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# Biochemical and hematological aspects of *Anaplasma marginale* infection in dairy cattle, central Goiás, Brazil

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**Abstract**
Anaplasmosis caused by the intraerythrocytic rickettsia *Anaplasma marginale* is scattered in cattle from tropical and subtropical areas in several regions of the world. Forty dairy cows, twenty clinically healthy (constituting the control group), and the other twenty with clinical signs suggestive of Anaplasmosis (infected group) were evaluated regarding to biochemical and hematological aspects in the mesoregion of central Goiás, state of Goiás, Brazil. Infected animals manifested parasitemia from 12% on, with severe anemia and development of clinical signs such as marked apathy and prostration, tachycardia and tachypnea, jaundice and fever. Significant statistical differences ($p$<0.05) were detected by decrease in the total number of red blood cells, hemoglobin and hematocrit; and increase in the mean corpuscular volume, monocytes and total protein. It is concluded that *Anaplasma marginale* from the region has the virulence to reproduce severe clinical conditions of Anaplasmosis for milch cows.

**Keywords:** *Anaplasma marginale* infection, dairy cattle, biochemical and hematological function, Goiás, Brazil

## 1. Introduction
The *Anaplasmataceae Anaplasma marginale* (*A. marginale*) is a Rickettsia inherent in the order Rickettsiales [1, 2], which causes Anaplasmosis in cattle from tropical and subtropical areas in several regions of the world [3]. This agent is considered an obligate intraerythrocytic hemoparasite [4], being responsible for the development of clinical signs such as lethargy, fever, anemia, jaundice, weight loss, decrease in milk production, and abortion [5, 6]. The organic lesions resulting from the pathogenesis worsen as the time of infection enlarges, due to the progressive increase in parasitemia [7].
The sudden increase in parasitemia progresses to hemolytic anemia and jaundice in its acute form [8]. It is suspected that the white blood series may also change, as in small ruminants infected by the hemoparasite *Babesia* sp. neutrophilia and/or lymphocytosis are verified [9, 10], special related to the release of pro-inflammatory cytokines [11]. Pathological lesions were additionally observed in the liver and muscles of cattle with Anaplasmosis [12]; and elevation of Aspartate aminotransferase (AST) and Alkaline Phosphatase (AP), which reinforces the possibility of liver injury [13, 14].
Considering these facts, there is a necessity for further investigation of the blood alterations caused by Anaplasmosis, such as the screening of hematological and biochemical parameters [9, 10, 14]. In addition, there are no studies developed in the center of Goiás on biochemical and hematological changes caused by *A. marginale* infection. It should be noted that due to the genetics of *A. marginale* and the immune defense of the animals – depending on the different geographic regions – there may be differences on its pathogenicity [2]. Thus, this work aimed to evaluate the biochemical and hematological alterations caused by *A. marginale* in dairy cattle located in the central region of the state of Goiás, Brazil.

## 2. Materials and Methods

The experimental protocols applied in this work are in accordance with the standards of care for animals in experiments approved by the Committee on Ethics in the Use of Animals (CEUA) of the University Center Brasília de Goiás, substantiated under number 017-MV/2021. 40 (forty) dairy cows aging from two to six years-old from the municipalities of São Luís de Montes Belos and Aurilândia, located in the mesoregion of central Goiás, state of Goiás, Brazil were allocated into two groups, the first with 20 (twenty) animals clinically healthy, constituting the control group; and the other 20 dairy cows represented the infected group, which showed clinical signs suggestive of Anaplasmosis. All animals in the study underwent a general physical examination and the research period (for sample collection and processing) was from October to December 2021.

All samples were obtained following the precepts of the National Committee for Clinical Laboratory Standard (NCCLS) [15] and analyzed in the Laboratory of Hematology and Biochemistry from the University Center Montes Belos. Blood samples for hemogram and biochemical tests were collected from the jugular vein by sterile 40 x 1.20mm hypodermic needles (BD®, Curitiba, PR, Brazil). To perform the blood count, 5mL of blood was collected in a tube containing the anticoagulant EDTA (LabSynth®, São Paulo, SP, Brazil); for carrying out biochemical tests, 10mL of blood without anticoagulant were collected to obtain the serum, which was processed by centrifugation at 5.000rpm for 10 minutes at 25ºC (Centrifuge Hettich Rotina 380R®, Newport Pagnell, Buckinghamshire, England). And, for the investigation of possible hemoparasites, blood samples were collected by puncturing the marginal ear vein.

Parasitaemia estimation was performed by direct examination of the agent in the blood of dairy cows, and blood smears were stained with Rapid Panotic (Laborclin®, Pinhais, PR, Brazil). After staining, was performed a microscopic examination (Zeiss®, Oberkochen, Germany) in 100 fields of the smear with an immersion objective (100X magnification). In each smear, from the count of 1000 red blood cells, the number of parasitized red blood cells was estimated, and the result was expressed in percentage (%) [16].

