Research, Society and Development, v. 14, n. 9, e8114949553, 2025
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v14i9.49553

Identification of gastrointestinal parasites in sheep in the coastal region of Northeast Brazil

Identificação de parasitos gastrointestinais em ovinos na região litorânea do Nordeste do Brasil

Identificación de parásitos gastrointestinales en ovinos de la región costera del Noreste de Brasil

Received: 09/10/2025 | Revised: 09/22/2025 | Accepted: 09/23/2025 | Published: 09/26/2025

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Abstract
The Northeast of Brazil is the region with the largest sheep herd, representing 60.6% of the country's animals. Sheep farming is of great social and economic importance, with parasitic infections being one of the biggest obstacles faced by producers, mainly affecting family-based producers. Given this, the objective of the study was to identify gastrointestinal parasites in sheep in the metropolitan region of Natal, Rio Grande do Norte, Brazil. Fecal samples were collected to evaluate the number of eggs and oocysts per gram of feces (EPG and OPG, respectively) and for coproculture. In addition, blood was collected from the animals to perform hematocrit tests to evaluate globular volume, as well as global and differential leukocyte counts to quantify eosinophils. Fifty-nine fecal samples were

analyzed, divided into groups of sheep raised on pasture (n=17), confined (n=22), confined without antiparasitic treatment (n=10), and confined after treatment (n=10). Blood was collected from the external jugular vein of these animals. The results showed a difference between pasture-raised and confined sheep in terms of EPG, OPG, hematocrit, and eosinophils. The helminth *Haemonchus contortus* was identified in the coproculture. Therefore, the form of management seems to influence the control of parasitism, while treatment had less influence than the management factor, which may be the result of the high rates of resistance to antiparasitic drugs already described in the literature.
Keywords: Sheep; Parasites; EPG; OPG.

Resumo
O Nordeste do Brasil é a região com o maior rebanho ovino, representando 60,6% dos animais do país. A ovinocultura é de grande importância social e econômica, sendo as infecções parasitárias um dos maiores obstáculos enfrentados pelos produtores, afetando principalmente os produtores familiares. Diante disso, o objetivo do estudo foi identificar parasitas gastrointestinais em ovinos na região metropolitana de Natal, Rio Grande do Norte, Brasil. Amostras fecais foram coletadas para avaliar o número de ovos e oocistos por grama de fezes (OPG e OPG, respectivamente) e para coprocultura. Além disso, foi coletado sangue dos animais para realização de exames de hematócrito para avaliação do volume globular, bem como contagem global e diferencial de leucócitos para quantificação de eosinófilos. Foram analisadas 59 amostras fecais, divididas em grupos de ovinos criados a pasto (n=17), confinados (n=22), confinados sem tratamento antiparasitário (n=10) e confinados após tratamento (n=10). Foi coletado sangue da veia jugular externa desses animais. Os resultados mostraram diferença entre ovinos criados em pasto e confinados em termos de OPG, OPG, hematócrito e eosinófilos. O helminto *Haemonchus* contortus foi identificado na coprocultura. Portanto, a forma de manejo parece influenciar o controle do parasitismo, enquanto o tratamento teve menor influência do que o fator manejo, o que pode ser resultado das altas taxas de resistência aos antiparasitários já descritas na literatura.
Palavras-chave: Ovinos; Parasitos; OPG; OOPG.

Resumen
El Nordeste de Brasil es la región con el mayor rebaño ovino, representando el 60,6% de los animales del país. La ganadería ovina es de gran importancia social y económica, siendo las infecciones parasitarias uno de los mayores obstáculos que enfrentan los productores, afectando principalmente a los productores familiares. Por ello, el objetivo del estudio fue identificar parásitos gastrointestinales en ovinos de la región metropolitana de Natal, Rio Grande do Norte, Brasil. Se recolectaron muestras fecales para evaluar el número de huevos y ooquistes por gramo de heces (HPG y OPG, respectivamente) y para coprocultivo. Además, se extrajo sangre de los animales para realizar pruebas de hematocrito para evaluar el volumen globular, así como recuentos leucocitarios globales y diferenciales para cuantificar los eosinófilos. Se analizaron cincuenta y nueve muestras fecales, divididas en grupos de ovejas criadas en pastura (n=17), confinadas (n=22), confinadas sin tratamiento antiparasitario (n=10) y confinadas después del tratamiento (n=10). La sangre se recolectó de la vena yugular externa de estos animales. Los resultados mostraron una diferencia entre las ovejas criadas en pastura y las ovinas en confinamiento en términos de HPG, OPG, hematocrito y eosinófilos. Se identificó el helminto *Haemonchus* contortus en el coprocultivo. Por lo tanto, el tipo de manejo parece influir en el control del parasitismo, mientras que el tratamiento tuvo menor influencia que el manejo, lo que podría deberse a las altas tasas de resistencia a los antiparasitarios ya descritas en la literatura.
Palabras clave: Ovejas; Parásitos; HPG; OOPG.

1. Introduction

Sheep farming is an economic activity that is present in several countries (Viana, 2008) with different climatic characteristics, ranging from tropical to subtropical (O´Connor et al., 2006) and temperate (Taylor et al., 2010) climates. Sheep were introduced to Brazil by the Portuguese when it was a colony, and today the country has approximately 21,79 million sheep, with a high concentration in the south and northeast regions, representing 20% and 71.2% of the national herd, respectively (Magalhães et al, 2024).

In the Northeast, Rio Grande do Norte ranks fifth in sheep production in the region, with the municipality of Apodi being the largest producer in 2023, according to data from the table of herd numbers by type of herd. However, the metropolitan region of Natal has 10 municipalities, among which those with the highest prevalence of sheep farming are Macaíba with 4,200 sheep and Monte Alegre with 2,787 of these animals (IBGE, 2023).

Sheep farming is of great economic and social importance, as it generates income for small and large producers, which is a factor that contributes to the permanence of people in the uncultivable lands of the northeastern semi-arid region, contributing both to economic development and to the growth of this activity in the region. In addition, it is an efficient crop due to its rapid production cycle and the animals' easy adaptation to the climate, providing an excellent source of protein for low-income populations and family-based sheep farmers, i.e., small producers who use this activity for their livelihood (Santello et al., 2006; Ahid et al. 2007; Arnoni, 2014).

However, breeders face one of the biggest and most serious problems in sheep farming: parasitic diseases caused by helminths and protozoa, which lead to high morbidity and mortality in animals and can cause developmental delays in young animals, reducing the productivity of small ruminants and, therefore, financial losses for producers (Vieira, 2008; Pimenta Neto, 2009).

In the study conducted by Macêdo (2015), using 252 sheep distributed across seven municipalities in Rio Grande do Norte, EPG and OOPG were performed to assess the parasite load between October 2012 and July 2014, with high positivity for helminth eggs found in 51% of samples, while *Eimeria* oocysts were found in 48% of the samples.

