

# Hematological, parasitological and biochemical parameters in sheep during the peripartum period

*Parámetros hematológicos, parasitológicos y bioquímicos en ovejas durante el parto*

*Parâmetros hematológicos, parasitológicos e bioquímicos de ovelhas durante o parto*

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## To cite this article:

David CMG, Costa RLD, Parren GAE, Rua MAS, Nordi ECP, Paz CCP, Quirino CR, Figueiredo RS, Bohland E.

Hematological, parasitological and biochemical parameters of sheep during peripartum. Rev Colomb Cienc Pecu 2020; 33(2): 81-95. DOI: <https://doi.org/10.17533/udea.rccp.v33n1a04>

## Abstract

**Background:** Pregnancy in all animal species requires special care and attention. During this period there is great variation in hematological and biochemical parameters. Many times these parameters are misinterpreted as being obtained from non-pregnant animals with unknown parasite status. **Objective:** To characterize the hematological, biochemical, and parasitological values within the peripartum period of Santa Ines sheep naturally infected. **Methods:** For this purpose, 45 ewes were used, with an average body weight of 50 kg. Data were collected during pregnancy, birth and, lactation. Measurements included body weight, body condition score (BCS), evaluation of conjunctive staining, and collection of blood and feces. **Results:** All variables changed throughout physiological stages. Weight and BCS were different between periods ( $p < 0.05$ ). Fecal egg counts (FEC) were higher in the peripartum period, with a predominance of *Haemonchus* ssp, 92.85%. Mean hematological and biochemical values varied throughout the study, ( $p > 0.05$ ). **Conclusion:** The influence of peripartum on the parameters studied should be considered and its use as a reference value. Regarding the physiological variations observed during peripartum, proper animal management could help minimizing the negative effects of parasitic infections on productivity.

**Keywords:** ewes; gestation; *Haemonchus contortus*; hematology; laboratory diagnosis; nematodes; *Ovis aries*; parasites; peripartum; Santa Ines; sheep.

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Received: March 12, 2018; accepted: August 22, 2019

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## Resumen

**Antecedentes:** La preñez en todas las especies animales requiere atención y cuidados especiales. En ese período existe gran variación en los parámetros hematológicos y bioquímicos. Muchas veces estos parámetros son interpretados erróneamente por ser obtenidos de animales no gestantes y con estatus parasitario desconocido. **Objetivo:** Caracterizar los valores hematológicos, bioquímicos y parasitológicos durante el periparto en ovejas Santa Inés infectadas naturalmente. **Métodos:** Se utilizaron 45 ovejas, con un peso corporal promedio de 50 kg. la recolección de muestras se realizó durante la gestación, el parto y la lactancia. Se evaluó el peso, la condición corporal, la tinción conjuntiva, y se recolectaron muestras de sangre y heces. **Resultados:** Todas las variables mostraron variaciones a lo largo de las etapas fisiológicas. La puntuación de peso y condición corporal (BCS) mostró diferencia entre períodos ( $p < 0,05$ ). Los recuentos de huevos fecales (FEC) tuvieron mayores promedios en el periparto, con predominio de *Haemonchus* ssp, 92,85%. Los valores medios hematológicos y bioquímicos variaron a lo largo del estudio ( $p < 0,05$ ). **Conclusiones:** Se recomienda considerar la influencia del periparto en los parámetros estudiados y su uso como valor de referencia. Con respecto a las variaciones fisiológicas observadas durante el periparto, el manejo adecuado de los animales podría ayudar a minimizar los efectos negativos de las infecciones parasitarias sobre la productividad.

**Palabras clave:** diagnóstico de laboratorio; gestación; *Haemonchus contortus*; hematología; periparto; nematodos; ovejas; *Ovis aries*; parásitos; Santa Inés.

## Resumo

**Antecedentes:** A prenhez em todas as espécies requer atenção e cuidados especiais. Neste período há grande variação dos parâmetros hematológicos e bioquímicos dos animais. Muitas vezes estes parâmetros são mal interpretados na realização de diagnósticos por serem baseados em parâmetros de animais saudáveis não gestantes, desconsiderando também a infecção parasitária. **Objetivo:** Caracterizar os valores hematológicos, bioquímicos e parasitológicos durante o periparto de ovelhas da raça Santa Inês naturalmente infectadas. **Métodos:** Foram utilizadas quarenta e cinco ovelhas, com peso corporal médio de 50 kg, os animais foram acompanhados durante a prenhez, parto e lactação. As análises compreendiam: pesagem, condição corporal, Famacha®, sangue e fezes. **Resultados:** Todas as variáveis apresentaram variações concomitantemente ao avanço da gestação, parto e lactação. O peso (PS) e o escore da condição corporal (ECC) apresentaram diferença estatística entre os períodos ( $p > 0,05$ ). As contagens de ovos por grama de fezes (FEC) apresentaram médias mais elevadas no periparto. Os valores hematológicos e bioquímicos também variaram ( $p > 0,05$ ). **Conclusão:** Devido ao resultado, sugere-se considerar a influência de periparto sobre os parâmetros estudados e a sua utilização como um valor de referência. Tendo em vista as variações fisiológicas devido o período do periparto e as infecções parasitárias o manejo dos animais deve ter como objetivo minimizar os impactos negativos na produção animal.

**Palavras-chave:** diagnóstico laboratorial; fenômeno do periparto; gestação; *Haemonchus contortus*; hematologia; nematoides; ovelhas; *Ovis aries*; parasitos; Santa Inês.

## Introduction

Sheep breeding is exploited on all continents, with wide dissemination mainly due to its ability to adapt to different climates, terrain and vegetation. Mutton production experienced 27% growth in Brazil over the last 20 years, despite the reduced demand for wool (MAPA, 2013).

The increase in mutton consumption has required greater production efficiency. Therefore, it is necessary to improve the management of herds through detailed characterization of breeds, breeding systems and critical phases, taking into account the different production regions. Nutritional and health problems are the main cause of the limited efficiency of ruminant production (Hernandez *et al.*, 2013). During the peripartum period, depression of the immune system is directly related to the physiological state of the animal (Silva *et al.*, 2003 to Nieto *et al.*, 2003) due to hormonal changes that occur near lambing and during lactation (Gennari *et al.*, 2002; Aya *et al.*, 2008). These changes are called "the peripartum phenomenon" (Amarante *et al.*, 2004; Rocha *et al.*, 2004).

