Time 2	17	Low	14.44	13.64	3.29	9.59	50.41	28.47	16.71	4.35	0.06^{a}
	6	Moderate	13.84	13.13	3.2	10.01	49.33	29.67	16.33	4.17	0.50^{b}
	19	High	15.96	13.42	3.26	8.97	49.79	29.68	16.00	4.21	0.37^{ab}
Probability			0.119	0.932	0.960	0.856	0.613	0.295	0.456	0.901	0.037

Means with different letters are significantly different ($p<0.05$). OPG: Oocysts per gram of feces; Hto : Hematocrit; RBC: Red blood cells; WBC: White blood cells; Neu : Neutrophils; Lin : Lymphocytes; Mon : Monocytes; Eosi : Eosinophils; Bas: Basophils.

(3.26×10^6/µL) compared to animals with a moderate parasite load (3.20×10^6/µL) ($p<0.05$). White blood cell values at T1 were higher with increased parasite load (9.15, 10.32 and 11.88 $\times 10^3$/µL). At T2 the amount of white blood cells in offspring with a high parasite load decreased (8.97×10^3/µL) ($p>0.05$).

According to Vale (1999) and Martínez (2009), iron is essential and vital in cellular activity, so an iron deficiency (iron deficiency anemia) exhibits a reduction in lymphocyte DNA synthesis and decreases the number of T cells. These results were documented in laboratory animals. Papale *et al.* (2008) show that iron is associated with the immune response against parasites, for this reason iron deficiency anemia impacts parasitic infestation. The results found at T2 indicate that the response of white blood cells against parasitic infection by a high load of *Eimeria* is deficient. Barrios *et al.* (2016) shows an average WBC of 18.02±7.7 $\times 10^3$/µL, with ranges of 7.2 to 43.6 $\times 10^3$/µL in alpaca calves with diarrhea. According to Barrios *et al.* (2016) and Bautista (2009) a significant decrease in leukocytes could be associated with parasitic factors that manage to evade and/or adapt to the leukocyte response. Arece *et al.* (2015) reported that parasitized sheep with greater amounts of eggs per gram, had lower WBC counts (6.51×10^3/ µL), than animals with lower HPG (10.57×10^3/µL).

The values of neutrophils, monocytes, eosinophils and basophils did not show variations with respect to the level of parasitic load at T1; implying that the level of parasitic infection in alpaca calves does not significantly alter the level of white blood cells. However, the values of lymphocytes in calves with a high level of parasitic load decreased to 30.15%. The level of neutrophils, lymphocytes, monocytes and eosinophils was not different at T2. However, the level of basophils decreased to 0.37%. Morales and Vargas (2018) reported that in cattle infected with gastrointestinal parasites, many with *Eimeria sp*. 41.46% of animals presented with monocytosis and 40.24% neutropenia. According to SUISA VET (2018) neutropenia could be due to the acute increase in tissue demand, as occurs in over-acute infections, such as gastrointestinal parasitosis since perforations and/or ruptures of the intestinal walls occur. Rodríguez (2017) mentions that monocytosis is the product of hemolysis as a result of parasitic proliferation, cellular debris, dead cells and hemoparasites. It is also known that infection in the body, nature of the causative agents, resistance by the animal and the localization of the inflammatory response might be possible causes of lower biochemical parameters and hematological parameters, except total leukocyte count (Sarkar *et al.,* 2016).

Albumins, globulins and total proteins

Table 4 shows the average total protein was 4.44 g/ dL at T1. At T2 the average value was higher, 4.80 g/dl ($p<0.05$). The average value found for albumin at T1 was 3.57 g/dl, lower than T2 which was 4.00 g/dl ($p<0.05$). The average globulin values at T1 were 0.87 g/ dL, similar to the average

Table 4: Biochemical parameters of young alpacas from the quimsachata annex at T1 and T2.

Time	Protein, g/dL	Albumin, g/dL	Globulin, g/dL
Time 1	4.44[b]	3.57[b]	0.87
Time 2	4.80[a]	4.00[a]	0.80
Probability	0.022	0.001	0.712

Means with different letters are significantly different (p<0.05).

Table 5: Biochemical parameters by level of infection in young alpacas from the quimsachata annex.