The main parasites associated with economic losses are those belonging to the genera *Haemonchus*, *Oesophagostomum*, *Trichostrongylus*, *Strongyloides*, and *Eimeria*, which consequently have a higher prevalence and intensity of infection (Costa & Vieira, 1984; Baima, 2015; Macêdo, 2015).

These parasites belong to two large phyla, Platyhelminthes (flatworms) and Nemathelminthes (cylindrical worms). The most prevalent protozoa in this type of infection belong to the phylum Apicomplexa, characterized by being obligate intracellular parasites and having an apical complex, a tapered termination that allows them to enter the host cell (Bowman, 2010; Monteiro, 2014). The biological characteristics of these parasites are mainly associated with their life cycle, as both protozoa and helminths have free-living (in the environment) and parasitic (in the host) life stages (Bowman, 2010).

Sheep suffer from common parasitic infections throughout their lives, and among the various species of parasites, *H. contortus*, a blood-feeding helminth that ingests large amounts of blood, up to 0.05 mL per day, is particularly prevalent. An infection with 5,000 worms, which settle in the animal's abomasum, can result in a daily loss of 250 mL of blood, causing paleness of the mucous membranes and skin. Another clinical sign is submandibular edema, caused by hypoproteinemia (Neves, 2010), in addition to severe anemia, confirmed by a hematocrit below 15%. There may also be cases of diarrhea in cases of mixed infection (Bowman, 2010). The decrease in the number of red blood cells in relation to the concentration of globular volume and hemoglobin leads to a decrease in oxygen to the body, which, consequently, causes necrosis of tissue cells (Feldman et al., 2000).

Another helminth that is quite common in sheep is *Trichostrongylus colubriformis*, which parasitizes the small intestine, causing protein exudation into the intestinal lumen, submandibular edema, hemorrhages, enteritis, and inflammation of the mucous membranes (Reineck, 1983). In mild infections, there is a decrease in growth rates and anorexia, while severe infections are characterized by weight loss and diarrhea. Nutritional deficiency and exposure of animals to many parasites in a short period of time are factors that lead to an increased risk of mortality in these animals (Taylor et al., 2010).

In addition to these helminths, protozoa of the genus *Eimeria* also deserve mention among gastrointestinal parasites of veterinary importance (Hansen & Perry, 1994), since the parasite lodges in the intestinal mucosa and causes a decrease in nutrient absorption in the host due to the parasitic action, resulting in decreased nutritional efficiency as well as diarrhea, weight loss, dehydration, and interference with peristaltic movements (Ahid, 2009; Bowman, 2010). *Eimeria ovinoidalis* and *Eimeria crandallis* are considered extremely pathogenic in sheep (Taylor et al., 2010), as they cause epithelial damage and atrophy of the microvillus membranes in the small intestine (Gregory & Catchipole, 1987). Severe infections of these two

species are responsible for disrupting the amphibious microbiota, allowing the growth of Gram-positive bacteria and aggravating diarrhea (Yakchaki & Golami, 2008).

The most serious effect of parasitic diseases on sheep farming is the death of animals associated with direct (ingestion of cells and tissues) or indirect (absorption of nutrients) exploitation and toxic actions with the elimination of catabolites in saliva (Silva, 2014), which can cause diarrhea, edema, anemia, weight loss, and loss of appetite, varying in different pathologies (Azevêdo et al., 2008; Silva, 2014) depending on the etiological agent, parasite load, age, diet, breed, and vulnerability of each animal (Urquhart et al., 1998).

Parasitic infections in sheep are usually mixed, meaning infection by more than one species of parasite, causing more harmful effects to the animal due to the debilitating action of the parasites, which in some cases result in the death of the sheep. As a result of prolonged infections, there is an increase in medication costs combined with a decrease in animal production. The main agents in mixed infections are *H. contortus, T. colubriformis, O. columbianum, Strongyloides spp., Cooperia spp., and Nematodirus spp.* (Amarante et al., 1997, Amarante et al., 2004).

Parasites are widely distributed geographically, mainly in tropical, subtropical, and temperate climates, due to their characteristics of adaptation, proliferation, and resistance to climatic conditions (Molento, 2005). Thus, infections have different incidence rates throughout the year, as each parasite adapts better to certain climatic periods, such as the prevalence of *H. contortus* during periods of high rainfall (Ramos et al., 2004). During the hot, dry summer, due to dry pasture, the infective larvae of *H. contortus* were unable to develop in the environment (Besier & Dunsmore, 1993).

In the Northeast, specifically in Rio Grande do Norte, there are two prevalent types of climates: tropical, which covers the coastal strip, and semi-arid in the interior of the state. In tropical regions, the parasitic population is prevalent throughout the year due to high humidity, especially in winter, the season when environmental conditions are most favorable, characterizing the seasonal growth of larvae (Taylor et al., 2010). In the semi-arid region, when it rains, infections caused by parasites appear in the herd, while in the dry season, the larvae are unable to continue their cycle in the environment due to solar radiation, high evapotranspiration, and reduced humidity. Due to adverse environmental conditions, the larvae tend to remain inside their hosts, with their development interrupted, becoming hypobiotic larvae (Vieira, 2008; Taylor et al., 2010).

In addition to climatic conditions and hypobiosis, other factors can influence the growth of the parasite population, such as parasite resistance, temperature, soil, type and management of pasture and animals, as well as species, breed, age, physiological and nutritional status (Ruas & Berne, 2001), host immunity, pasture contamination, elimination of a larger number of eggs in the peripartum period, among others (Costa et al., 2011).

In many places, parasite control is carried out by strategically using antiparasitic drugs for both curative and prophylactic treatment, with intervals of 30 or 45 days (González-Garduño et al., 2014). In view of this, the continuous and improper use of antiparasitic drugs is responsible for the emergence of parasite resistance (Molento & Prichard, 1999).

Most anthelmintics were created in the 1960s and are still widely used today in parasite control, allowing producers to increase their herds due to the reduction in gastrointestinal helminth infections. Given this, these drugs are distributed according to the mechanism of action of the medication on nematodes, and may be narrow-spectrum, such as salicylanilides/substituted phenols and organophosphates (Amarante, 2007), in addition to broad-spectrum drugs involving benzimidazoles, imidazothiazoles, and macrocyclic lactones, the latter consisting of avermectins (ivermectin, abamectin, doramectin, and selamectin) and milbemycins (milbemycin and moxidectin) (Delayte et al., 2006).

Currently, there are integrated programs for parasite control that aim to delay the onset of resistance to anthelmintics, with the use of treatments based on epidemiology, adequate pasture cleaning, and the technique of grazing with alternating

species, mainly between sheep and cattle, aiming at pasture decontamination following the principle of parasitic specificity, as cattle ingest the infective larvae of sheep parasites, killing them.

Besides, eliminating unnecessary deworming (Mota, 2003; Vieira, 2008) as proposed by the FAMACHA (FMC) method, a technique widely used in the control of *H. contortus*, selecting the animals most in need of treatment through microhematocrit analysis, visualization of submandibular edema, and mucosal staining. The latter may be anemic, depending on the degree of infection by *H. contortus*, and is classified, with the aid of the FMC standard card, on a scale from one (bright red) to five (pale, almost white), indicative of severe anemia.