Nutrients and energy requirements increase during pre-natal and early lactation, concomitant with a reduction in dry matter intake due to rumen compression by the fetus and production of hormones involved in pregnancy (Rodrigues *et al.*, 2006). This nutritional challenge is also associated with increased susceptibility to gastrointestinal parasites. An increase in the number of nematode eggs per gram of feces in sheep during peripartum can occur due to inadequate supply of dietary protein (Donaldson *et al.*, 1998 and 2001; Houdijk *et al.*, 2001).

Although a number of studies have investigated the association between nutrition and gastrointestinal infections in sheep (Hoste *et al.*, 2005) few have focused on the potential variations of hematological, biochemical and parasitological parameters associated with peripartum, a critical stage for productive and reproductive efficiency. This knowledge is necessary to improve management practices,

particularly because of the large heterogeneity of values within this species due to differences of breed, gender, age, nutrition, management systems (Kerr, 2003) and physiological status (Cardoso *et al.*, 2010).

In this study, we characterized the hematological, biochemical and parasitological values in the peripartum period in Santa Ines sheep, the main native breed used in Brazil and one of great interest in other South American countries.

## Materials and Methods

### *Ethical Considerations*

This study was conducted and approved by the ethics committee for animal research at Instituto de Zootecnia (finding no. 142/2011).

### *Local*

This study was conducted at Centro de Pesquisa e Desenvolvimento de Zootecnia Diversificada, at Instituto de Zootecnia, of Agência Paulista de Tecnologia dos Agronegócios (APTA), da Secretaria de Agricultura e Abastecimento, located in Nova Odessa, São Paulo state (22°42' S and 47°18' W).

According to the Köppen classification, the climate in the Nova Odessa region is Cwa, characterized as tropical with relatively dry and hot summers and rainy winters. Average rainfall is 1,317 mm per year and the temperature is between 10 °C and 35 °C, with an average of 26 °C. The average annual relative humidity is approximately 75%.

### *Animals and management*

A total of 70 Santa Ines ewes kept under a natural breeding system were tested for pregnancy by ultrasound. Of these, we used 45 sheep diagnosed with close gestational age and 50 kg average body weight. Sheep were kept under rotational grazing on *Panicum maximum* Jacq. cv. Aruana and cv. Tanzania pastures, with ad libitum access to water and mineral mixture. During late pregnancy, sheep were confined in

paddocks and supplemented with corn silage *ad libitum* and 0.300 kg concentrate (16% crude protein). They remained in the paddocks until the lambs were weaned at 3 months of age. Feed samples were taken every 15 days and frozen at -20 °C for later analysis.

#### *Sample collection and evaluation of animals*

Blood and stool samples were collected every 14 days during five months, for a total of 10 collections. At this time, sheep were also weighed and evaluated for body condition score and conjunctival staining. Blood samples were also collected for hematological and biochemical analyses on the day of lambing. Body condition score was determined by palpation of the lower back (Sanudo and Sierra, 1986) using a half-point scoring (Pugh, 2004). Parasitological and hematological analyses were performed at the Laboratory for Fecal Analysis and Laboratory for Clinical Analysis, respectively, of Instituto de Zootecnia de Nova Odessa/APTA.

#### *Parasitological examinations*

Stool samples were collected directly from the rectum for egg counting (eggs per gram of feces; EPG) according to the modified technique by Gordon and Whitlock (1939). Fecal culture of infective larvae was conducted for identification of parasite genera (Roberts and O'Sullivan, 1950).

#### *Hematological analysis*

Blood samples from all animals were collected by venipuncture of the jugular vein in Vacutainer tubes (5 ml) containing ethylenediaminetetraacetic acid (EDTA). Packed cell volume analysis (PVC) was conducted after microhematocrit centrifugation. Hemoglobin (Hb) concentration was measured by the cyanmethemoglobin method using a semi-automatic biochemical analyzer (Bio 200 L Bioplus) using commercial kits for hemoglobin determination (Labtest). Total plasma protein (TPP) was determined by the refraction technique. Total leukocyte count (Tot Leuk) and erythrocyte count (RBC) were determined

manually in a Neubauer hemocytometer (Schalm and Carroll, 1986). Tests identified blood smears and differential count of segmented neutrophils (Seg Neut), lymphocytes (Lymph), eosinophils (Eosin), monocytes (Mono), basophils (Baso) and rods (Bast) (Schalm and Carroll, 1986). No rods were observed, so this parameter was removed from the statistical analysis.

#### *Biochemical analysis*

The blood samples were subjected to the following biochemical analyses: globulin, albumin, serum protein (Ser Pt), urea, creatinine, total bilirubin (BT), direct bilirubin (BD), indirect bilirubin (BI), glucose (Gluko), cholesterol, AST (AST), calcium, iron (Fe), and phosphorus. The tests were performed in a Bio 200L semiautomatic biochemical analyzer (Bioplus, São Paulo, Brazil) using commercial kits (Labtest, Lagoa Santa, Minas Gerais, Brazil).

#### *Statistical analysis*

The assumption of normal distribution of all variables was verified. The variables fibrinogen, total leukocytes, segmented neutrophils, lymphocytes, eosinophils, monocytes, trichostrongylid, *Strongyloides* spp, *Moniezia* spp and *Eimeria* spp which did not meet the assumption of normality were transformed. The data were transformed for the FAMACHA and fibrinogen variables by raising to the -0.2 power. Square roots were used for total leukocytes and erythrocytes, and the other non-normal variables were log10 transformed. The means are presented in the original scale.

Analysis of variance (ANOVA) was conducted using the PROC GLM routine of SAS for all variables (processed or not) considering repeated measures. The model and analysis considered the effect of weight as a covariate in addition to the effects of treatment and time and their interaction. The means were compared by the Tukey test considering 5% probability for Type I error.

## Results

### Feeding and performance

Sheep ate a daily average of 1.85 kg dry matter and 177 g crude protein at the beginning of the experiment, with consumption decreasing in the peripartum month (September) and increasing linearly until the end of lactation (November), when it reached 2 kg dry matter and 200 g crude protein (Figure 1).