For the erythrogram, red blood cell count and determination of hemoglobin concentration were performed using an automated cell counter (Bio 2900 v*et a*lere®, Waltham, Massachusetts, USA); to determine the hematocrit, the blood was centrifuged at 12.500rpm for 5 minutes at 25ºC; plasma protein was measured using a refractometer (RZ Contec®, Bologna, Italy); Mean Corpuscular Volume (MCV) and Mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulas hematocrit X 10 / number of red blood cells and hematocrit / hemoglobin X 100, respectively, in order to determine the type of anemia [16].

For the leukogram, total leukocyte count was performed on the automatic cell counter, and the leukocyte differential was performed on a blood smear stained with Rapid Panotic [16].

The serum samples were submitted to biochemical examination through the kinetic or colorimetric method in a semiautomatic biochemical analyzer (BIO-200®, São Paulo, SP, Brazil) where the enzymes aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), albumin and total protein were measured to verify possible liver alterations; and the urea and creatinine metabolites to verify animals renal function [16].

Results were expressed as mean ± standard error. Windows version of the Graph Pad Prism 5.01 software was used to perform statistical tests. And, the Student's t test with *P* Values < 0.05 was applied.

## 3. Results and Discussion

The blood smear examination identified the causative agents of Anaplasmosis on its clinical form. All 20 infected animals manifested red blood cells containing small spherical dark dots located peripherally, consistent with intraerythrocyte corpuscles formed by Rickettsia [4, 13, 16] (Figure 1). Likewise, anisocytosis and polychromasia could be observed, corroborating a regenerative process of erythrocytes in the bone marrow [17].

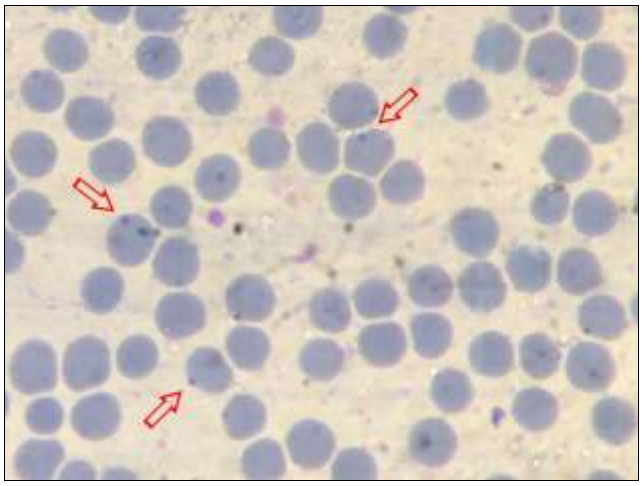

**Fig 1:** Blood smear showing intraerythrocyte corpuscles consistent with *Anaplasma marginale* (arrows). 100X magnification.

Studies have shown that clinical signs of Anaplasmosis are only observed after the parasitaemia to exceed 15%, and that in the acute phase of the disease, parasitaemia can vary from 15% to 48% [18]. However, the dairy cows of this study exhibited severe anemia and the development of clinical signs within 12% of infected red blood cells.

As clinical signs shown by positive animals for *A. marginale* were marked apathy and prostration, in consonance with the literature [2, 6, 14, 19]; tachycardia and tachypnea (average of 100 heartbeats/minute and 60 respiratory movements/minute, respectively), considered as compensatory mechanisms to prevent tissue damage resulting from hypoxemia [20]; jaundice, which has been associated with extravascular hemolysis [2]; and fever (mean rectal temperature of 40°C) [17, 18].

In the erythrogram, it could be seen a significant decrease ($p$<0.01) in the total number of red blood cells, hemoglobin and hematocrit (Table 1), revealing the severe anemia caused by *A. marginale*. Such reductions were greater than those observed in previously published studies [14]. It should be noted that the intracellular destruction of red blood cells infected by the agent occurs mainly in the spleen. However, the participation of the reticuloendothelial system of the liver is also described. After erythrocyte phagocytosis by macrophages – in these compartments – there is a reduction in the number of circulating red blood cells, which may exceed the bone marrow compensation threshold, which explains the reduction in hematocrit and hemoglobin [16, 21].

The anemia observed in the test group was classified as macrocytic normochromic due to the significant increase in the MCV ($p$<0.05) and the non-change in the MCHC, respectively. Also, it was considered regenerative due to the increase in MCV, associated with anisocytosis and

polychromasia. In contrast, other authors have described that the anemia caused by hemoparasites is hypochromic macrocytic [9, 23]. Lastly, in this work, the MCHC did not change, which suggests that the parasitized dairy cows were able to compensate with the production of young red blood cells without the relevant decrease in hemoglobin concentration [17, 18].