Based on this method, animals classified as 4 and 5 undergo treatment. For this reason, the FAMACHA method reduces spending on antiparasitic drugs due to the decrease in the number of doses and animals to be treated (Bath & Van Wyk, 2001; Molento et al., 2004). Another way to control the use of antiparasitics is to employ management practices aimed at strengthening the herd's immunity, investing in vaccines (Grønvold et al., 1996) and an energy- and protein-rich diet (Torres-Acosta & Hoste, 2008).

Another integrated program is biological control, used as a prophylactic measure against nematodes, in which helminth antagonists are used, causing the reduction of the number of worms in the soil and reducing the chances of infection for the host (Grønvold et al., 1996; Waller & Faedo, 1996). As it is a sustainable way to combat parasitic infections by nematodes, biological control also reduces the use of antiparasitic drugs, leading to a decrease in the emergence of parasitic resistance (Fontenot et al., 2003).

The main objective of this study was to identify gastrointestinal parasites in fecal samples of sheep from the metropolitan region of Natal, Rio Grande do Norte, Brazil.

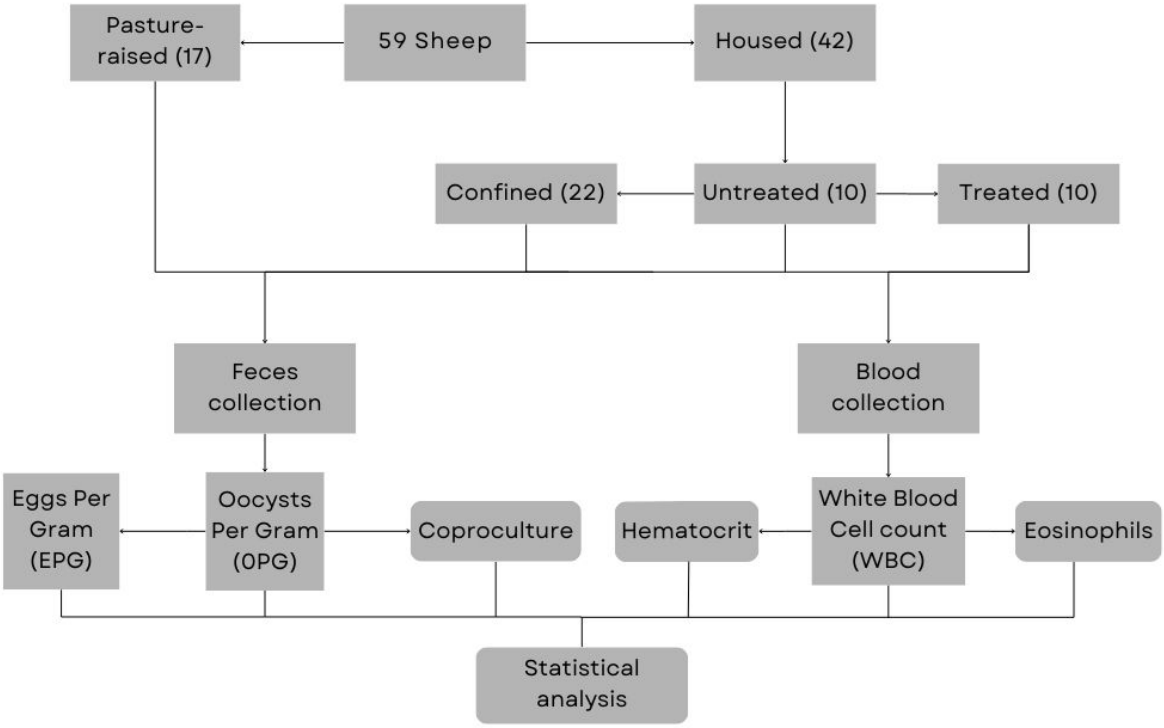
2. Methodology

Experimental research was conducted, of a mixed nature with part of the study in the field and part in the laboratory (Pereira et al., 2018), in a qualitative investigation for the identification of worms and quantitative in the statistical treatment of the data (Shitsuka et al., 2014; Vieira, 2021). In this study, Santa Inês sheep (*Ovis aries*) approximately one year old were used, raised under different management conditions, on pasture and in confinement, at the Agricultural School of Jundiaí – Federal University of Rio Grande do Norte, located in coastal region, in the city of Macaíba/RN.

A total of 59 sheep were used in the experiment: 17 pasture-raised and 42 confined. The confined animals were divided into two groups (untreated and treated). The groups underwent fecal and blood collection stages according to the experimental design in Figure 1.

The first group (n=17) consisted of animals raised on pasture using mineral salt with monensin, the second group (n=22) consisted of confined animals that were part of a nutritional experiment, in which the animals in the study were subjected to a protein diet. The third group (n=10) corresponds to confined animals without antiparasitic treatment for a period of 90 days, and the fourth group (n=10) consists of the same animals as the third group, but after 15 days of treatment. It is worth noting that some animals in the latter group were grazing before confinement.

Figure 1 - The stool and blood samples were processed as detailed in the experimental design of the study.



Source: Research data (2025).

This study followed the guidelines of the National Council of Experimentation (*Conselho Nacional de Controle de Experimentação Animal* - CONCEA) and was approved by the Ethics Committee (Comissão de Ética no Uso de Animais da Universidade Potiguar - CEUA/UnP), under protocol number 014/2017.

2.1 Collection and Analysis of Fecal Samples

In this study, 10 fecal samples were collected from each group in the experiment directly from the rectal ampoule and immediately refrigerated to maintain sample integrity and egg viability, with the aim of obtaining reliable results (Taylor, 2010).

The quantification of eggs and oocysts in the fecal samples was performed through laboratory tests to count eggs per gram of feces (EPG) and oocysts per gram of feces (OPG), respectively, using the Mac Master technique described by Gordon and Whitlock (1939) (Ueno & Gonçalves, 1998), which consists of dissolving two grams of feces in 60mL of saturated sodium chloride solution and filtering the solution with gauze to retain debris. The filtered solution was left to stand for five minutes to allow the eggs and oocysts, which have a lower density than the saturated solution, to float. After this time, the surface portions of the filtered solution were collected with a Pasteur pipette, enough to fill both sides of the Mac Master chamber, for analysis under a microscope at 400x magnification (Roberts & O'Sullivan, 1950).

When counting the Mac Master chamber, the number of eggs counted on each side of the chamber is added together and multiplied by 100. The average is then obtained from the result obtained from the two quadrants. In this way, the degree of infection (severe, moderate, or mild) is classified by the number of eggs found in the feces, but the parameter for the number of eggs for this assessment varies among different species of parasites (Roberts & O'Sullivan, 1950).