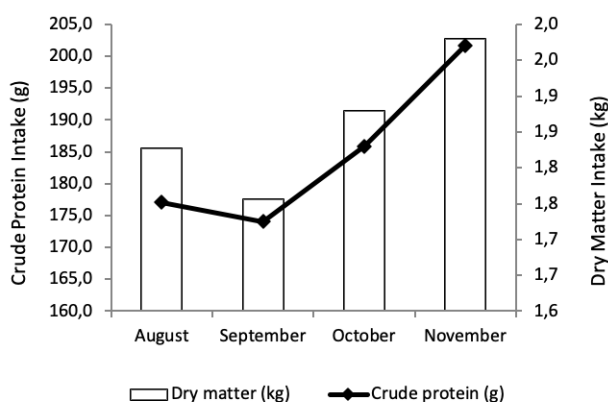


Figure 1. Dry matter and crude protein intake of Santa Ines ewes.

Body weight increased significantly from the beginning of the study until lambing, when it was the highest. Subsequently, there was a sharp and significant drop in weight from day 1-13 after birth, when body weight returned to a value similar to that observed >30 days before birth. A further reduction in weight was also observed from days 14-27 (lactation), after which it remained constant. The analysis of BCS showed a significant reduction associated with the peripartum period, which was particularly accentuated during the peak of lactation (up to 83 days after birth). From day 84 onwards, BCS was not different from that during pregnancy (Table 1).

### Parasitological analysis

Table 2 shows the results of the endoparasite analysis. The mean FAMACHA score varied from 1.2 (1-13 days) to 1.7 (<-30 days), with no difference between periods ( $p>0.05$ ). Trichostrongylid FEC was, however, significantly higher during gestation and peripartum than during lactation, with a particularly sharp reduction in FEC towards the end of the latter period.

Table 1. Least square means of body weight and body condition score (BCS) of Santa Ines ewes.

|            | Days   | BW                | BCS                |
|------------|--------|-------------------|--------------------|
| Pregnancy  | < -30  | 48.1 <sup>a</sup> | 2.9 <sup>a</sup>   |
|            | >14-30 | 52.3 <sup>b</sup> | 2.8 <sup>ab</sup>  |
| Peripartum | >1-13  | 53.8 <sup>c</sup> | 2.6 <sup>bcd</sup> |
|            | Partum | .                 | .                  |
|            | 1-13   | 47.7 <sup>a</sup> | 2.6 <sup>be</sup>  |
| Lactation  | ≥14-27 | 45.6 <sup>d</sup> | 2.5 <sup>ce</sup>  |
|            | ≥28-55 | 44.7 <sup>d</sup> | 2.5 <sup>ce</sup>  |
|            | ≥56-83 | 45.5 <sup>d</sup> | 2.5 <sup>be</sup>  |
|            | >84    | 44.9 <sup>d</sup> | 2.6 <sup>ade</sup> |
|            | SE     | ±1.1              | ±0.1               |

Different superscript letters (<sup>a, b, c, d, e</sup>) within the same column indicate significant differences at  $p<0.05$ .



**Table 2.** Least square means of FAMACHA® ( $\pm$  standard error) and parasitological variables of Santa Ines ewes ( $\pm$  standard error).

|            |              | FAMACHA*      | Tricho                                   | Strong                            | Moni SD                         | Eime SD                                    |
|------------|--------------|---------------|--|-----------------------------------|---------------------------------|--|
| Pregnancy  | >30          | 1.7 $\pm$ 0.1 | 2885 $\pm$ 290 <sup>a</sup>              | 11.8 $\pm$ 90.2 <sup>ab</sup>     | 195.6 $\pm$ 297.50 <sup>a</sup> | 34.2 $\pm$ 130.3 <sup>a</sup>              |
|            | >14-30       | 1.5 $\pm$ 0.1 | 3296 $\pm$ 32 <sup>a</sup>               | 35.7 $\pm$ 104.0 <sup>ab</sup>    | 228.6 $\pm$ 315.68 <sup>a</sup> | 14.3 $\pm$ 78.3 <sup>a</sup>               |
|            | >1-13        | 1.4 $\pm$ 0.1 | 2659 $\pm$ 315 <sup>ab</sup>             | 180.6 $\pm$ 101.6 <sup>c</sup>    | 34.1 $\pm$ 120.45 <sup>b</sup>  | 4.5 $\pm$ 14.5 <sup>a</sup>                |
| Peripartum | Day 0        | 1.1 $\pm$ 0.4 | 2324 $\pm$ 1119 <sup>ac</sup>            | 836.5 $\pm$ 388.8 <sup>acde</sup> | 0 $\pm$ 0.0 <sup>b</sup>        | 0 $\pm$ 0 <sup>ab</sup>                    |
|            | 1-13         | 1.2 $\pm$ 0.1 | 2457 $\pm$ 318 <sup>a</sup> <sup>d</sup> | 758.1 $\pm$ 102.8 <sup>d</sup>    | 0 $\pm$ 0.0 <sup>b</sup>        | 25.6 $\pm$ 152.5 <sup>a</sup>              |
|            | $\geq$ 14-27 | 1.4 $\pm$ 0.1 | 1923 $\pm$ 318 <sup>bcd</sup>            | 436.5 $\pm$ 102.8 <sup>d</sup>    | 0 $\pm$ 0.0 <sup>b</sup>        | 31.4 $\pm$ 87.3 <sup>a</sup>               |
| Lactation  | >28-55       | 1.3 $\pm$ 0.1 | 1128 $\pm$ 243 <sup>c</sup>              | 340.6 $\pm$ 72.0 <sup>e</sup>     | 2.2 $\pm$ 12.8 <sup>b</sup>     | 544.4 $\pm$ 3665 <sup>b</sup>              |
|            | >56-83       | 1.3 $\pm$ 0.1 | 483 $\pm$ 250 <sup>e</sup>               | 72.1 $\pm$ 74.9 <sup>e</sup>      | 42.8 $\pm$ 336.1 <sup>b</sup>   | 681.1 $\pm$ 1842 <sup>c</sup>              |
|            | >84          | 1.4 $\pm$ 0.1 | 273 $\pm$ 375 <sup>f</sup>               | 3.8 $\pm$ 123.2 <sup>b</sup>      | 18.3 $\pm$ 74.8 <sup>b</sup>    | 78.3 $\pm$ 133.1 <sup>a</sup> <sup>b</sup> |

\*In those cases where ER, SE were used. Otherwise, different superscript letters (a, b, c, d, e) within the same column indicate significant differences at  $p < 0.05$  (Tukey-Kramer). Trichostrongylides (Tricho), *Strongyloides* spp (Strong), *Moniezia* spp (Moni), *Eimeria* spp (Eime).