**Table 1:** Biochemical and hematological levels of dairy cows from control group and infected group.

| Parameters | Control group (N = 20) | Infected group (N = 20) | Reference ranges [22] |
|---|---|---|---|
| Red Cells | 7.41±0.39 ** | 3.65±0.2 | 5-10 (x $10^6$/µL) |
| Hemoglobin | 10.01±0.32 ** | 6.14±0.72 | 8-15 (g/dL) |
| Hematocrit | 31.33±0.87 ** | 18.20±1.33 | 24-46 (%) |
| MCV | 42.71±1.61 ** | 53.55±3.12 | 40-60 (fL) |
| MCHC | 32.85±0.41 | 33.44±1.13 | 30-36 (%) |
| Total Leukocyte | 9,331.55±479.82 | 13,789.36±2541.62 | 4,000-12,000/µL |
| Lymphocytes | 5,434.17±656.71 | 5,843.96±881.53 | 2,500-7,500/µL |
| Segmented neutrophils | 2,991.47±201.03 | 5,583.14±1122.77 | 600-4,000/µL |
| Monocytes | 471.33±206.20 * | 1299.52±555.12 | 25-840/µL |
| Eosinophils | 744.08±088.14 | 799.75±301.09 | 0-2,400/µL |
| Auer rod | 0.00 | 58.48±50.22 | 0-120/µL |
| AST | 101.77±5.14 | 101.51±9.09 | 48-100 (UI/L) |
| GGT | 32.66±1.41 | 39.89±8.95 | 6.1-17.4 (UI/L) |
| Albumin | 2.53±0.06 | 2.69±0.20 | 3.03-3.55 (g/dL) |
| Total Protein | 5.88±0.41 ** | 7.83±0.20 | 6.7-7.4 (g/dL) |
| Urea | 49.14±1.99 | 46.66±6.09 | 20-30 (mg/dL) |
| Creatinine | 0.71±0.05 | 1.11±0.64 | 1-2 (mg/dL) |

Student's t test. * $p<0.05$; ** $p<0.01$

In the leukogram, the total number of leukocytes remained without significant change (Table 1). However, monocytosis was observed ($p<0.05$), which may be a consequence of pro-inflammatory cytokines release by activated macrophages [9, 20]. However, these same authors reinforce the occurrence of associated neutrophilia and lymphocytosis, which was not observed in the present study. This aspect is questioned because, as described by Stockham and Scott (2011) [24], both polymorphonuclear and mononuclear lineages are activated in the bone marrow. It is believed that the antigenic stimulus might have been more specific on the monocytic lineage because there is a greater demand on these cells for the erythrogaphocytosis mechanism [17, 18].

Concerning serum biochemistry, in the evaluation of renal function, urea and creatinine values did not change significantly when compared to the control group (Table 1). These results disagree to Coskun *et al.* (2012) [14], who observed a significant creatinine increase in cattle naturally infected with *A. marginale*, even with higher hematocrit and red blood cell concentration than in this study. Thus, it is believed that affected dairy cows showed greater resistance to the hypoxemia process.

In the assessment of liver function, serum AST, GGT and albumin values also showed no significant change (Table 1). These outcomes are in disagreement with approaches of two previous works [14, 19], who found elevation of AST, Alkaline Phosphatase (AP) and GGT in cattle naturally infected with *A. marginale*, possibly related to liver damage both at hepatocytes and canalicular levels [17].

Total protein increased significantly ($p<0.01$) in parasitized animals (Table 1), which could be related to the antigenic stimulus of the hemoparasite and subsequent increase in the concentration of blood globulins, which are part of one of the fractions of the total protein [9, 16, 20]. Hemoconcentration due to dehydration in infected animals may contribute to its increase [18].

The observed variations in the serum values of renal and hepatic function markers (although not mostly significant), between the two groups, may be related to the difference in nutrition, management and genetics between the studied dairy cows [25].

Finally, it should be highlighted this study has an epidemiological importance as it is the first to identify Rickettsia in the mesoregion of central Goiás, state of Goiás, Brazil. In addition, it reinforces the laboratory diagnosis importance, biochemical and hematological tests for the diagnosis of Anaplasmosis and evaluation of organic lesions, respectively.

## 4. Conclusion

Based on clinical signs, parasitemia and changes in the red blood series, it is concluded that *A. marginale* from the region has the virulence to reproduce the clinical conditions of Anaplasmosis in adult cattle. Thus, this disease can be considered a risk for milch cows in the center of Goiás. Furthermore, *A. marginale* did not cause significant changes in the parameters used to assess liver and kidney function, even with the infected animals developing severe anemia. A condition that has been related to an increased tolerance to anemia by the dairy cows.

## 5. Acknowledgement

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## 6. Declaration of interest statement

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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