The qualitative assessment was performed by coproculture, a technique that allows the identification of larvae that originate from the hatching of eggs found in the EPG exam, which allows the identification of the species of nematode(s)

present in the animals by viewing the morphology of the head and tail of the larva (Figura 2). The fecal sample was deposited in a beaker and mixed with sawdust to facilitate aeration, then moistened with distilled water and covered with plastic wrap containing holes in its surface to allow air to enter, simulating the environment in which the parasite lives in its free-living stage. The coproculture needs to be moistened daily and kept at room temperature for 21 days. After this period, distilled water was added to half of the beaker, which was inverted under the Petri dish, and after one hour, the volume of water with the larvae around the beaker was transferred to another Petri dish for visualization under a magnifying glass. subsequently, slides were prepared with a drop of the sample, Lugol's iodine, and a coverslip, followed by visualization under a microscope at 10x and 40x magnification to verify the larval morphology (Roberts & O'Sullivan, 1950).

2.2 Hematocrit

Hematocrit is used to assess the percentage of globular volume; low levels may be indicative of anemia in ruminants (Macedo et al., 2015). To determine hematocrit, blood samples were collected from the jugular vein using a 5 mL syringe and SR brand needle and then placed in a tube containing Vacuplast® brand ethylenediaminetetraacetic acid (EDTA). Hematocrit values will be determined by the microhematocrit methodology, which consists of filling capillary tubes (Precision®) with blood and placing them in a microcentrifuge at 10,000 rpm for five minutes (Bath et al., 2001). The reading is performed with the aid of the microhematocrit card, knowing that the reference value for sheep is 24 to 50%.

2.3 White Blood Cell Count

The global leukocyte count is a quantitative method that aims to provide the total number of leukocytes in the animal. For the global leukocyte count procedure, a 1:20 dilution of whole blood with Turk's diluting solution was performed, which lyses red blood cells and stains the leukocyte nucleus. The procedure is performed by pipetting 380 µL of Turk's solution into the hemolysis tube and 20 µL of blood. The solution is then homogenized by aspirating and expelling the liquid from the tip and inverting it for two minutes. Subsequently, the Neubauer chamber is filled with the previously prepared content, and, after cell sedimentation, the reading is performed at 400x magnification in the four quadrants of the upper and lower extremities (Vivas, 2017).

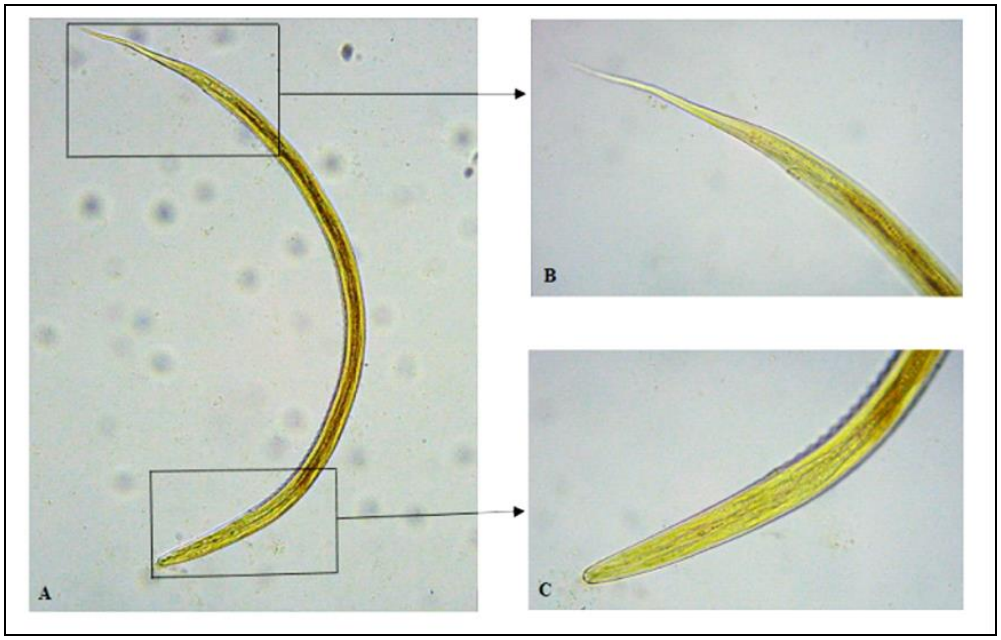
Thus, to obtain the overall leukocyte count, the calculation to obtain leukocytes per ml of blood is performed using the following formula:

$$\textbf{Leukocytes per µL of blood} = \frac{\textbf{Number of leukocytes x 20 x 10}}{\textbf{4}}$$

According to a formula, 20 is dilution conversion factor, the number 10 corresponds to the depth of the chamber and 4 is number of fields counted.

The illustration shows the larval stage of *Haemonchus contortus* in its infective phase. Panel (A) shows the entire larva, emphasizing its morphological characteristics. Panel (B) details the medium-sized sheath, which gradually tapers toward the terminal region of the tail, a feature commonly used to distinguish this species. Finally, panel (C) presents the anterior portion of the larva, where the narrow and rounded head can be observed, an important diagnostic trait in parasitological identification.

Figure 2 - Larva in the infective stage of *H. contortus* (A); medium-sized sheath tapering at the end of the tail (B); and narrow, rounded head (C).



Source: Research data (2025).

2.4 Differential Leukocyte Count

Leukocytes originate from precursor cells in the bone marrow and are divided into two groups: granulocytes (neutrophils, eosinophils, and basophils) and agranulocytes (lymphocytes and monocytes). The differential white blood cell count aims to quantify the different white blood cells separately, providing relative results in percentage and absolute results in mm^3 of blood (Rosa et al., 2012).

The differential white blood cell count is performed using a blood smear. The slides are stained using the Romanowsky method with rapid panoptic staining (Renybab), which consists of immersing the slides with a continuous up and down movement for 30 seconds in three solutions, the first being a fixative (0.1% triarylmethane), the second being 0.1% xanthene dye, and the third being 0.1% basic thiazine dye to stain the leukocytes (Renylab, 2012). In the latter, immersion occurs for 20 seconds. The slides are then washed with running water to remove excess dye and left to dry at room temperature. After drying, they are placed under a microscope and, using an immersion objective, 100 leukocytes are counted to obtain the relative value of the differential leukocyte count.

With the values of the total leukocyte count and the relative differential leukocyte count, it is possible to determine the absolute values of the differential leukocyte count, which will provide the number of leukocytes per microliter of blood.