Unexpectedly, Trichostrongylid FEC was not significantly higher on the lambing date. *Strongyloides* spp. FEC increased from gestation to birth, with accentuated increase on the birth date, and subsequent reduction afterwards. The *Moniezia* spp. count showed the highest values in the first two gestational periods, while *Eimeria* counts had the lowest values in the first six periods (pregnancy, peripartum and lactation up to 27 days postpartum), differing from later lactation periods.

The coproculture results showed average counts of Trichostrongylidae, which is the parasite genus of greatest interest, with predominance of *Haemonchus* ssp (92.85%).

#### Hematological profile

The hematological variables showed significant variations across the gestational period (Table 3). As expected, there was a gradual increase in packed cell volume over the course of the study, and a decrease in mean corpuscular volume (MVC). The lowest values of hemoglobin concentration (Hb), total plasma protein (TPP), and mean corpuscular hemoglobin concentration (MCHC) were observed near the birth date.

This date was also associated with the highest values of leucocytes, neutrophils and lymphocytes (Table 4). Eosinophils and monocytes were, conversely, particularly low at birth.

#### Blood biochemistry

All blood biochemistry analyses, regarding metabolites, enzymes (Tables 5 and 6) and minerals (Table 7), were performed in a Bio 200L semiautomatic biochemical analyzer (Bioplus, São Paulo, Brazil), with Labtest commercial kits (Labtest, Lagoa Santa, Minas Gerais, Brazil). Blood levels of metabolites, globulin, albumin, total serum protein, urea, creatinine, glucose (Table 5) total bilirubin, direct bilirubin, indirect bilirubin, cholesterol and AST differed significantly ( $p < 0.05$ ) with the different physiological conditions (Table 6). The globulin values ranged from 3.88 ( $\geq$ 14-27 days) to 4.85 ( $>$  84 days), with a significant increase over the course of lactation. The values of albumin and serum protein were significantly lower in the birth date, although similar to the average associated with day 84 onwards. The values of urea (Tab 5) decreased from gestation to peripartum, subsequently increasing over the lactation period. There were no clear trends in the variation of creatinine values.

Sheep had lower levels of total, direct and indirect bilirubin on lambing day ( $p>0.05$ ) compared to all other periods, except for the direct bilirubin, which was similar to that of the period  $> 84$  days postpartum (Table 6).

Cholesterol and AST had constant variations during all periods, with respective amplitudes of

$48.2 \pm 1.8$  (1-13 days) to  $60.8 \pm 1.8$  ( $> 1-13$ ) and  $82.6 \pm 3.7$  ( $\geq 14-27$ ) to  $111.0 \pm 4.4$  ( $> 84$ ).

The serum levels of calcium, phosphorus and iron showed significant differences ( $p<0.0001$ ) between the periods of pregnancy, birth and lactation. The mean changes in calcium, phosphorus and iron were independent of the physiological condition.

**Table 3.** Mean ( $\pm$  standard error) of hematological variables of Santa Ines ewes, according to physiological stage.

|                   |              | RBC<br>( $10^6/\mu\text{L}$ ) | PCV (%)                | Hb (g/dL)             | TPP(g/dL)           | MCV (fL)              | MCH                   | MCHC (%)              |
|-------------------|--------------|-------------------------------|------------------------|-----------------------|---------------------|-----------------------|-----------------------|-----------------------|
| <i>Pregnancy</i>  | $>30$        | $7.18 \pm 0.2^a$              | $27.41 \pm 0.60^{ab}$  | $10.01 \pm 0.2^{ab}$  | $6.7 \pm 0.09^a$    | $39.96 \pm 0.65^a$    | $3.57 \pm 0.059^a$    | $35.73 \pm 0.59^a$    |
|                   | $>14-30$     | $7.51 \pm 0.2^{ab}$           | $27.06 \pm 0.59^{ad}$  | $10.17 \pm 0.3^{acd}$ | $6.5 \pm 0.08^{bc}$ | $36.71 \pm 0.72^b$    | $3.82 \pm 0.066^{bc}$ | $38.23 \pm 0.66^{bd}$ |
|                   | $>1-13$      | $7.86 \pm 0.2^{bc}$           | $28.03 \pm 0.59^b$     | $10.57 \pm 0.3^{ade}$ | $6.5 \pm 0.09^{ab}$ | $36.09 \pm 0.73^b$    | $3.78 \pm 0.067^{bc}$ | $37.81 \pm 0.67^{bd}$ |
| <i>Peripartum</i> | Dia 0        | $7.86 \pm 0.2^{cd}$           | $28.88 \pm 0.59^c$     | $10.00 \pm 0.3^{abc}$ | $6.4 \pm 0.09^b$    | $36.21 \pm 0.72^b$    | $3.47 \pm 0.068^a$    | $34.73 \pm 0.68^a$    |
|                   | 1-13         | $8.39 \pm 0.2^{cd}$           | $28.25 \pm 0.50^{bc}$  | $10.71 \pm 0.3^{de}$  | $6.6 \pm 0.09^a$    | $33.87 \pm 0.73^c$    | $3.85 \pm 0.068^{bc}$ | $38.54 \pm 0.68^{bd}$ |
|                   | $\geq 14-27$ | $8.13 \pm 0.2^{ce}$           | $26.56 \pm 0.59^a$     | $10.10 \pm 0.3^{ac}$  | $6.7 \pm 0.09^a$    | $32.95 \pm 0.72^{cd}$ | $3.78 \pm 0.067^{bc}$ | $37.78 \pm 0.67^b$    |
| <i>Lactation</i>  | $>28-55$     | $8.41 \pm 0.2^{de}$           | $26.83 \pm 0.55^a$     | $10.00 \pm 0.2^{bc}$  | $6.7 \pm 0.08^a$    | $31.98 \pm 0.54^d$    | $3.75 \pm 0.048^b$    | $37.55 \pm 0.48^b$    |
|                   | $>56-83$     | $9.14 \pm 0.2^f$              | $27.46 \pm 0.56^{ab}$  | $11.55 \pm 0.2^{ef}$  | $6.8 \pm 0.08^a$    | $30.56 \pm 0.55^e$    | $4.26 \pm 0.049^d$    | $42.61 \pm 0.49^c$    |
|                   | $>84$        | $9.04 \pm 0.3^f$              | $28.23 \pm 0.65^{bcd}$ | $11.06 \pm 0.3^e$     | $6.7 \pm 0.10^a$    | $31.40 \pm 0.87^{de}$ | $3.98 \pm 0.080^c$    | $39.83 \pm 0.80^d$    |

Average followed by different superscript letters (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup>, <sup>e</sup>) within the same column differ by the Tukey's test ( $p<0.05$ ). (RBC) erythrocytes, (PCV) packed cell volume, (Hb) hemoglobin concentration, (TPP) total plasma protein, (MCV) mean corpuscular volume, (MCH) mean corpuscular hemoglobin, (MCHC) mean corpuscular hemoglobin concentration.