Month	OPG Load	Total protein, g/dl	Albumin, g/dl	Globulin, g/dl
	Low	4.44	3.88	0.56
Time 1	Moderate	5.22	3.66	1.57
	High	4.35	3.53	0.82
Probability		0.109	0.322	0.180
	Low	4.77	3.97	0.80
Time 2	Moderate	4.80	4.02	0.78
	High	4.83	4.01	0.82
Probability		0.969	0.986	0.993

OPG: Oocysts per gram of feces.

value of 0.80 g/dL recorded at T2 (p>0.05). Table 5 shows that the level of parasite load did not influence the parameters of albumin, protein and globulins at T1 or T2 (p>0.05).

The level of parasite load did not influence the parameters of albumin, protein and globulins. However, it should be noted that the levels of total protein, albumin and globulin at both time points were below the normal reference values. Woodward (2015) points out that various gastrointestinal diseases, including parasitic infections, cause changes in the mucosa, as well as lesions in the intestinal epithelial cells and cause the rupture of lymphatic vessels in the intestinal lumen; leading to the loss of proteins through the vessels and the interstitial space of the intestinal mucosa. Total protein is affected by other infections (Ekisi *et al.,* 2021).

The decrease in normal albumin values is related to hypoproteinemia due to diarrheal intestinal processes, lack of appetite and malabsorption of nutrients, which can give rise to cases of ascites and edema (Gyles *et al.,* 2010). Globulins increase as an immune response against parasites. However, the average value of globulins found in this research is outside the range of values reported by other authors. This hypoglobulinemia is related to enteropathies in which proteins are lost due to parasitic infestations, hemorrhages and malabsorption syndromes.

Correlation between the burden of *Eimeria* and some hematological parameters

Table 6 shows the significant negative correlation (-0.42) between the level of red blood cells and the level of oocysts at T1 and the positive correlation (0.31) between neutrophils and OPG levels. At T1 and T2 there was a moderate, highly significant inverse correlation (-0.39 and -0.43) between OPG level and monocytes.

A similar result was reported by Arece *et al.* (2015), who found a high negative evaluation of -0.63 with significance (p<0.01) between the egg count per gram of feces and the RBC values in sheep. As the eggs per gram level increased the red blood cell count values decreased. Although they are not eimeria but the influence on the RBC count is similar in the month of T1, At T2 neutrophils were increasing with the OPG levels, presenting a positive pressure of 0.31. This increase in neutrophils occurs because they are the main cells recruited in the submucosa during the infection process with the aim of fighting and resolving the infection. According to Barrios *et al.* (2016) and Bautista (2009) the levels of leukocytes are increased due to the invasion of foreign agents, such as parasites.

Table 6: Correlation between the parasitic load of *Eimeria* oocysts and hematological and biochemical variables.

	OPG (Time 1)	OPG (Time 2)
RBC	-0.42**	0.09
Neu,%	0.31	0.24
Mon,%	-0.39**	-0.43**

OPG: Oocysts per gram of feces; RBC: Red blood cells; Neu: Neutrophils; Mon: Monocytes, ** significant at 0.01.

CONCLUSION

The parasitic load of *Eimeria spp*. oocysts in young alpacas negatively impacts hematological parameters, including hematocrit, red and white blood cells and various blood cell types. However, no significant changes were observed in the levels of albumin, globulin, or total protein. These findings highlight the need to implement control and prevention measures to ensure the health and well-being of newborn alpacas.

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Disclaimers

The opinions and conclusions expressed in this article are the sole responsibility of the authors and do not necessarily reflect the position or stance of their respective home or associated institutions. The authors guarantee the truthfulness and accuracy of the information provided, but assume no responsibility for any damage or loss, direct or indirect, that may arise from the use or application of this content.

Informed consent

Sample collection was carried out in collaboration with the INIA, which is the institution responsible for the animals and which authorized and supervised the obtaining of samples. No harm or suffering was caused to any animal during the collection of samples and they were carried out following protocols that prioritized the welfare of the animals, in addition to the approval of the ethics committee of the Faculty of Veterinary Medicine and Animal Husbandry of the National University of the Altiplano with Ethics Certificate No. 02-2020-FMVZ-UNA-PUNO.

Conflict of interest

The authors have declared no conflict of interest regarding the publication of this article.

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