2.5 Statistical Analysis

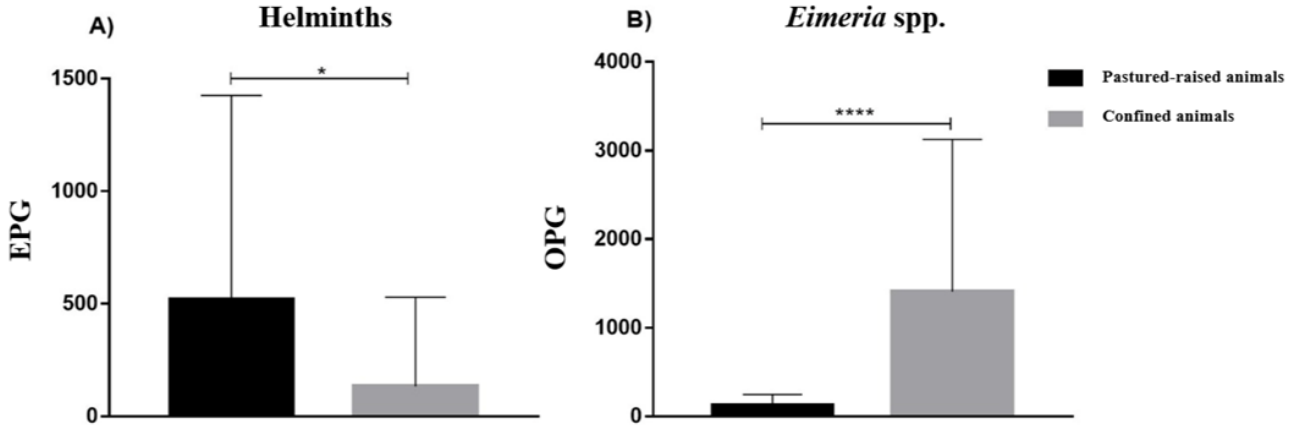
Microsoft Excel® software was used to organize the data collected in spreadsheets, and statistical analysis was subsequently performed using PRISM® 7 (GraphPad, San Diego, CA, USA). In this software, we initially analyzed whether the data were normally distributed using the D'Agostino-Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov tests. In the case of normally distributed data, we used parametric tests (Anova and t-test), while for data that did not obtain a normal distribution, the analyses were nonparametric (Kruskal Wallis and Mann-Whitney). Correlation tests were performed using Spearman's nonparametric test. Data were considered significant when the P value was less than or equal to 0.05.

3. Results and Discussion

Animals raised under different management systems have different parasite prevalences, with helminth infection being more common in pasture-raised animals (Siqueira et al., 1993; Silva et al., 2013) and protozoan *Eimeria* spp. predominating in confined animals (Kimberling, 1988; Ekawasti et al., 2021; Liu et al., 2024).

Given this, we evaluated groups under different management systems, measuring the EPG of pasture-raised animals with a higher helminth egg index compared to confined animals (Figure 3A). In the OPG (Figure 3B), the opposite is observed: sheep in the confined group had a higher prevalence of the protozoan of the genus *Eimeria* spp. due to the management system used for these animals.

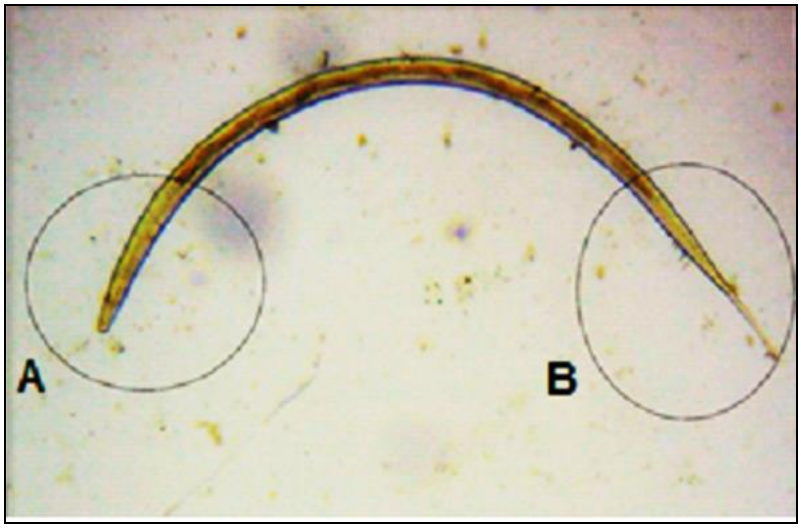
Figure 3 - Egg count per gram of feces (A) and oocyst count per gram of feces (B) performed using the MacMaster technique on sheep raised on pasture (n=17) and confined (n=22). Data are expressed as mean and standard deviation. *P ≤ 0.05. ****P ≤ 0.0001.



Source: Research data (2025).

The evaluation of coproculture samples is important for species identification following the identification key described by Taylor et al. (2010). In the analyzed samples from all study groups, the helminth *Haemonchus contortus* was identified (Figure 4), through the morphology of the larva's head, narrow and rounded (Figure 4A), and characteristics of the sheath present in the tail, which is medium in size and tapers at the terminal portion (Figure 4B).

Figure 4 - Larva in the infective stage of *H. contortus*. Narrow, rounded head (A) and medium, tapering sheath at the end of the tail (B).

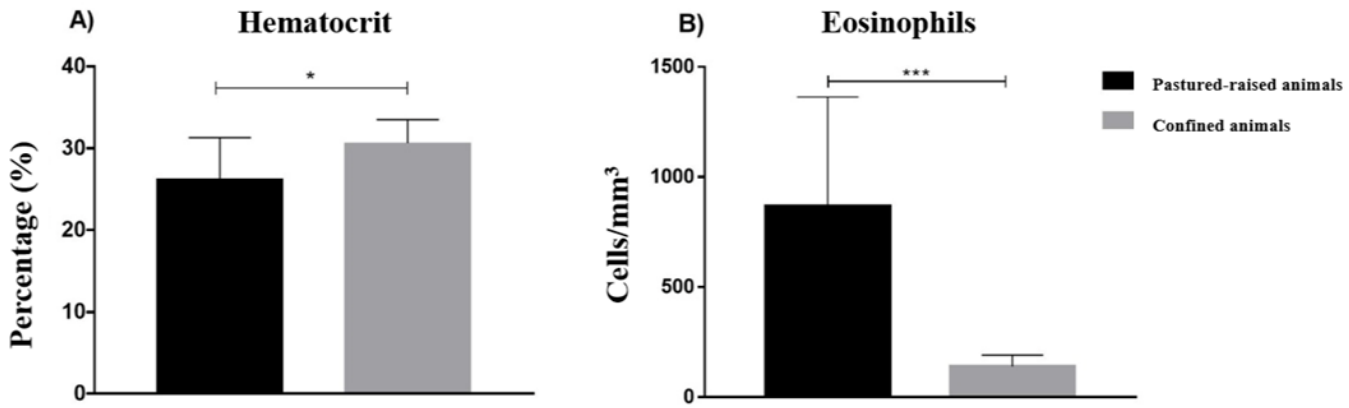


Source: Research data (2025).

Due to the high levels of infection by *H. contortus*, suspected cases of anemia are always strong. For this reason, microhematocrit was performed, and the group of animals grazing presented lower hematocrit when compared to the confined group (Figure 5A).

Additionally, total and differential white blood cell counts were performed to assess the presence or absence of infection or inflammation and the characteristics of the infection based on the type of cell that is elevated, respectively. With the overall white blood cell count, no difference was observed between the groups (Figure 5B), but the opposite was noted in the differential white blood cell count, where there was a difference in the number of eosinophils between the groups, showing that the immune system of grazing animals is likely acting to combat infection, since eosinophils are granulocytic cells of the immune system that are elevated in parasitic infections and allergic processes.