**Table 4.** Least square mean ( $\pm$  standard error) of fibrinogen (Fibri), total leukocytes (Tot Leuk), segmented neutrophils (Seg Neut), lymphocytes (Lymph), eosinophils (Eosin), and monocytes (Mono) of Santa Ines ewes according to physiological stage.

|                   |              | Fibri.          | Tot.Leuk.<br>( $/\mu\text{L}$ ) | Seg Neut<br>( $/\mu\text{L}$ ) | Lymph<br>( $/\mu\text{L}$ ) | Eosin<br>( $/\mu\text{L}$ ) | Mono<br>( $/\mu\text{L}$ ) |
|-------------------|--------------|-----------------|---------------------------------|--------------------------------|-----------------------------|-----------------------------|----------------------------|
| <i>Pregnancy</i>  | $>30$        | $1.22 \pm 0.03$ | $9161 \pm 348^a$                | $4883 \pm 2634^a$              | $3580 \pm 841^a$            | $373 \pm 65^a$              | $491 \pm 27^a$             |
|                   | $>14-30$     | $1.26 \pm 0.02$ | $8751 \pm 371^a$                | $4854 \pm 2954^a$              | $3021 \pm 938^b$            | $481 \pm 71^a$              | $332 \pm 30^b$             |
|                   | $>1-13$      | $1.25 \pm 0.02$ | $9385 \pm 374^a$                | $6227 \pm 2954^b$              | $2325 \pm 938^c$            | $589 \pm 71^b$              | $193 \pm 30^c$             |
| <i>Peripartum</i> | Dia 0        | $1.22 \pm 0.02$ | $10918 \pm 371^b$               | $18466 \pm 3061^c$             | $5860 \pm 972^b$            | $269 \pm 73^c$              | $68 \pm 31^{cd}$           |
|                   | 1-13         | $1.24 \pm 0.02$ | $9480 \pm 374^a$                | $6390 \pm 2954^b$              | $2388 \pm 938^{bcd}$        | $527 \pm 71^{ab}$           | $110 \pm 30^{ce}$          |
|                   | $\geq 14-27$ | $1.25 \pm 0.02$ | $9404 \pm 371^a$                | $6719 \pm 2989^b$              | $2199 \pm 949^c$            | $503 \pm 71^{ab}$           | $96 \pm 31^{cf}$           |
| <i>Lactation</i>  | $>28-55$     | $1.26 \pm 0.02$ | $8931 \pm 305^a$                | $6341 \pm 2157^b$              | $2158 \pm 690^c$            | $484 \pm 54^{ad}$           | $91 \pm 22^{def}$          |
|                   | $>56-83$     | $1.26 \pm 0.02$ | $9289 \pm 309^a$                | $6183 \pm 2168^b$              | $2184 \pm 694^c$            | $666 \pm 55^{bd}$           | $101 \pm 22^{def}$         |
|                   | $>84$        | $1.23 \pm 0.03$ | $9347 \pm 429^a$                | $5544 \pm 3525^b$              | $2723 \pm 1119^{bd}$        | $197 \pm 83^c$              | $133 \pm 36^{cdef}$        |

Different superscript letters (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup>, <sup>e</sup>) within the same column indicate significant differences at  $p<0.05$  (Tukey-Kramer).

**Table 5.** Mean ( $\pm$  standard error) of globulin, albumin, serum protein (Ser Pt), urea, creatinine (Creatinin), and glucose (Gluko) of Santa Ines ewes according to physiological stage.

|                   |              | Globulin<br>(g/dL) | Albumin<br>(g/dL)   | Ser Pt.<br>(g/dL)  | Urea<br>(mg/dL)                 | Creatinin<br>(mg/dL)            | Gluko<br>(mg/dL)               |
|-------------------|--------------|--------------------|---------------------|--------------------|---------------------------------|---------------------------------|--------------------------------|
| <i>Pregnancy</i>  | >30          | 4.06 <sup>ab</sup> | 2.41 <sup>ab</sup>  | 6.41 <sup>a</sup>  | 40.09 $\pm$ 1.85 <sup>a</sup>   | 1.01 $\pm$ 0.024 <sup>ab</sup>  | 47.3 $\pm$ 1.06 <sup>a</sup>   |
|                   | >14-30       | 4.00 <sup>b</sup>  | 2.51 <sup>a</sup>   | 6.53 <sup>ab</sup> | 39.79 $\pm$ 2.19 <sup>a</sup>   | 1.05 $\pm$ 0.027 <sup>a</sup>   | 46.3 $\pm$ 1.23 <sup>a</sup>   |
|                   | >1-13        | 4.04 <sup>b</sup>  | 2.49 <sup>a</sup>   | 6.51 <sup>a</sup>  | 28.54 $\pm$ 2.23 <sup>b</sup>   | 1.14 $\pm$ 0.027 <sup>c</sup>   | 48.6 $\pm$ 1.23 <sup>ab</sup>  |
| <i>Peripartum</i> | Dia 0        | 3.90 <sup>b</sup>  | 2.09 <sup>c</sup>   | 5.97 <sup>c</sup>  | 24.180 $\pm$ 2.22 <sup>bc</sup> | 1.13 $\pm$ 0.027 <sup>c</sup>   | .                              |
|                   | 1-13         | 3.99 <sup>b</sup>  | 2.36 <sup>bd</sup>  | 6.29 <sup>a</sup>  | 21.65 $\pm$ 2.24 <sup>c</sup>   | 0.99 $\pm$ 0.027 <sup>ad</sup>  | 51.5 $\pm$ 1.25 <sup>bcd</sup> |
|                   | $\geq$ 14-27 | 3.88 <sup>b</sup>  | 2.43 <sup>adf</sup> | 6.37 <sup>a</sup>  | 26.69 $\pm$ 2.22 <sup>bc</sup>  | 0.90 $\pm$ 0.027 <sup>e</sup>   | 58.6 $\pm$ 1.23 <sup>c</sup>   |
| <i>Lactation</i>  | >28-55       | 4.29 <sup>a</sup>  | 2.47 <sup>a</sup>   | 6.76 <sup>bd</sup> | 33.16 $\pm$ 1.63 <sup>b</sup>   | 0.92 $\pm$ 0.021 <sup>ef</sup>  | 55.0 $\pm$ 0.95 <sup>e</sup>   |
|                   | >56-83       | 4.60 <sup>c</sup>  | 2.34 <sup>bef</sup> | 6.94 <sup>c</sup>  | 49.57 $\pm$ 1.64 <sup>d</sup>   | 0.97 $\pm$ 0.022 <sup>bdf</sup> | 52.9 $\pm$ 0.95 <sup>de</sup>  |
|                   | >84          | 4.85 <sup>d</sup>  | 2.07 <sup>c</sup>   | 6.96 <sup>de</sup> | 52.91 $\pm$ 2.52 <sup>d</sup>   | 0.97 $\pm$ 0.033 <sup>bde</sup> | 50.2 $\pm$ 1.4 <sup>bd</sup>   |
|                   | SE           | $\pm$ 0.1          | $\pm$ 0.1           | $\pm$ 0.1          | -                               | -                               | -                              |