Figure 5 - (A) Hematocrit performed using the microhematocrit methodology. (B) Quantification of eosinophils performed using differential leukocyte counts in pasture-fed (n=15) and confined (n=8) animals. Data are expressed as mean and standard deviation. *P ≤ 0.05. ***P ≤ 0.001.

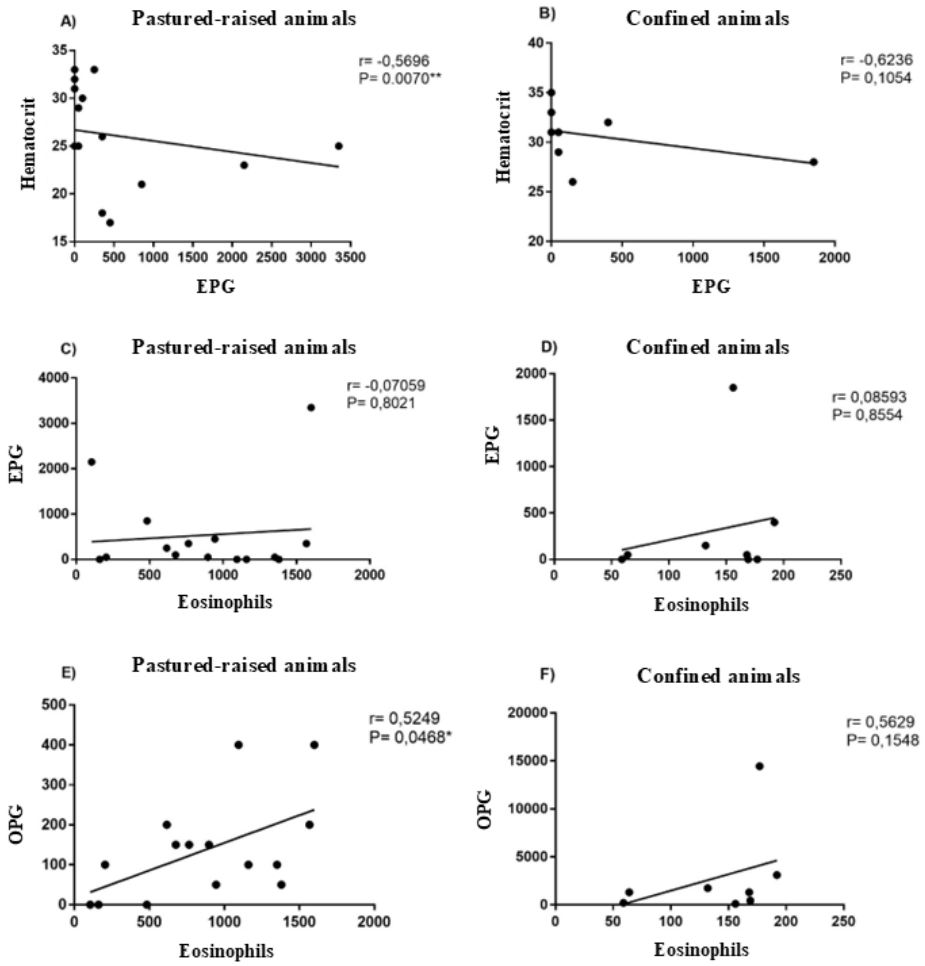


Source: Research data (2025).

Given these results, we evaluated the correlation between EPG and hematocrit in the groups of sheep raised on pasture (6A) and confined (6B), which showed a negative correlation, indicating that the higher the EPG, the lower the hematocrit value obtained, confirming the exploitative action of *H. contortus*. In confined animals (6B), there was no difference.

There was no difference in the correlation between EPG and eosinophils in pasture-raised sheep (6C) and confined sheep (6D). Meanwhile, OPG and eosinophils in pasture-raised (6E) showed a positive correlation, demonstrating that the higher the OPG value, the greater the number of eosinophils in the bloodstream to fight infection. However, there was no difference among the group of confined animals (6F).

Figure 6 - Correlations performed between hematocrit, EPG, OPG and eosinophil parameters in groups of animals on pasture (Figures 6A, C and E) (n=15) and confined animals (Figures 6B, D and F) (n=8).



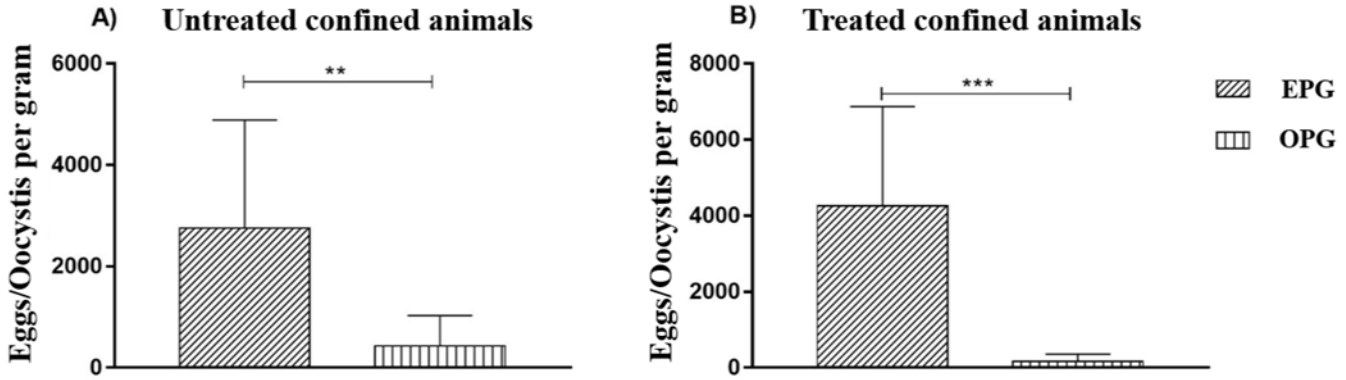
Source: Research data (2025).

Given the results, a second collection was performed on animals under extensive and confined systems to assess the parasite load before and after treatment with Cydectin®, a broad-spectrum drug belonging to the class of macrocyclic lactones (milbemycins), whose active ingredient is moxidectin. However, in the collection before treatment, the grazing animals had low EPG and OPG and, consequently, were not treated, continuing the research with the confined animals before and after treatment.

Initially, the EPG of untreated and treated confined animals was evaluated, as was the OPG, but there was no difference in either graph. However, it was found that the animals before treatment (Figure 7A) and after treatment (Figure 7B) had a higher EPG index compared to OPG, that is, *H. contortus* infection is prevalent in both groups.

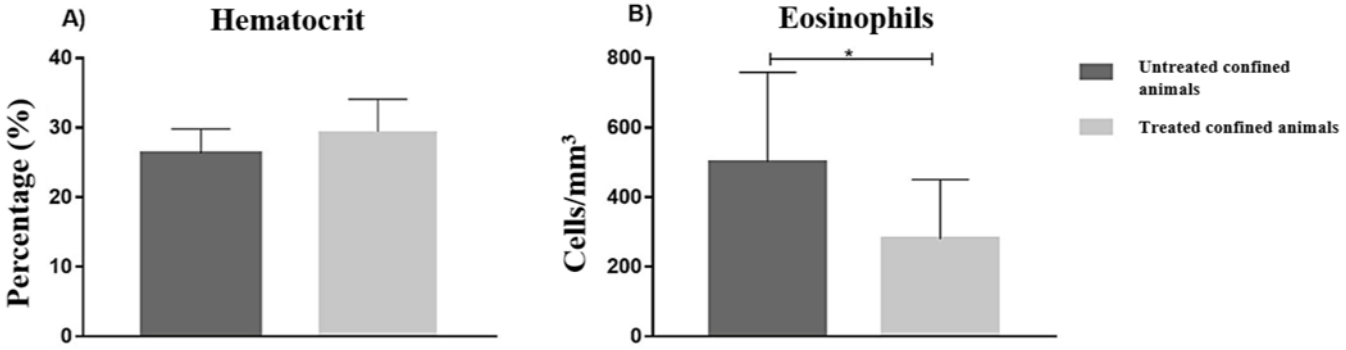
In the analysis of blood samples, hematocrit measurements showed no statistical difference between untreated and treated confined animals (Figure 8A). The overall leukocyte counts results showed no statistical difference between the two groups. However, the eosinophil count was significant, indicating that eosinophil levels were higher in the untreated group (Figure 8B).

Figure 7 - Egg count per gram of feces and oocyst count per gram of feces performed by the Mac Master technique, of sheep raised in confinement before (A) and after treatment (B) (n=10). Data are expressed as mean and standard deviation. **P ≤ 0.01. ***P ≤ 0.001.



Source: Research data (2025).

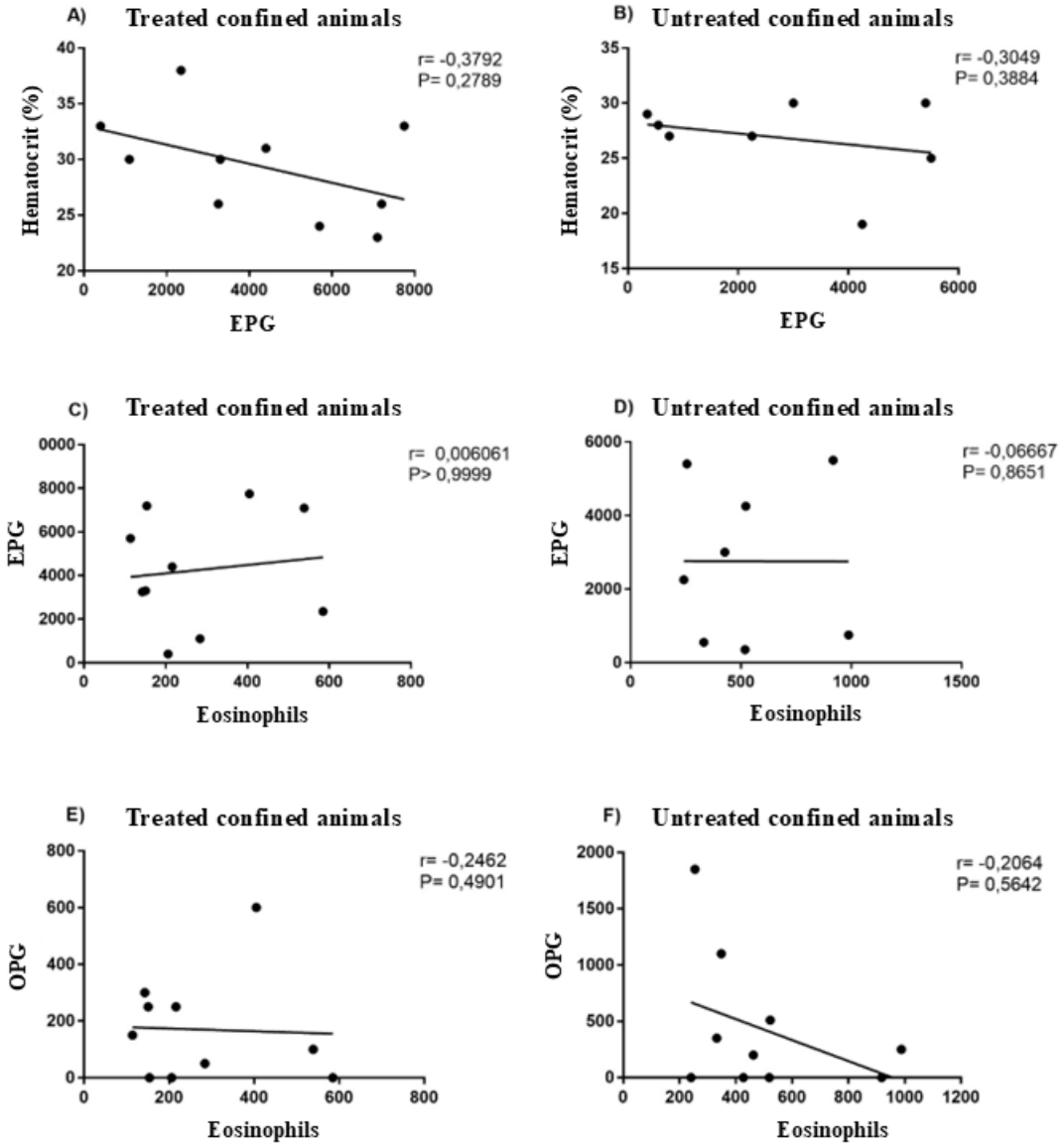
Figure 8 - Hematocrit (A) performed by the microhematocrit methodology and quantification of eosinophils through the absolute differential leukocyte count in untreated (n=10) and treated (n=10) confined animals. Data are expressed as mean and standard deviation. *P ≤ 0.05.



Source: Research data (2025).

Given the findings in the second analysis, we verified the splendid results among the data obtained. However, there was no difference in the graphs created between hematocrit and EPG, EPG and eosinophils, as well as OPG and eosinophils of the treated (Figure 9A, C and E) and untreated (Figure 9B, D and F) animals.

Figure 9 - Correlations performed between hematocrit, EPG, OPG and eosinophil parameters in groups of confined animals without treatment (Figures 9A, C and E) (n=10) and confined animals treated (Figures 9B, D and F) (n=10).



Source: Research data (2025).

The economic, social, and health damage caused by gastrointestinal parasites, associated with the high prevalence of intestinal parasites such as the helminth *H. contortus*, is quite common in Brazil (Amarante et al., 1992; Vieira & Cavalcante, 1999; Ramos et al., 2004). To control this parasitic disease, it is important to implement parasite control measures and avoid unnecessary use of antiparasitic drugs. Therefore, it is important to perform tests to assess the parasite load and health conditions of the animal, such as the presence of anemia.