Different superscript letters (a, b, c, d, e) within the same column indicate significant differences at  $p < 0.05$  (Tukey-Kramer).

**Table 6.** Mean ( $\pm$  standard error) of total bilirubin (BT), direct bilirubin (BD), indirect bilirubin (BI), cholesterol, and AST of Santa Ines ewes according to physiological stage.

|                   |              | BT(mg/dL)                     | BD(mg/dL)                      | BI(mg/dL)                       | Cholesterol(mg/dL)             | AST (U/L)                      |
|-------------------|--------------|-------------------------------|--------------------------------|---------------------------------|--------------------------------|--------------------------------|
| <i>Pregnancy</i>  | >30          | 0.240 $\pm$ 0.03 <sup>a</sup> | 0.094 $\pm$ 0.01 <sup>ac</sup> | 0.101 $\pm$ 0.02 <sup>a</sup>   | 54.48 $\pm$ 1.6 <sup>ab</sup>  | 83.61 $\pm$ 3.5 <sup>acd</sup> |
|                   | >14-30       | 0.181 $\pm$ 0.03 <sup>a</sup> | 0.068 $\pm$ 0.01 <sup>ad</sup> | 0.125 $\pm$ 0.03 <sup>bc</sup>  | 56.99 $\pm$ 1.8 <sup>ac</sup>  | 97.47 $\pm$ 3.7 <sup>b</sup>   |
|                   | >1-13        | 0.258 $\pm$ 0.03 <sup>a</sup> | 0.072 $\pm$ 0.01 <sup>ad</sup> | 0.193 $\pm$ 0.03 <sup>b</sup>   | 60.82 $\pm$ 1.8 <sup>c</sup>   | 95.13 $\pm$ 3.7 <sup>b</sup>   |
| <i>Peripartum</i> | Dia 0        | 0.023 $\pm$ 0.03 <sup>b</sup> | 0.007 $\pm$ 0.01 <sup>b</sup>  | 0.020 $\pm$ 0.03 <sup>d</sup>   | 49.81 $\pm$ 1.7 <sup>de</sup>  | 92.95 $\pm$ 3.7 <sup>b</sup>   |
|                   | 1-13         | 0.458 $\pm$ 0.03 <sup>c</sup> | 0.122 $\pm$ 0.01 <sup>c</sup>  | 0.326 $\pm$ 0.03 <sup>e</sup>   | 48.16 $\pm$ 1.8 <sup>df</sup>  | 83.34 $\pm$ 3.7 <sup>cd</sup>  |
|                   | $\geq$ 14-27 | 0.364 $\pm$ 0.03 <sup>d</sup> | 0.074 $\pm$ 0.01 <sup>ad</sup> | 0.295 $\pm$ 0.03 <sup>e</sup>   | 51.44 $\pm$ 1.8 <sup>bd</sup>  | 82.57 $\pm$ 3.7 <sup>cd</sup>  |
| <i>Lactation</i>  | >28-55       | 0.249 $\pm$ 0.02 <sup>a</sup> | 0.083 $\pm$ 0.01 <sup>ad</sup> | 0.166 $\pm$ 0.02 <sup>cf</sup>  | 52.63 $\pm$ 1.4 <sup>beg</sup> | 94.57 $\pm$ 3.2 <sup>b</sup>   |
|                   | >56-83       | 0.254 $\pm$ 0.02 <sup>a</sup> | 0.076 $\pm$ 0.01 <sup>ad</sup> | 0.183 $\pm$ 0.02 <sup>bf</sup>  | 55.28 $\pm$ 1.4 <sup>ag</sup>  | 99.20 $\pm$ 3.2 <sup>ab</sup>  |
|                   | >84          | 0.210 $\pm$ 0.04 <sup>a</sup> | 0.047 $\pm$ 0.02 <sup>bd</sup> | 0.173 $\pm$ 0.03 <sup>abf</sup> | 49.64 $\pm$ 2.1 <sup>bef</sup> | 111.0 $\pm$ 4.3 <sup>c</sup>   |

Different superscript letters (a, b, c, d, e) within the same column indicate significant differences at  $p < 0.05$  (Tukey-Kramer).