The Brazilian Santa Inês sheep breed is a cross between the Moarada Nova and Bergamácia breeds (Jardim, 1987). The resulting breed is more resistant to gastrointestinal nematodes when compared to other foreign breeds such as Sulffock, lle de France, and Pool Dorset (Moraes et al., 2000; Bueno et al., 2002; Bricarello et al., 2003; Amarante et al., 2004; Rocha et al., 2004).

Several studies report that parasite infection occurs during rainy periods extending into the beginning of the dry season, as there is insufficient humidity for the development of infective larvae in the environment during other times of the year (Pinheiro, 2011), with a decrease in the infection rate during these periods. However, Souza et al. (2013) report that in addition to H. contortus parasitizing animals in tropical climates, it is also capable of causing infections in semi-arid regions, indicating that the helminth can grow in high temperatures and low humidity. Studies show that the etiology of infection in sheep is influenced by the type of management to which these animals are subjected, with a higher prevalence of helminths in grazing animals (Siqueira et al., 1993) and protozoa in young, confined sheep (Kimberling, 1998; (Vieira, 2005; Silva et al., 2011).

Another influencing factor is the presence of animals that are resistant or tolerant to parasitic diseases in the herd. It is known that in cases of resistance, the immune response limits the establishment of the parasite in the animal, while tolerance indicates that the animal can live with the parasite without affecting its productivity, but the parasite's cycle in the animal continues and these animals remain a source of infection for susceptible animals (Amarante et al., 2007; Basseto et al., 2009), although they do not live long when exposed to a high parasite load. Given this, the response produced by the host depends on two factors: one intrinsic and genetically regulated, and the other environmental, related to the nutritional conditions of the animals, since in sheep with a protein-rich diet, greater resistance to nematode infection is noted (Knox & Steel, 1996; Bricarello et al., 2003).

Given the above, the high EPG observed in pasture-fed animals is due to their greater susceptibility to parasitic infections caused by helminths (Siqueira et al., 1993). Consequently, the animals themselves are the sources of environmental contamination, as it is in the pasture that eggs, excreted in the animal's feces, develop into infective larvae (Oliveira-Sequera, 2001; Amarante, 2004). This process is facilitated by the region's climatic conditions, particularly high temperature and humidity, increasing helminth infections in animals kept in this type of management (Santos et al., 2006). As a result of the period that the animals in the confined groups before and after treatment with Cydectin® were kept on pasture before confinement, it is assumed that this is when nematode infection occurred, increasing the EPG observed in these sheep.

The presence of *H. contortus*, a nematode identified in stool culture samples, is consistent with studies by Costa & Vieira, 1984; Charles, 1989; Charles, 1995; Arosemana et al., 1999; Silva et al., 2003, which indicate the prevalence of this helminth in the Northeast region. It also corroborates the study by Souza et al., 2009, conducted in Lajes, Rio Grande do Norte, which also found a greater predominance of *H. contortus* in sheep. Unlike the groups mentioned above, the OPG of confined sheep was higher, common in young sheep and those raised in confinement (Vieira, 2005; Silva et al, 2011). This management system appears to have prevented helminth infection, as the oocyst is lightweight and can be transported from pasture to confinement pens by the wind, depositing in the feed and infecting the animals; the same does not occur with helminth eggs. In addition, there is the lack of use of mineral salt with monensin, an antiparasitic that selects the rumen microbiota, supplements the animal's minerals, and acts to control coccidiosis caused by protozoa of the genus *Eimeria*.

Given this information, the high rates of infection by *H. contortus*, a hematophagous helminth, raise suspicions of anemia. For this purpose, hematocrit was measured, which decreases due to the spoliative action of *H. contortus* (Van Wyk et al., 1997; Kaplan et al., 2004; Amarante et al., 2004; Bricarello et al., 2004), thus corroborating the data obtained from the OPG and stool culture of the study. Regarding the hematocrit of confined animals before and after treatment, there was no difference, indicating that the treatment or its absence did not influence the anemic state of the animals, as most sheep presented hematocrit within the reference value (24 to 50%).

Regarding eosinophils, an important cell in host defense against parasitic infections, they increase in such a situation. They were detected in greater numbers in the bloodstream of animals in the pasture-fed and confined groups before treatment,

demonstrating that the immune systems of these groups are working to combat the helminth infection. In confined sheep after treatment, it was demonstrated that the medication, or its non-use, did not influence the number of eosinophils in the blood of these animals, suggesting resistance to the anthelmintic used.

Anthelmintic therapy is a necessary resource, but given the progressive increase in antiparasitic resistance across available chemical groups (Nari & Eddi, 2002), the future availability of new antiparasitic drugs is affected both by this growing number of resistance cases and by the increasing costs of research and development for new drugs (Coles et al., 2006). The literature has already reported the occurrence of helminth resistance to several groups of antiparasitic drugs in sheep (Sangster, 1999; Thomaz-Soccol et al., 2004; Coles et al., 2006). Therefore, resistance of *H. contortus* in Santa Inês sheep to Cydectin®, a medication used to deworm the sheep in this experiment, which contains moxidectin as its active ingredient, was described (Rosalinski et al., 2007; Chagas et al., 2014). Thus, even though the animals were medicated, they still had higher-than-expected EPG after treatment, even higher than the pre-treatment group, which may indicate parasite resistance to the drug used.

According to Amarante (2004) and Cenci et al. (2007), most infections in sheep are mixed, meaning the animal can be infested by more than one parasite simultaneously. This may be explained by the correlation graph between OPG and eosinophils, which indicated that the higher the OPG, the greater the number of eosinophils in the blood, suggesting interference due to mixed infections between gastrointestinal worms and *Eimeria* spp. (Souza, 2009), as observed in this study, since a combination of high EPG and low OPG was observed in these animals. Thus, the increase in eosinophils was possibly due to high EPG, not necessarily due to OPG.

4. Conclusion

The results demonstrate that EPG and OPG were effective tools for assessing parasite load, enabling the identification of helminths and protozoa. Among the helminths identified, the species *Haemonchus contortus* stood out, whose high parasite load in animals kept on pasture was directly associated with the reduction in hematocrit values, suggesting a negative impact on the hematological status of these individuals. Furthermore, eosinophil counts were increased in both the pasture and feedlot-fed groups before treatment was instituted. Correlation analysis revealed that, in animals kept on pasture, there was a negative association between EPG and hematocrit, as well as a positive association between OPG and eosinophils, demonstrating the relationship between infection intensity and hematological responses. However, the treatment employed did not result in a significant reduction in EPG and OPG values. Therefore, management through confinement demonstrated importance in controlling infection, reinforcing the relevance of integrated management strategies to mitigate parasitic effects on animal health.

Acknowledgment

We would like to acknowledge the support of the Jundiaí Agricultural School (Macaíba, Rio Grande do Norte), on behalf of Prof. Dr. Valdi de Lima Júnior and Dr. Jorge Motta for their contributions and Dr. Antônia Cláudia Jácome da Câmara, from the Federal University of Rio Grande do Norte (Natal, Rio Grande do Norte), for her collaboration in the support laboratory.

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