**Table 7.** Mean ( $\pm$  standard error) of calcium, phosphorus, iron (Fe) of Santa Ines ewes according to the physiological period.

|            |              | Calcium<br>(mg/dL)  | Phosphorus<br>(mg/dL) | Fe<br>( $\mu$ g/dL)   |
|------------|--------------|---------------------|-----------------------|-----------------------|
| Pregnancy  | >30          | 8.05 $\pm$ 0.162ab  | 5.29 $\pm$ 0.22a      | 197.53 $\pm$ 7.04abd  |
|            | >14-30       | 8.43 $\pm$ 0.194a   | 4.72 $\pm$ 0.24b      | 207.44 $\pm$ 10.27abd |
|            | >1-13        | 8.22 $\pm$ 0.239ab  | 4.72 $\pm$ 0.24b      | 197.16 $\pm$ 10.08abd |
| Peripartum | Dia 0        | 9.02 $\pm$ 0.239cde | 4.93 $\pm$ 0.24ab     | .                     |
|            | 1-13         | 9.51 $\pm$ 0.251cf  | 5.29 $\pm$ 0.24a      | 183.35 $\pm$ 9.71abc  |
|            | $\geq$ 14-27 | 7.79 $\pm$ 0.233b   | 5.02 $\pm$ 0.24ab     | 220.28 $\pm$ 10.07d   |
| Lactation  | >28-55       | 9.03 $\pm$ 0.144cg  | 4.75 $\pm$ 0.20b      | 185.37 $\pm$ 6.80bc   |
|            | >56-83       | 9.38 $\pm$ 0.150dfg | 5.05 $\pm$ 0.20ab     | 168.17 $\pm$ 6.67c    |
|            | >84          | 8.46 $\pm$ 0.265ae  | 4.98 $\pm$ 0.28ab     | 175.92 $\pm$ 9.64c    |

Different superscript letters (<sup>a, b, c, d, e</sup>) within the same column indicate significant differences at  $p < 0.05$  (Tukey-Kramer).

## Discussion

Previous research suggests that feed intake of sheep and other ruminants is often insufficient in the final period of gestation and early lactation. Our results show that although a decrease in dry matter and crude protein intake occurred in these stages, consumption was sufficient to meet the animals' requirements in these physiological states (NRC, 2006) in confined conditions. Accordingly, we observed relatively small weight and body condition score variations.

Although the peripartum period is often associated with increased susceptibility to gastrointestinal parasitic infections, there were no significant variations in trichostrongylid counts between gestational and peripartum periods. *Haemonchus* spp. genus are highly pathogenic blood-suckers, causing a decrease in the packed cell volume and hence increasing the ocular mucosa coloration score (FAMACHA). *Haemonchus* spp. had high mean prevalence (92.85%), while Trichostrongylid egg counts decreased during the experiment. The FAMACHA score was constant during different physiological conditions. It is unlikely that this low association between Trichostrongylid

FEC and the FAMACHA score resulted from a subjective assignment of this score since the association between PCV and FAMACHA was high. Therefore, the weak association between FAMACHA score and Trichostrongylid count may have resulted from the fact that sheep were already infected before the experiment. Although the Trichostrongylid FEC was high until the peripartum period and decreased with the onset of lactation, the appropriate protein intake might have allowed maintenance of PCV values predominantly within the range considered normal for sheep (27 to 45%; Pugh, 2005) throughout the experiment. The only exception was a small decrease in PCV just after the beginning of lactation, but was followed by swift recovery to values within the normal range. Other parasites with lower pathogenicity than *Haemonchus* spp. (Afonso *et al.*, 2013) were also identified, such as *Trichostrongyloidea* (*Trichostrongylus*, *Cooperia* and *Oesophagostomum*), *Strongyloides* spp., *Eimeria* spp. and *Moniezia* spp. *Moniezia* and *Eimeria* FEC were higher in the pregnancy and lactation periods, respectively, probably due to the management used. Better food, confined animals are less exposed to infection by *Moniezia* (albeit more exposed to infection by *Eimeria* due to trough feeding). Although *Haemonchus* spp. can cause severe anemia and

hypoproteinemia (Faria Junior *et al.*, 2002), depression, loss of body condition, reduced productivity, and even death (Kaplan *et al.*, 2004), no animal died or had to be dewormed before or during our experiment.

As expected, there was a variation in the hematological profile of animals between periods. The values of hematological variables were predominantly below the normal range for the species, possibly due to the negative action that hormone variation has on hematological variables (Kerr, 2003). Hematological parameters change easily and fast during peripartum (Cardoso *et al.*, 2010) and the PCV decreases as pregnancy progresses due to the physiological hemodilution in this period (Brito *et al.*, 2006). Recent studies suggest that even healthy Santa Ines sheep have lower erythrocyte, hemoglobin and PCV levels and higher MCHC values (Salviano *et al.*, 2013) compared to the reference values for the species found in the literature (Schalm, 1981; Harvey and Meyer, 1998; Pugh, 2005). Our results showed increased amounts of red blood cells and hemoglobin from pregnancy until the end of lactation. However, we did not observe lower PCV values during gestation, as reported in the literature (Brito *et al.*, 2006). We also found higher MCHC values in relation to the literature, including those suggested by Salviano *et al.* (2013) for non-pregnant sheep. These findings reinforce the hypothesis that breed can significantly affect hematological patterns, and emphasizes the need for studies on specific breeds considering age, sex and physiological condition to avoid misinterpretation of results.

During pregnancy, hormone action on the immune system (Kerr, 2003) is necessary to suppress the defense mechanisms and thus promote survival and development of the fetus (Lloyd, 1983). The low total leukocyte levels observed before delivery may be related to this factor, while the high level on the day of delivery may be related to the migration of these cells into the uterus, where there is tissue injury caused by birth.

Segmented neutrophils act as a first line of defense against infection. Many researchers have investigated the activity of these leukocytes close to delivery (Guidry *et al.*, 1976; Fonteque *et al.*, 2013). The level of segmented neutrophils increased as delivery approached, peaking on day 0 (delivery), with a gradual decrease afterward. This subsequent reduction may be related to the migration of these cells to injury sites, corroborating the results of Fontequete *et al.* (2013), who observed decrease in total leukocytes and segmented neutrophils during postpartum. However, they also noted increased segmented neutrophils and total leukocytes after delivery, indicating the likely need to replenish the number of circulatory cells due to their flow to the uterus. We did not observe this pattern, probably because sheep were parasitized, which kept the immune system active.

The observed decrease in lymphocytes and monocytes before delivery, starting 30 days beforehand and declining sharply 15 days before delivery, may have occurred because of migration of cells to the uterus and/or abomasum. Migration of leukocytes to the abomasum occurs to enable antigen recognition of the nematode, followed by stimulation of the immune system to generate the appropriate response to fight parasites (Douch, 1990). The activation of larvae, previously in hypobiosis, happens due to immunosuppression in response to circulating cortisol in the days before delivery. The number of EPG also increased in these same periods. Lymphocyte concentration was lower during postpartum than on the delivery day, whereas monocytes showed higher values 15 days after delivery and after more than 84 days of lactation. The high score in the first period after delivery may be related to peak lactation and attempt of the body to restore immunity after the stress period caused by constant hormonal changes.

Eosinophil counts increased gradually until the day of delivery, decreased considerably in the delivery day, and then rose gradually in the postpartum period until 84 days in milk, after which the concentrations declined. These results are similar to those reported by Fonteque

*et al.* (2013) who found that stress caused by birthing in goats is overcome in the first week after delivery, with more stable counts, a fact confirmed in our study. However, these findings contrast with those of Sabag *et al.* (1978) who noted a decreasing number of circulating eosinophils due to the action of cortisol associated with inhibition of eosinophilopoiesis in the marrow and/or migration of these cells to other tissues.

The globulin profile behaved proportionally to the nutritional requirement in each physiological condition, with a progressive increase from the peak of lactation ( $\geq 14$ -27 days) until the end of lactation, when the demand decreased due to lower milk production. Moreover, our results showed a decrease of serum albumin and protein values in the peripartum period, specifically on the day of birth, with subsequent increase during lactation. This decline can be expected to occur starting in the last third of gestation until peak lactation, due to three main factors. First, there is a decrease in consumption of dry matter and crude protein in late pregnancy and early lactation. Second, there is a larger requirement of the fetus in this stage, with 70% of growth happening in the last 50 days of pregnancy (Russell, 1979). Finally, there is higher nutritional requirement until peak milk production (Houdijk, 2008), with changes in hormone concentrations to promote gluconeogenesis and mobilization of adipose tissue to provide energy to the fetus and the mammary glands (Rodrigues *et al.* 2007). However, the decrease in serum albumin and protein levels occurred only on the day of delivery, although food consumption (DM and CP) apparently met the animals' requirements, as demonstrated by maintenance of body condition throughout the experiment. The specific reasons for the reduction in these parameters only on the lambing day remain to be determined.

The urea profile is directly related to protein intake and renal function; changes in its level indicate possible kidney disorders.

As expected, THE UREA VALUES FOUND IN THIS STUDY FOLLOWED the protein intake pattern in the different physiological stages, with decrease from gestation to peripartum periods and subsequent increase in the lactation period. Variations in creatinine were not related to these periods and were always below the normal range for the species (1.2 to 1.7 mg/dL).

Calcium values increased from the gestation peripartum. There was a decrease in the level of this mineral during peak milk production ( $\geq 14$ -27) in relation to peripartum, which might be related to milk production requirements. However, endocrine control of calcium means calcium is not a good nutritional indicator, although this mineral is closely associated with metabolism in the peripartum period (Gonzalez, 2000). The small variation in serum phosphorus profile was not related to the physiological condition. However, in some stages of gestation, peripartum and lactation, values lower than the normal ones for other sheep breeds were noted (5.1 to 7.3 mg/dL, Gonzalez *et al.*, 2006). In the postpartum period, this mineral -which is an indirect indicator of energy metabolism- can be used in the form of adenosine triphosphate, which would cause a decrease in its concentration (Feijó *et al.*, 2014).

According to Borjesson *et al.* (2000), glucose concentration in ruminants is readily altered with stress and excitement due to catecholamine release. Since the assessed periods can be considered stressful, especially peripartum, the changes noted can be considered normal. However, ruminants have an excellent mechanism for maintenance of glucose, keeping it at relatively constant concentrations. This makes observations of large changes unlikely, because only very severe dietary restrictions would lead to hypoglycemia (Peixoto *et al.*, 2010).

An increase in AST levels is associated with liver and/or muscle damage. However, our results were within the normal range for the species and in accordance with those reported by Avellanet *et al.* (2007) for the Xisqueta breed (66.0 to 194.0 U/L). Similarly,

increased concentration of blood bilirubin is most commonly caused by hemolysis or liver diseases (Raposo *et al.*, 2004). Batista *et al.* (2009), studying healthy sheep in two age groups (3-5 years and over 5 years), found mean values of total bilirubin of 0.046 and 0.037 mg/dL, direct bilirubin of 0.020 and 0.016 mg/dL, and indirect bilirubin of 0.026 and 0.021 mg/dL, respectively. However, according to Pugh (2005), normal values for the total and direct bilirubin are between 0.1 to 0.5 mg/dL and 0.0 to 0.27 mg/dL, respectively. Thus, despite the variation found in different physiological periods, just total bilirubin on the delivery day had a lower average than what is considered normal for the species.

There was little variation in cholesterol levels throughout the experiment, with lower values in the peripartum and at the end of lactation -when the animals were already at a lower nutritional level to promote drying of milk. Ribeiro *et al.* (2004) found no difference in cholesterol levels between empty and pregnant ewes and suggested that blood metabolites do not vary with the reproductive status of well-fed sheep. However, Peixoto *et al.* (2010) reported that formation of steroid hormones during gestation causes part of the cholesterol in the blood to be reabsorbed, decreasing its level at that stage.

Mendonça *et al.* (2005) reported that serum changes of some minerals, including iron, can result from infectious diseases of ruminants, including mastitis. Kaneko *et al.* (1997) considered serum iron values between 166 and 221 mg/dL to be normal for the species. Despite the variations in periods, the concentrations remained within the normal range.

The results of this study indicate that the peripartum period exerts an influence on the hematological, parasitological and biochemical parameters of sheep. Nonetheless, correct knowledge and interpretation of physiological parameters during this phase can prevent mistaken diagnosis and provide adequate support during this stage.

## Declarations

### *Funding*

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (Finance Code 001), and by the Foundation for Research Support of the State of São Paulo (Proc. Fapesp 2009-54564-7).

### *Conflicts of interest*

The authors declare they have no conflicts of interest with regard to the work presented in this report.

### *Author contributions*

Caroline M G David - collected the data and wrote the manuscript.

Ricardo L D Costa - conception of the study; administered the project and reviewed the manuscript.

Guadalupe A E Parren - collected the data.

Miguel A S Rua - collected the data.

Ellen C P Nordi - collected the data.

Cláudia C P Paz - statistical analysis.

Celia R Quirino - critical Reading of the manuscript.

Rogério S Figueiredo - collected the data and conducted laboratory analysis.

Elizabeth Bohland - laboratory analysis.

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